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Effect of extended heat stress in dairy cows on productive and behavioral traits

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HIGHLIGHTS

1. Heat stress and strong heat waves affect productivity and behavior of dairy cows.
2. Rest time and milk yield are the two traits mostly affected by increase in Temperature-Humidity Index.
3. Heat stress has a different impact on parity and lactation stage on several traits.
4. Rest time can be used as parameter to describe the effects of heat stress in dairy cattle.
5. Heat stress increases activity and compromises milk delivery and production.

Abstract

This study evaluates the response of dairy cows to short and extended heat stressing conditions (from 1 to 28 days), as expressed in changes in their behavior. Due to climate change, heat stress and strong heat waves are frequently affecting productivity and behavior of dairy cows. In the five years under study from 2018 to 2022, two were characterized by extremely strong heat waves occurring in the region analyzed in this study (Northern Italy). The dairy cattle farm involved in this study is located in Northern Italy and includes about 1

600 Holstein Friesian lactating dairy cows. Phenotypic data were provided by the Afimilk system and compromised behavioral and productive traits. Behavioral traits analyzed were: activity, rest time, rest bouts, rest ratio, rest per bout and restlessness. Production traits were daily milk yield, average milking time, somatic cells count, fat percentage, protein percentage and lactose percentage. Climate data came from the NASA/POWER database. Heat stress was analyzed considering Temperature-Humidity Index (THI) averaged over 28 different time windows of continuous heat stress. Results showed that rest time and milk yield were the two traits most affected by the increased THI. Rest time was immediately affected by high THI, showing a marked decrease already from 1d window and maintaining this all over the other windows. Furthermore, results show that rest time and rest ratio were only slightly negatively correlated with milk yield (-0.14 and -0.15). In addition, heat stress has a different effect depending on parity and lactation stages on the studied traits. In conclusion, the results indicate that heat stress increases activity and compromises milk production, rest time and milk quality traits. Results further suggest that rest time can be a better parameter than activity to describe the effects of heat stress on dairy cattle. The novel approach used in this study is based on the use of different time windows (up to 28 days) before the emergence of undesired THI and allows to identify the traits that are immediately influenced by the undesirable THI values and those that are influenced only after a prolonged heat stress period.

Keywords

Cattle, heat stress, Temperature-Humidity Index, milk yield, activity traits

Implications

The response of dairy cows as expressed in changes in their behavior and milk production to prolonged heat has been explored in this study. Climate change and strong heat waves are becoming more frequent. Our results indicate that heat stress increases activity and compromises rest time and milk production. Furthermore, heat stress has a different impact on parity and lactation stages on all the studied traits. Considering that climate change could cause a steady increase in temperatures in the next decades, management improvements are fundamental as forced ventilation and cooling, barn isolation and the integration of a genetic approach.

Introduction

In recent years, several improvements to dairy cattle management, health and welfare have been achieved improving animal wellbeing (Bell et al., 2011; Halachmi et al., 2019; von Keyserlingk & Weary, 2017). Among the most important problems that remain unsolved, the effect of heat stress can be listed (Mikovits et al., 2019). Heat stress has a huge number of acute or chronic effects which can persist life-long (Bernabucci et al., 2014; Polsky & von Keyserlingk, 2017; West, 2003). Among the acute effects, behavioral adaptation strategies of cows such as reduced feed ingestion and increased standing time can be observed, as well as higher resting time in shaded areas and higher drinking volumes, which directly influence milk productivity negatively. Increased susceptibility to illnesses and reduced fertility are instead more related to the long-term effects (Tao et al., 2020). Heat stress is considered a big issue, due to the increase of extreme heat events (Bonaldo et al., 2023).

To cope with it, two major actions can be undertaken, one is acting on the barn micro-environment and consequently on the animals' health, welfare and behavior, and the other is based on the identification of more tolerant animals (Bernabucci et al., 2014; Cheruiyot et al., 2022; Hayes et al., 2009). The building facility, shading curtains, ventilation and cooling systems have become more important (Brown-Brandl et al., 2005; Berman, 2019; Gunn et al., 2019; Schütz et al., 2009; Pinto et al., 2019). Since several authors have demonstrated the detrimental effect of heat stress on cows and genetic improvement can only be effective on the long term, improving the living environment can be a practicable solution. The monitoring of the internal barn environment and the adoption of microclimatic control strategies can be performed by installing sensors able to measure temperature and relative humidity, and to activate forced ventilation and cooling systems based on the resulting Temperature-Humidity Index (THI) (Bohmanova et al., 2007). Such instruments have been widely studied and validated (Stygar et al., 2021; Bar et al., 2019; Zhang et al., 2021). It is nowadays assured that farms able to control the barn climate can take advantage of improved health, welfare, and productivity in a cost-effective manner (Balaine et al., 2020; Rojo-Gimeno et al., 2019). Generally, in countries where the production system is composed of many small herd size farms, there is a low diffusion of this technology (Lora et al., 2020). Furthermore, building size can be limited by the geopedological conformation of the country with many mountain areas. As an example, in 2021, 74% of total dairy cows in Italy were farmed in the Northern regions of the country (CLAL, 2023), which is recognized as a highly intensive livestock area when referring to the plain areas (Lovarelli et al., 2022). In the whole country, slightly more than 1.6 million dairy cows are farmed (CLAL, 2023). However, the average herd size is still quite small in most of the regions, and only in recent years (after 2016) the average herd size has been increasing. In the plains (Po' Valley) of Northern Italy more than 50% of farms currently herd between 100-500 heads, while about 20% of farms host more than 500 heads (ISMEA, 2023). In these areas, technology is more widespread than in other regions, especially for what concerns tools dedicated to the microclimate control, the milking operations and the animals' behavior (Abeni et al., 2019; Lora et al., 2020).

Although controlling the barn environment is the most direct and fast action to complete, it may not be sufficient to prevent heat stress. This could happen when a building is not properly designed and when environmental conditions are too extreme (Bonaldo et al., 2023) or animals' management is not accurate (Das et al., 2015; Polsky & von Keyserlingk, 2017).

In this context, the aim of this study is to evaluate the relationship between environmental conditions monitored in a dairy cattle farm and the behavioral measures: by first understanding the impact of increasingly heat stressing conditions on the behavioral traits and how these reflect on the productivity of the animals based on the analysis of prolonged heat stress up to 28 time windows prior to the recorded measures. This approach, based on the adoption of up to 28-d time windows, allows a complete assessment of the effects of prolonged heat stress on dairy cows. Furthermore, the analysis of different behavioral and milk production traits allows to understand which traits are immediately influenced by undesirable THI values, and which are affected only after a prolonged period.

Material and methods

Farm description

Data used in this study came from a large commercial facility located in the Po Valley, North of Italy. This dairy cattle farm was monitored from 2018 until 2022, following a positive agreement with the farmer (data owner). The study was approved by the Ethics committee of the University of Milan, number OPBA_67_2021 of 16/07/2021.

