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# **Modeling heat stress in dairy cattle using high spatio-temporal resolution observational and grid-interpolated weather data**

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*Dedicated to my late father*

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# GENERAL INTRODUCTION

## Background

The global climate change is on the path of increasing temperature with global warming likely to reach 1.5°C between the years 2030 and 2052 if it continues at the current rate (IPCC, 2018). The projections for Europe are temperature increase in all seasons (Kjellström *et al.*, 2018). In the Alpine region, it is projected that the changes in 2 m air temperature will be positive in all seasons, with an average warming of 0.25°C per decade in the first half of the 21<sup>st</sup> century (Gobiet *et al.*, 2014). In the northern parts of Germany, temperatures are expected to rise by 2°C and precipitation decline by up to 15% in summer months by the year 2050 (Gauly and Ammer, 2020). These events will be in addition to changes in seasonal cycles of precipitation, humidity and global radiation including frequency of hot days and heat waves. In Africa, temperatures are expected to rise faster than the global average during the 21<sup>st</sup> century (Niang *et al.*, 2014). Maximum and minimum temperatures over equatorial eastern Africa are projected to increase by 2°C above the 1981 – 2000 average by mid-century (*ibid.*).

The direct and indirect effects of global warming together with increased frequency of extreme weather events will have serious implications for livestock production, even in moderate climate zones like Central Europe (Gauly and Ammer, 2020). When exposed to temperatures above their thermal comfort zones, livestock suffer heat stress. Already, the frequency of days and heat wave events which cause heat stress in dairy cattle have been reported to increase (Dunn *et al.*, 2014, Nidumolu *et al.*, 2014). Heat stress negatively affects milk yield and alters milk components including protein, fat, solid-non-fat, casein and lactose contents (Hammami *et al.*, 2015). Reduced milk yield is the most negative effect of heat stress because its economic results are visible after a few days (Herbut *et al.*, 2018). In addition to changing the quantity of milk components, heat stress affects milk protein fractions particularly casein which in return affect cheese making properties (Bernabucci *et al.*, 2015).

To assess the animals reaction to changing environmental heat stress indicators, two methods have been developed. The first are a variety of different temperature humidity indices (THI) expressed in absolute units that define the thermal comfort of the cows and the second are algorithms which define the temperature as experienced by the animal (Herbut *et al.*, 2018). The THI, a combination of temperature and humidity initially developed by Thom (1959), remain the most common heat stress indicator for cattle and other livestock species. Over the years the formulae for calculating THI have been modified and corrected numerous times by

various authors. Many other indices have been developed based on measurement of meteorological factors and their interaction with the animals (Gaughan *et al.*, 2008, Herbut *et al.*, 2018, Wang *et al.*, 2018). A literature review was carried out on the developments in animal performance records and weather information and the applications of climatic indices as heat stress indicators.

It is expected that dairy cattle raised in the tropical conditions of Kenya are exposed to heat stress due to high temperatures for a significant duration through the year. Dairy production is largely undertaken in the highlands where the climate, infrastructure and market access are favourable (Kosgey *et al.*, 2011). Among the environmental constraints to production are the cyclic hot and dry seasons occurring in December to March and July to August (Ojango *et al.*, 2019). To increase animal productivity in such conditions in the face of climate change, simultaneous interventions are required in the areas of animal feed, health and genetics (Marshall *et al.*, 2019). On the genetics front, breeding for resilience - defined as “the capacity of an animal to be minimally affected by disturbances or to rapidly return to the state pertained before exposure to a disturbance” (Berghof *et al.*, 2018), could be the much needed intervention. Given that heat stress is one of the main environmental stresses under Kenyan production systems, routine evaluation that considers heat stress would be the best way to prevent deterioration of heat tolerance as recommended by Bohmanova *et al.* (2008).

Depending on model complexity, the population and individual variations on the onset of heat stress can be evaluated at three levels: i) the value of THI at which mean yield begin to decline as well as the extent of the decline, ii) assuming a single THI threshold beyond which individual variation is estimated and iii) individual variation for onset of heat stress and extent of decline (Sanchez *et al.*, 2009). A simplistic model to quantify heat stress is linear regression of production traits on weather variables. Initial studies on genetic components of heat stress used a general linear model with regression on a function of THI (Ravagnolo and Misztal, 2000, Ravagnolo *et al.*, 2000). In linear regression, the weather variable may be treated as categorical and Least Square Means estimated which indicate the fluctuation in milk yield (Ravagnolo *et al.*, 2000). Methodologies that assume a single THI threshold apply the so called broken-line models. Broken-line models assume that production remains constant within a thermoneutral zone where no response to increasing temperature is observed and after this threshold heat stress sets in and production decreases linearly. Several studies have applied the broken-line approach in genetic studies for milk production traits (Ravagnolo and Misztal, 2000, Carabano *et al.*, 2016). Models that determine the THI value at which performance begin to drop and also the

degree to which performance decline after some unknown THI threshold have been developed using random regression and spline polynomial functions (Sanchez *et al.*, 2009, Carabano *et al.*, 2016). Such models have been found to provide more flexible patterns than the broken line models in fitting milk production response to increasing heat loads in different production systems (Brügemann *et al.*, 2012, Carabano *et al.*, 2016). Furthermore, modelling random animal effects enables determination of individual animal variation on the onset and persistence of heat stress and heat tolerant animals can be identified and selected.

The theory of factor analysis (FA) models for structuring covariance matrices of additive genetic effects have been discussed in the context of multivariate mixed models (de Los Campos and Gianola, 2007, Meyer, 2009). The studies vouch for FA application in analysis of data on genotypes with different expression in different environments i.e. genotype  $\times$  environment interaction studies