This dairy cattle farm is one of the largest in the country. The farm has about 1,600 lactating Holstein Friesian dairy cows, with an average milk yield of 40 kg/d per cow and a total production of about 13 000 kg of milk per cow per lactation. Lactating cows (almost 1 200) are housed in deep bedded cubicles in a loose-housing system, while dry and post-calving cows lie on permanent straw litter. The farm is equipped with a ventilation and cooling system consisting of different type of fans based on the different areas (Dual Blade, Dryer and Ceiling fans Air2 systems, TDM, San Paolo, Brescia, Italy) and a cooling system in the feeding area. The Dual Blade fan is composed of two aluminum blades. The fan is fixed with an inclination of 70 degrees towards the ground and moves air at a speed higher than 3 m/s directly towards the animals. The fan is used in combination with the use of water. In a first phase, the animals are sparkled with a large drop system, when the water stops, the fan will start and dry the animals' skin, thus lowering their body temperature. Both ventilation and sprinklers start automatically with the TEN Air2 control unit that runs based on the barn temperature measured by environmental sensors. The fans start at the temperature of 17.7°C and reach the maximum power at 24.8°C. The cooling system with the use of water starts at 29.3°C and reaches the maximum power of functioning at 35.4°C. Concerning the herd management, the Afimilk system (Afifarm 5.5 System, TDM, San Paolo, Brescia, Italy) is adopted. This softwareincludes data on animal behavior and milking. The first is monitored through pedometers (Afitag II, TDM, San Paolo, Brescia, Italy) that serve to monitor the cow behavior individually (e.g. activity, rest time, rest bouts), allowing to identify estrus cycles. The milking parlor is equipped with a milk meter with the related control unit (Milk Module and Afimilk MPC, TDM, San Paolo, Brescia, Italy), which records milk production and milk parameters per cow per milking (e.g. milk electric conductivity to early alert about the possible emergence of mastitis). Milking takes place in two independent ‘25+25’ parallel milking parlor and cows are milked three times per day.

Data set

Environmental data

Climate data used for this study was obtained from two different sources. First, data on temperature and relative humidity were collected on an hourly basis from six weather stations installed in the barn (YGRO-10 and ST-10K, FR Systems, Brescia, Italy). Second, from the National Aeronautics and Space Administration/Prediction of Worldwide Energy Resources database (NASA/POWER, https://power.larc.nasa.gov). Data were obtained for temperature (**Temp**) and relative humidity (**RH**). The Temperature-Humidity Index (**THI**) was calculated based on the equation by Vitali et al. (2009).

Two sources of information (farm data and NASA data) were considered as environmental descriptors, but eventually the NASA/POWER data was used for this study since the farm data showed a large number of missing records due to technical problems during the 5-year study. However, a correlation of 0.99 was found between the two data sources for THI at least for the records present from the farm weather stations (results not shown). This high correlation was due to the fact that for the majority of the days the ventilation system was not activated. Thus, NASA/POWER climate data was extracted and made available for over

5 years (from 2018 to 2022). In total, 43 824 records were available on the environmental micro-environment.

Herd data

Regarding the monitoring of animal behavior and milk production, all data were provided by the Afimilk system. Daily data was used for the analysis. The daily animal behavior measures were available for: activity time as the number of steps per hours (**Activity**), rest time as the total time spent laying (**RestTime**), number of rest bouts (**RestBout**) and rest time per bout (**RestPerBout**). Then, rest ratio (**RestRatio**) is the mean time (in %) of rest time per session and between milkings, and restlessness (**RestLessNess**) is provided by Afimilk as the ratio of activity time on rest time (see table S1 for further detail). Production traits were registered on a per-milking basis and then processed to obtain daily milk yield **(Yield**) and average milking time (**MilkingTime**). Routine test-day monitoring also provided information for somatic cells count (**SCC**), fat percentage (**Fat%**), protein percentage (**Pro%**) and lactose percentage (**Lact%**). Measures of SCC, Fat%, Pro%, Lact% were recorded monthly and adhered to the International Committee for Animal Recording standards. A description of each behavioral measure is reported in supplementary table S1.

Data on animal behavior and production were collected data from 01/01/2020 to 10/12/2022. Data editing included removing of outlier records, with a procedure reported in supplementary table S2. Only records falling between 6 and 600 days in milk were retained for analyses. In total, after editing, 1 555 668 records from 3 317 cows were available for behavioral measures and milk yield, while less than 30 000 records were available for milk quality measures on the same cows. The dataset contained 746 950 records from first lactation, 515 173 records from second lactation and 293 545 records from third and later lactations.

Statistical analysis

Data handling, calculation of descriptive statistics, statistical analysis and production of graphs were all performed in the R statistical environment (R Core Team, 2022), version 4.1.3. First Temp, RH and THI were summarized, then merged to the phenotypic records creating a dataset reporting ‘one record per cow per day’. Each of the records showed information for animal behavioral and productive measures in different columns. An additional column reported THI as a climatic indicator. Based on that, twenty-eight different time windows were created, averaging THI during 1) the day of recording (test-date), 2) the day of recording and the previous day, 3) the day of recording and the two previous days, 4) the day of recording and the three previous days, and so on until the 27 days prior to test date. Each time window defined a different climatic indicator, for a total of twenty-eight (continuous) indicators. These were then converted to class indicators, with fourteen classes being formed, with specific breaking values taken and adapted from Lovarelli et al. (2021). The values are reported in supplementary table S3.

The relative impact of each of the 28 climatic indicators on each behavioral and productive measure was tested. A linear mixed model was fitted, such as:

$$y_{ijklmn} = LAC_i + SOL_j + CLIM_k + LAC_i * CLIM_k + LAC_i * SOL_j + SOL_j * CLIM_k + LAC_i * SOL_j * CLIM_k + cow_l + td_{m:k} + err_{ijklmn}$$

Where y_{ijklmn} is the behavioral or productive measure, LAC_i is the fixed effect of the i^{th} lactation number (3 classes: first, second and third and later), SOL_j is the fixed effect of the j^{th} SOL (10 monthly classes, with the last class being open to 600 days in milk), $CLIM_k$ is the fixed effect of the k^{th} class of climatic indicator (14 classes, as defined above), cow_l is the random effect of the l^{th} cow, $td_{m:k}$ is the random effect of the m^{th} test-date (nested within class of climatic indicator), err_{ijklmn} is the residual error. The model also included all two-way and three-way interactions among the fixed effects. The solutions for each random effect (cow, test-date and residual) were considered normally distributed with mean equal to '0' and variance equal to values estimated from the data. All models were implemented in R using the "lmer" function of the "lme4" package (Bates et al., 2015).

Each of the twenty-eight climatic indicator was tested for its effect on each behavioral and productive measure (eight in total), leading to 224 model runs. Since one single climatic indicator was to be chosen for the analysis a specific behavioral or productive measure, the following procedure was applied. First, each of the 224 models was implemented and the Deviance Information Criterion (DIC) was stored. For this step, a Maximum Likelihood estimator was used (option 'REML=FALSE' in *lmer*). The trait-specific climatic indicator (i.e. time window length) was chosen for giving the strongest decrease in DIC while increasing the time window length by 1 day. For each trait, the DIC values were ranked according to an increasing time window length, and difference in DIC value was calculated for each 1 d increase in time window length. The climatic indicator (time window length) which generated the largest change (decrease) in DIC was chosen as the most appropriate descriptor. All the generated DIC values together with the chosen indicator are reported in Supplementary Table S4. Then, the model was implemented again with the chosen indicator using a Restricted Maximum Likelihood estimator (option 'REML=TRUE' in *lmer*) in order to obtain unbiased variance components. The solutions were then calculated using values of variance components from this last run. The Analysis of Variance (type 3) table was generated using the native 'anova' R function (R Core Team, 2022). Least Square Mean estimates (LSM) were then calculated for all the fixed effects in the model using R-package 'emmeans' (Lenth, 2022), *Tukey* contrast values and significance were calculated using function 'cld' from R-package 'multcomp' (Hothorn et al., 2008). All graphs were produced using R-package 'ggplot2' (Wickham, 2016). Statistical models used in the analysis and relative R code are available as supplementary material S1.

Results and discussion

Descriptive statistics

The study was based on a 5-year timeframe (from 2018 to 2022), where environmental and herd data was collected on a daily basis on a large dairy cattle farm in Northern Italy. Environmental data was available from 2018 to 2022, while herd data from 2020 to 2022. In this period, both 2019 (THI yearly mean equal to 60.3) and 2022 (THI yearly mean equal to 62.3) were two years characterized by particulary warm summer weather, affecting the THI and consequently animal production and behavior. As climate change is moving towards the direction of increased temperatures and strong heat waves (Bonaldo et al., 2023), the inclusion of these two years in the analysis brings insights in the future management of heat stress in dairy cattle.

Table 1 shows the mean monthly THI per each year. The most undesirable conditions can be observed from May to September. In particular, July and August show mean THI values above the threshold of 72, and this occurs constantly for a considerable part of each month. In July, except for 2020 (21 days) and 2021 (26 days), in 2018, 2019 and 2022, between 29-31 days recorded mean THI above 72. In August this occurred for 26, 29, 25, 19 and 28 days, from 2018 to 2022, respectively.

Table 2 shows the descriptive statistics of the whole herd in the monitored period. Average milk production resulted 40±10.4 kg/d, with an average milking time of 336±104 seconds/cow. Behavioral traits and milk yield were all recorded on a large number of time points allowing further detailed analyses on the effect of heat stress. Instead, milk quality data were excluded from further analyses as few records were available. Calving events were evenly distributed across the year despite some reduction during the spring, with September and November being the months with the highest calving rate (~11%) and April and May the months with the lowest (5-6%, results not show in table).

Table 3 reports the Pearson correlation matrix among the tested traits. All correlations are significant. As expected, the correlation between milk yield and quality traits is in line with current literature results (Martinez-Castillero et al., 2021). Furthermore, all traits associated with activity or rest are strongly correlated. MilkingTime is positively correlated with MilkYield (0.27). Restlessness is positively related to Activity (0.60) and negatively related (-0.65) to RestTime, as expected.

Considering the findings by several authors (Allen et al., 2015; Herbut et al., 2021; West, 2003), heat stress is not only an instantaneous condition, but can endanger cows with lifelong chronic effects. Following Ouellet et al. (2019), we analyzed every possible stressing condition taking into account intervals from 1 to 27 days of continuous heat stress based on temperature, relative humidity and THI.

The effect of environmental conditions (i.e. temperature, relative humidity and THI) was included in the model described above, based on the Deviance Information Criterion (DIC) reported in supplementary table S4. Results of analysis of variance identified that all effects included in the model were highly significant.

As THI described heat stress effects more than the other environmental variables of temperature and relative humidity, the focus of analyses was on THI (data not shown). Figure 1 shows Least Square Means for the six main traits that were analyzed considering THI averaged over the 3 to 18-days window depending on the trait. Interestingly traits are affected differently based on the duration of the heat stress. Therefore, studying the proper time window for each trait results essential to acquire a correct view of the effect of THI on the herd traits. Regarding milk yield, already some decades ago, Linville and Pardue (1985) found that milk yield was negatively affected by THI>74 during the precedent 4 days and by THI>80 during the precedent day. Heinicke et al. (2019) studied the cow behavior over the 3 days before the test, showing that when the heat load was prolonged, the activity response of cows reduced, especially in multiparous cows. In our study, long time windows (up to 28 days) have been analyzed to evaluate the effect of THI over productive and behavioral traits, allowing to identifying the single best time window to study each trait.

RestTime and MilkYield were the two traits most affected by the increased THI over the 18 and 15-days window respectively.

For MilkYield we observe a decrease in production in both high and low values of THI, suggesting the occurrence of both heat and cold stress. Least square means of milk production were estimated to be 34.9±0.12, 40.3±0.12 and 41.5±0.13 kg/d respectively on first, second and third lactation (results not shown in figures) all showing significant differences given by the lactation number, which reflects on what reported in figure 2, showing MilkYield per parity (1st, 2nd and 3+ lactations) per stage of lactation (SOL) considering the different THI classes. This figure shows that 1st lactation cows are less disturbed by THI increases over all lactation stages, while 2nd and 3+ lactation cows highlight a stronger reduction in the production of milk from the THI classes n. 13 (THI 72-75) and n. 14 (THI 75-77). This results in line with most literature findings (Bernabucci et al., 2014; West, 2003) and can be explained by the lower milk production and by the lower metabolic heat production of primiparous than multiparous cows (Becker et al., 2020), however contradictory results are also available (Leliveld et al. 2023) indicating that further research is still needed. Further details of the number of records per lactation, lactation stage and THI class are reported in Supplementary Table S5.

Regarding the other production traits, we can conclude that milking time follows the same trend of milk yield as THI increases, showing a less regular pattern.

As far as the behavioral traits of Figure 1, RestTime decreased already at THI between 51-54 points and continues to reduce radically at the increase of THI. Primiparous cows rest more than 2nd and 3+ lactating cows, especially during the first lactating months (Figure 3). Least square means of RestTime were estimated to be 672.8 ± 1.52, 657.4 ± 1.52 and 657.2± 1.60 respectively on first, second and third lactation with primiparous cows showing significant difference based on pairwise contrasts. From SOL beyond the 10th month of lactation, the decrease of RestTime based on increased THI is less evident than in the previous months of lactation, probably due to a higher susceptibility of cows during the first lactation stages or due to the carry-over effect of metabolic stress during the lactation. Moreover, this effect could be also due to the physiological decrease in milk production at the end of lactation, as when cows are more productive they are also more susceptible to heat stress and hence spend more time resting (Balaine et al., 2020). The difference in RestTime between a condition of low/medium THI and when THI exceeds 63 points is more evident and all cows show a very similar response to hot weather. With the possible increase of heat wave events, rest time appears to be one of the most affected traits. This itself represents an alarming signal for the farmer, who can decide to act with management solutions that are more in line with cow requirements (e.g., prefer cooler areas for 2nd and 3+ lactating cows, reduce calving at the beginning of summers, etc.).

Concerning the other behavior traits, Activity increases markedly from THI between 60-63 points; instead, when THI is above 72 for a prolonged time it decreases slightly, probably due to the start of the ventilation system. Primiparous cows are more active than secondiparous cows and, especially, in later lactations. Regarding the number of rest bouts (RestBouts), it increased with THI at 54-57 points and from THI between 60-63 they maintain estimates higher than 11.5, so this class can be considered as the starting point of a thermal distress condition of cows. Differently from Activity, rest time and RestBouts do not seem to be positively influenced by the ventilation effect when THI is larger than 72. Instead, the RestPerBout reduces significantly when THI in the barn increases, similarly with the rest time. RestRatio showed a pattern similar to RestTime, being derived from it, likewise

Restlessness showed a pattern similar to Activity. Results for these measures are reported in supplementary Figure S1.

To highlight the effect of a prolonged duration of each THI class, Figure 4 and Figure 5 show the variation of MilkYield and RestTime, respectively, at the different THI classes when considering windows of daily (1 day) or prolonged (from 2 to 6 days) duration of THI.

Figure 4 (top left graph) reports the MilkYield when daily THI is considered, whereas Figure 4 (remaining graphs) shows how MilkYield changes if the THI class is prolonged for a longer interval. In THI windows of 1 and 2 days, the average MilkYield in the most extreme THI class (75-77) is above 38 kg/d, while when THI lies between75-77 in the windows of 5d or 6d, the average MilkYield is close to 37.5 kg/d.

Figure 5 shows that RestTime is immediately affected by high THI, showing a noticeable decrease already from 1d window and maintaining this all over the other windows. Furthermore, the significant reduction of RestTime occurs already at THI between 48-51 points. Then, other steps can be identified, with marked reductions at THI reaching 57-60 points, then at THI between 66-69 and finally at THI between 75-77. As conclusion we can see that 48-51 THI points can be seen as a putative cut-off level of tolerance.

Figure 6 shows THI and milk yield over the 3 months of summer (June, July and August) in the last three years of the study (2020, 2021 and 2022). The model used here was based on THI conditions lasting for at least 7 days. Depending on the severity of the selected class, this condition can occur more or less frequently. In summer, it is very frequent to observe THI above 72 points for a long period, whereas THI above 75 points is still quite infrequent to be observed for such a long time, based on the data on the last five years (Table 1). However, in the studied farm, this negative trend is increasing in frequency and duration, therefore we can expect that the effect of the most extremely hot classes will become more evident in the future.

Options to deal with such stressing conditions can be identified. The first includes the use of powerful and properly designed ventilation and cooling systems, which is currently the most practical solution for farmers. This solution has also been widely studied, commonly resulting in the importance of forced ventilation and cooling, which is cost-effective even if it brings additional costs to the natural ventilation systems (Honig et al., 2012; Pinto et al., 2019). Furthermore, shaded areas and unpaved spaces can be useful (Bar et al., 2019; Schütz et al., 2009). In agreement with Leliveld et al. (2023), an interesting option could be to group animals in the barn based on their susceptibility to heat stress, giving to more susceptible cows the most comfortable spaces (e.g., cooler areas, areas where forced ventilation or cooling systems can be started at earlier stages of THI levels than others). The second option includes the design of new barns, characterized by closed environments with cross or tunnel ventilation systems (Pakari & Ghani, 2021) or with positive-pressure precision ventilation systems (Jung et al., 2023), which can help to control much more the internal barn environment but can be expensive (Mondaca & Cook, 2019) and are still quite far from the traditional Italian farming practices. Finally, the last opportunity that has been of raising interest is the selection of dairy cows for heat stress. In the last years several studies have already been done on the genetic determination of heat tolerance in dairy cattle (Bernabucci et al., 2014; Cheruiyot et al., 2022; Nguyen et al., 2016), but further research is needed based on detailed daily phenotypic records that can surely gasp all the individual variability in heat tolerance and recovery time after strong heat waves.

One of the main features of our results is the availability of 3 years of records related to milk and behavior traits and 5 years of environmental information allowing to draw solid conclusions. One further novelty of our study was the use of extended time windows that allowed to understand that some traits are rapidly affected by heat stress, while others require more days to be affected. Although the study was based on a one single farm, the farm hosted a large number of animals (more than 3 000 cows over three years) that were all kept under the same management and environmental conditions that highlight individual response to heat stress, also depending on lactation stage or parity.

Conclusions

In conclusion, the results of our study indicate that heat stress increases activity and compromises rest time, milk production and milk delivery. Furthermore, heat stress has a different impact on parity and lactation stages on all the studied traits. Considering that climate change could cause a steady increase in temperatures in the next decades, the novel approach used in this study to evaluate the effects of short or prolonged undesirable THI values up to 28 days for behavioral and productive traits of dairy cows is very interesting, because it can support the identification of the most susceptible traits immediately affected by excessive THI values as well as of those traits more able to respond to prolonged heat stress conditions. Hence, in the near future, improvements able to cope with such stressing conditions are fundamental, among which can be listed the forced ventilation and cooling, barn isolation and the integration of a genetic approach for heat stress tolerance selection in geographical areas more prone to experience increases in THI values and duration.

Ethics approval

The study was approved by the Ethics committee of the University of Milan, number OPBA_67_2021 of 16/07/2021.

Data and model availability statement

None of the data were deposited in an official repository. The data of this study belongs to a commercial dairy farm and can be made available only under request and agreement. The authors can be contacted for specific requests. Statistical models used in the analysis and relative R code are available as supplementary files of this manuscript.

Declaration of generative AI and AI-assisted technologies in the writing process

The authors did not use any artificial intelligence assisted technologies in the writing process

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M. Guarino: Writing – review & editing, Funding acquisition, Project administration, supervision. **A. Arazi:** data curation, writing-review & editing. **F. Tiezzi**: Data curation, conceptualization, data analysis &writing-review & editing.

Declaration of interest

None.

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Table 1. Mean Temperature-Humidity Index (THI) values and number of days (x) with THI mean above 72 points per month for the monitored period (2018-2022) in the dairy cattle study. The mean THI value and sum of days per year are also reported.

Month	2018		2019		2020		2021		2022	
	Mean THI	Days x>72	Mean THI	Days x>72	Mean THI	Days x>72	Mean THI	Days x>72	Mean THI	Days x>72
January	48.1	.	44.5	.	47.1	.	43.7	.	46.3	.
February	44.6	.	49.5	.	51.3	.	49.6	.	50.0	.
March	49.5	.	55.4	.	52.3	.	52.8	.	51.8	.
April	61.4	.	58.2	.	59.0	.	55.8	.	57.9	.
May	65.6	.	61.0	.	65.1	.	62.1	.	66.9	.
June	71.1	5	73.5	9	68.5	5	71.6	8	73.2	1

July	75.0	24	76.0	23	73.0	15	73.5	18	76.0	17
August	74.8	21	74.6	23	74.3	17	72.8	9	73.7	23
September	69.4	1	68.3	3	68.2	1	68.9	.	66.2	15
October	62.4	.	61.9	.	58.9	.	58.9	.	64.0	.
November	53.2	.	52.2	.	53.3	.	52.2	.	56.0	.
December	46.4	.	48.0	.	45.4	.	45.5	.	.	.
Yearly	60.2	51	60.3	58	59.7	38	59.0	35	62.3	56

Table 2. Sample size and descriptive statistics (mean, median, SD, minimum and maximum values) of the monitored productive and behavioral traits measured in the dairy cattle herd under study.

Trait	N	Mean	SD	Median	Min	Max
Milk yield (kg/d)	1 494 125	39.57	10.4	39.39	0.21	80
Milking Time (s)	1 490 876	336.34	104.73	314	61	1 000
Activity (step/h)	1 677 003	164.23	47.74	156.00	20	500
Rest Time (min/session)	1 621 382	672.07	138.43	679	102	1 199
Rest Bout (n./session)	1 621 366	11.16	3.4	11	1	30
Rest Ratio (%)	1 621 540	46.68	9.58	47	1	80
Restlessness	1 611 434	25.88	17.28	22	10	788

Rest Per Bout (min/session)	1 621 646	65.22	22.95	62	0	250
SCS (Somatic Cell Scores)	29 830	2.9	0.55	2.8	1	4.7
Fat percentage (%)	26 151	3.95	0.94	3.84	0.10	7.50
Protein percentage (%)	16 669	3.25	0.35	3.21	2.11	5.51
Lactose percentage (%)	16 666	4.77	0.21	4.81	3.01	5.52

Table 3: Pearson correlation values among the recorded variables in the dairy cattle herd under study.

Item	Milk Yield	Milking Time	Activity	Rest Time	RestBouts	Rest Ratio	Restlessness	RestPerBout	Somatic Cell Score	Fat %	Protein%	Lactose%
MilkYield		0.27	-0.08	-0.14	-0.05	-0.15	0.02	-0.06	-0.13	-0.27	-0.46	0.15
MilkingTime	0.27		0.03	-0.06	0.01	-0.06	0.05	-0.04	-0.01	-0.19	-0.17	-0.02
Activity	-0.08	0.03		-0.31	-0.09	-0.30	0.60	-0.07	0.04	0.02	0.04	-0.01
RestTime	-0.14	-0.06	-0.31		0.20	**0.99**	**-0.65**	0.39	0.06	0.10	0.21	-0.01
RestBouts	-0.05	0.01	-0.09	0.20		0.20	-0.14	**-0.73**	-0.01	0.01	-0.05	0.03
RestRatio	-0.15	-0.06	-0.30	**0.99**	0.20		**-0.65**	0.39	0.06	0.10	0.21	-0.01

Restlessness	0.02	0.05	0.60	**-0.65**	-0.14	**-0.65**		-0.26	0.00	-0.05	-0.08	0.01
RestPerBout	-0.06	-0.04	-0.07	0.39	**-0.73**	0.39	-0.26		0.06	0.06	0.16	-0.04
Somatic Cell Score	-0.13	-0.01	0.04	0.06	-0.01	0.06	0.00	0.06		0.08	0.15	-0.33
Fat %	-0.27	-0.19	0.02	0.10	0.01	0.10	-0.05	0.06	0.08		0.30	-0.14
Protein %	-0.46	-0.17	0.04	0.21	-0.05	0.21	-0.08	0.16	0.15	0.30		-0.10
Lactose %	0.15	-0.02	-0.01	-0.01	0.03	-0.01	0.01	-0.04	-0.33	-0.14	-0.10	

Figure 1. Least Square Means for the climatic Temperature-Humidity Index (THI) effect on the studied traits in the dairy cattle herd under study.

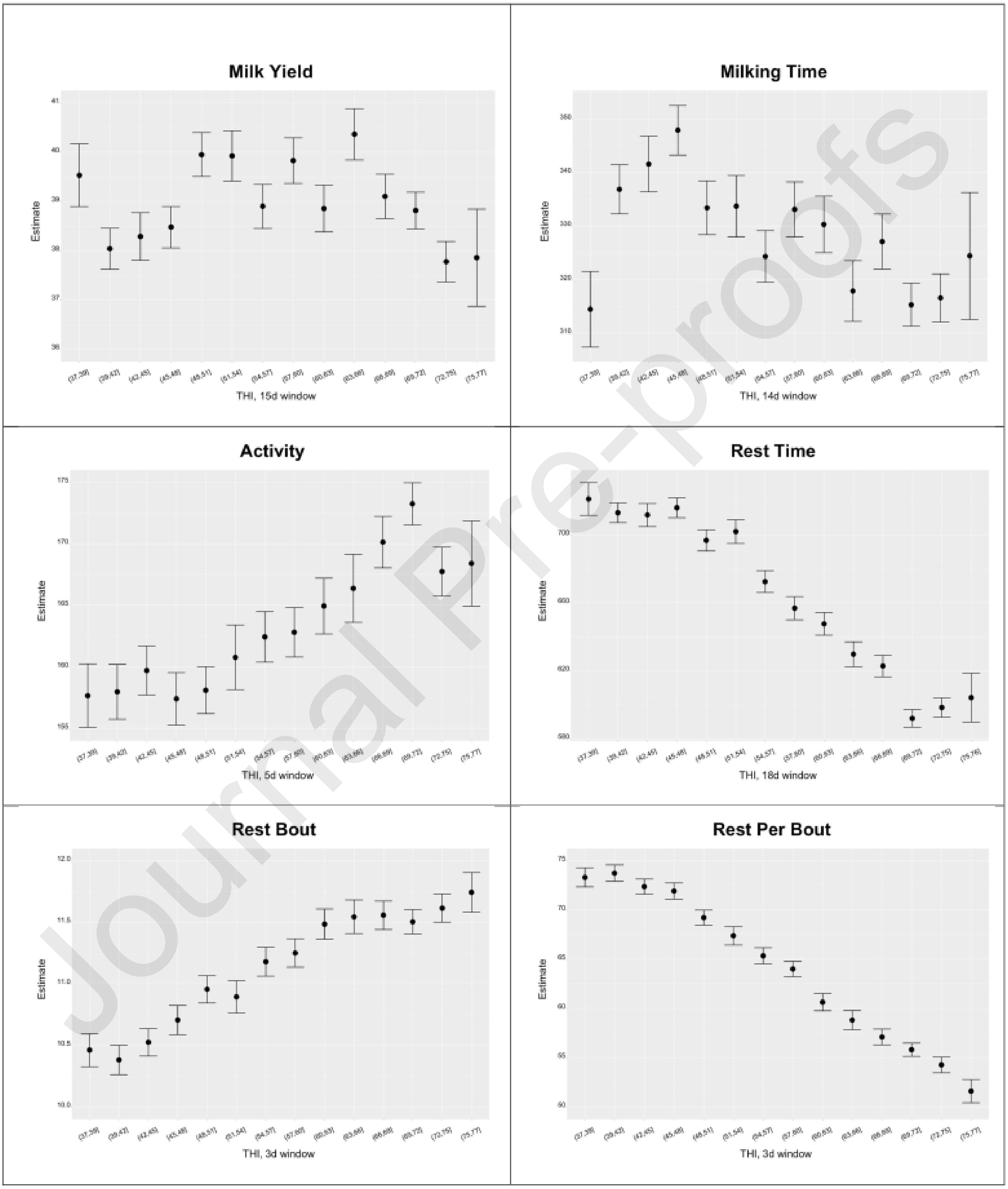
Figure 2. Estimates of milk yield of primiparous (top), 2nd lactation (middle) and 3+ lactation (bottom) cows at different stages of lactation (SOL) and Temperature-Humidity Index (THI) classes.

Figure 3. Estimates of rest time of primiparous (top), 2nd lactation (middle) and 3+ lactation (bottom) cows at different stages of lactation (SOL) and Temperature-Humidity Index (THI) classes.

Figure 4. Estimates of mean milk yield as affected by Temperature-Humidity Index (THI) measured over 1-day to 6-day windows traits in the dairy cattle herd under study.

Figure 5. Estimates of Rest Time as affected by Temperature-Humidity Index (THI) measured over 1-day to 6-day windows traits in the dairy cattle herd under study.

Figure 6. Temperature-Humidity Index (THI) and milk yield over the 3 months of summer (June, July and August) in the last three years of the study (2020, 2021 and 2022) in the dairy cattle herd under study.


Milk Yield
Estimate
36
37
38
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40
41
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THI, 15d window
Milking Time
Estimate
310
320
330
340
350
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THI, 14d window
Activity
Estimate
155
160
165
170
175
(37,39]
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THI, 5d window
Rest Time
Estimate
580
620
660
700
(37,39]
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THI, 18d window
Rest Bout
Estimate
10.0
10.5
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THI, 3d window
Rest Per Bout
Estimate
50
55
60
65
70
75
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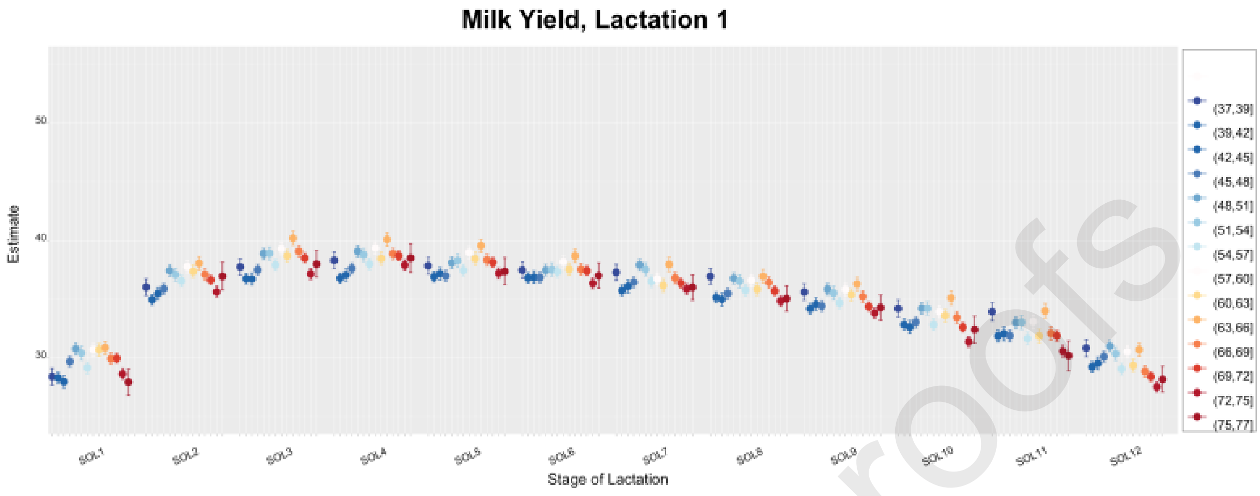
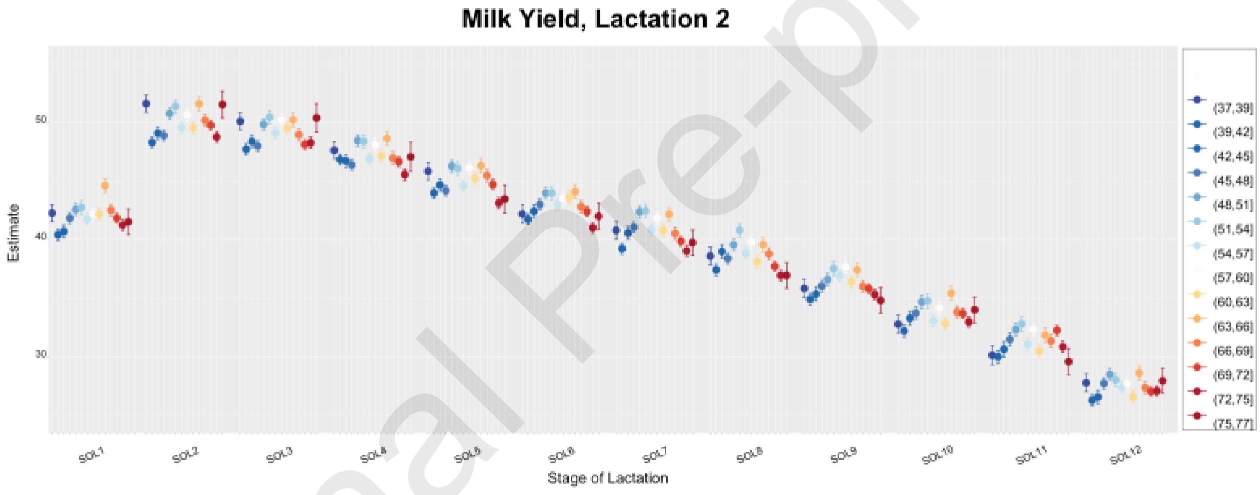
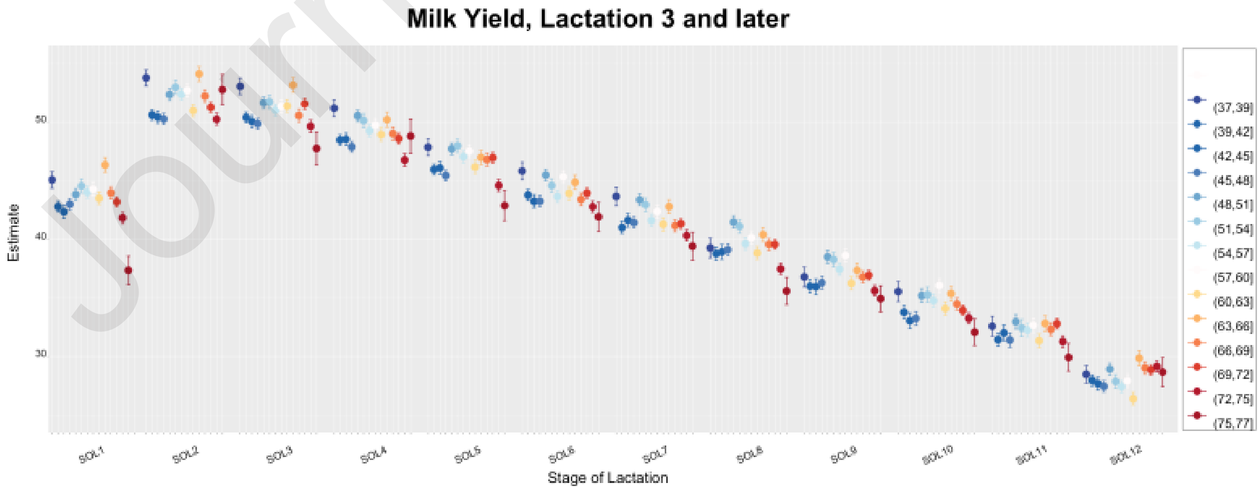
THI, 3d window



Milk Yield, Lactation 1



Milk Yield, Lactation 2



Milk Yield, Lactation 3 and later


Rest Time, Lactation 1
Estimate
800
700
600
500
SOL1
SOL2
SOL3
SOL4
SOL5
SOL6
SOL7
SOL8
SOL9
SOL10
SOL11
SOL12
Stage of Lactation
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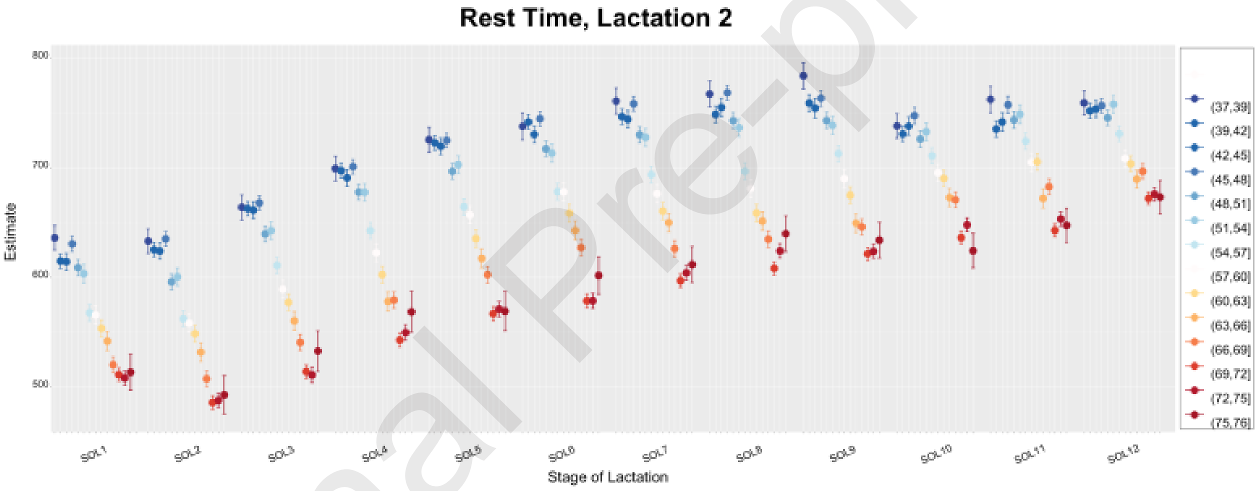
Rest Time, Lactation 2
Estimate
800
700
600
500
SOL1
SOL2
SOL3
SOL4
SOL5
SOL6
SOL7
SOL8
SOL9
SOL10
SOL11
SOL12
Stage of Lactation
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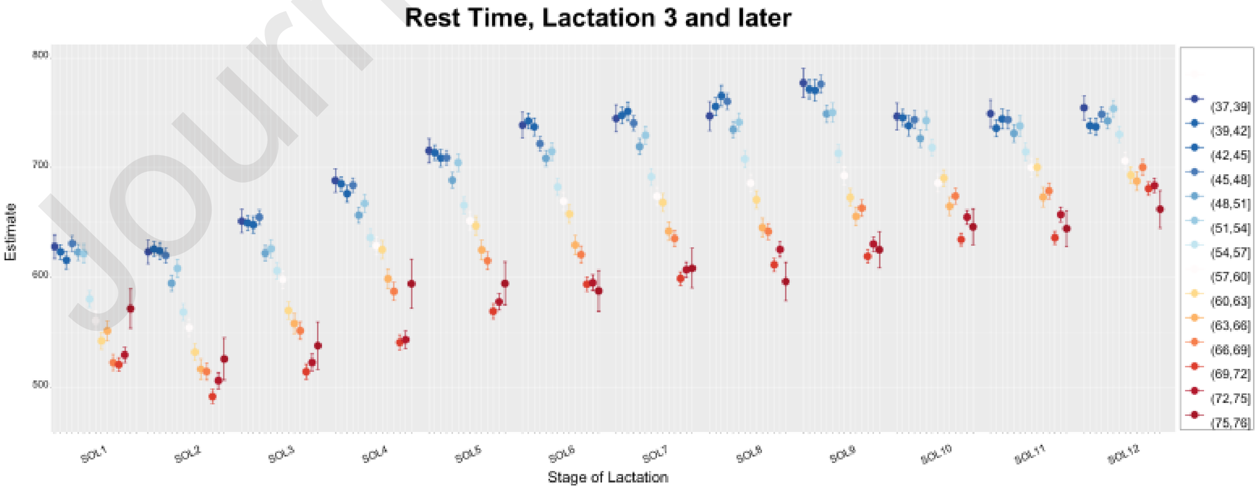
Rest Time, Lactation 3 and later
Estimate
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Stage of Lactation
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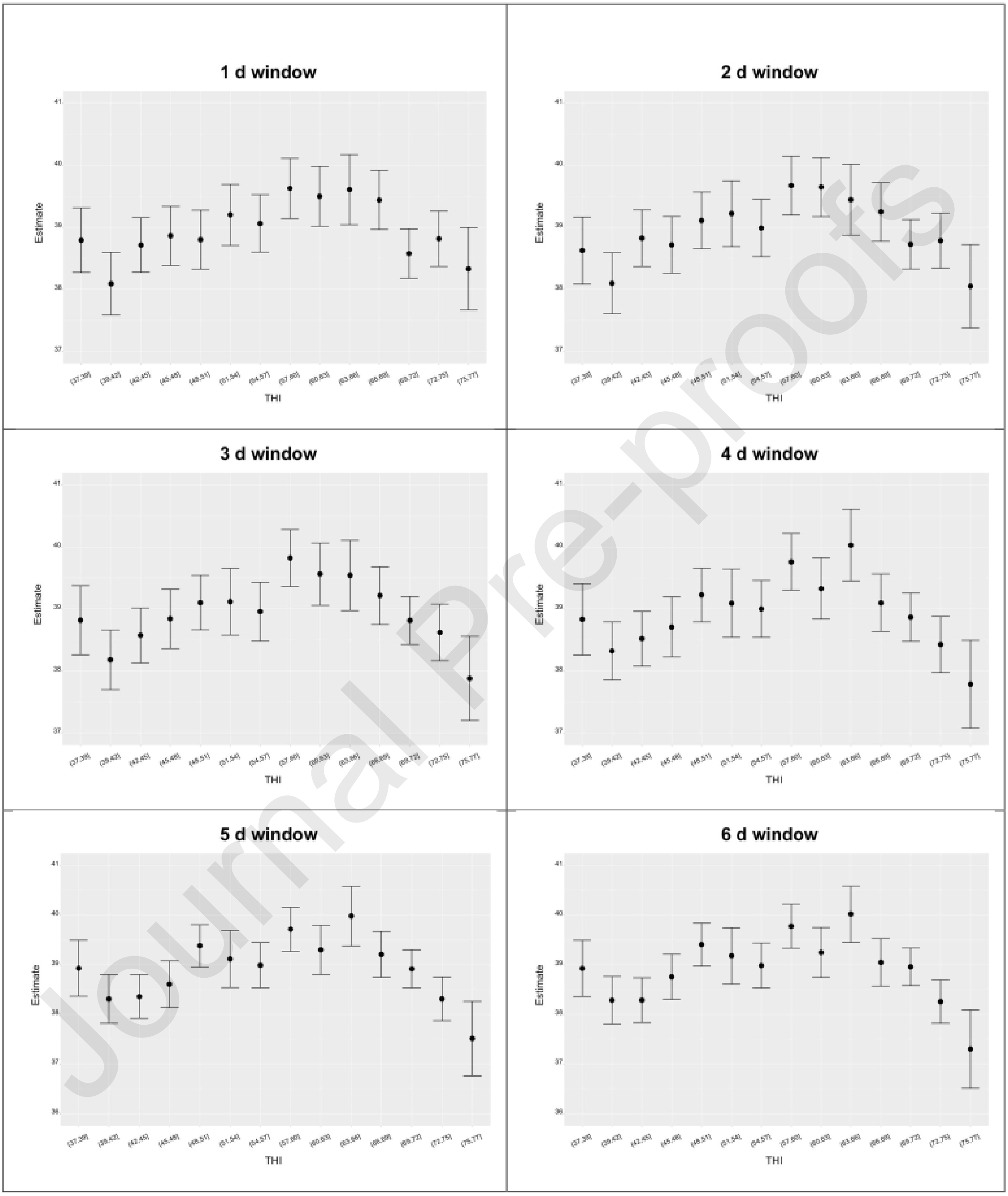
1 d window
2 d window
3 d window
4 d window
5 d window
6 d window
Estimate
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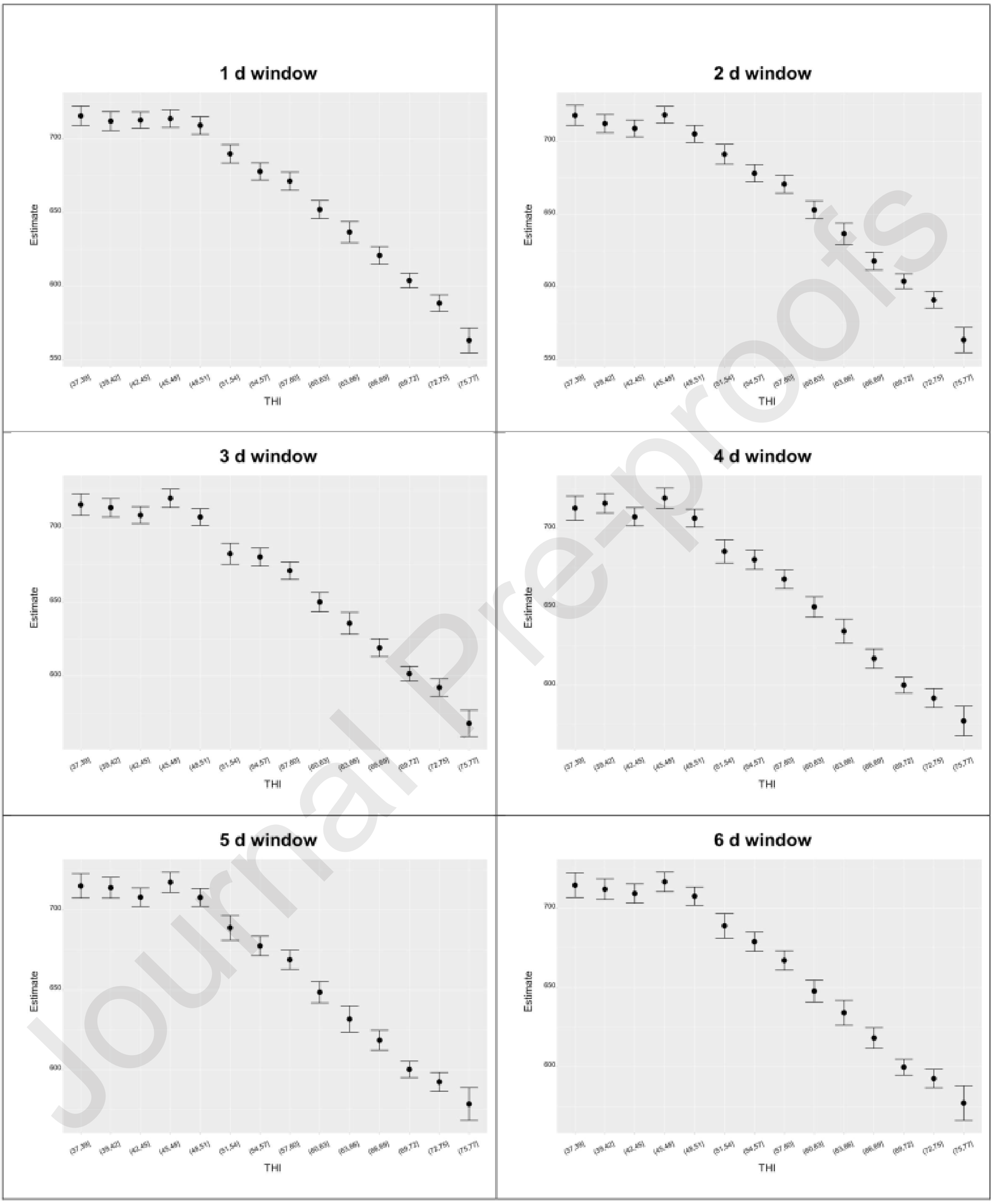
THI


1 d window
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Estimate
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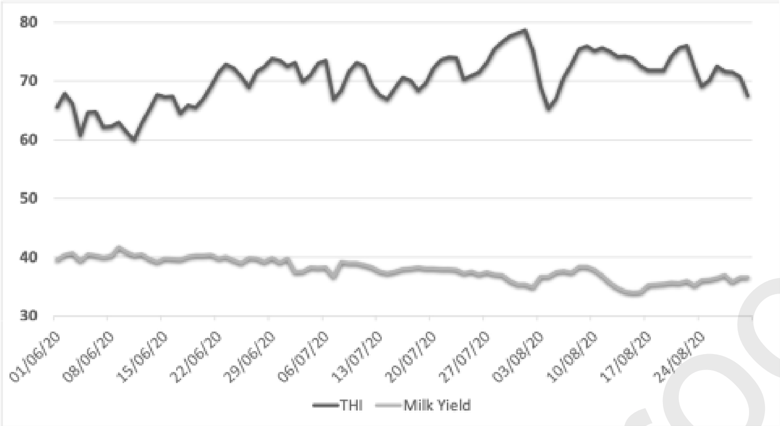
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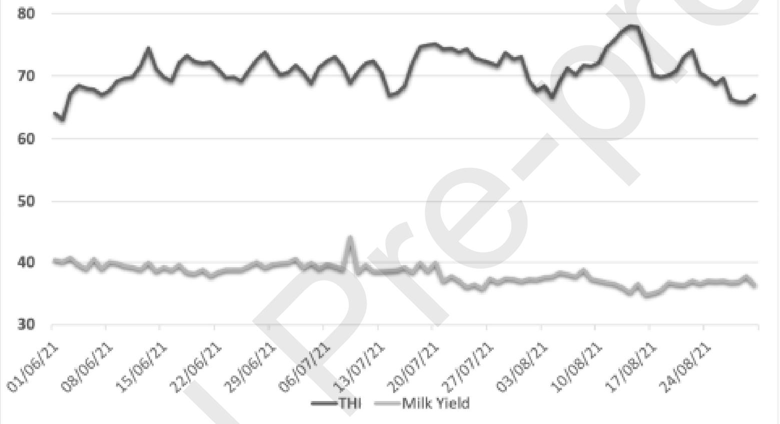
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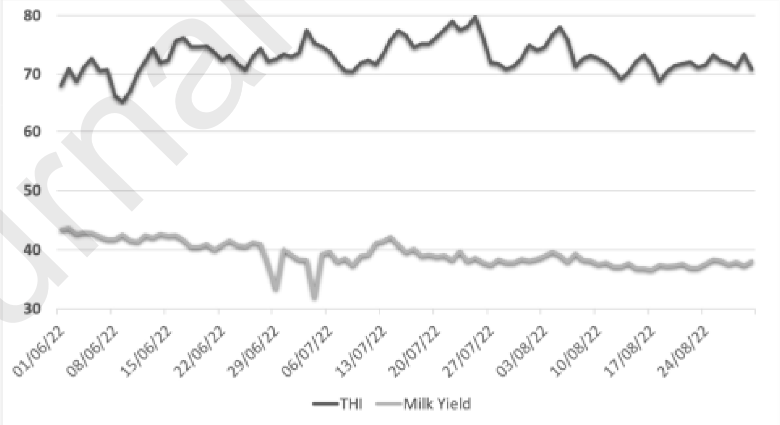
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Milk Yield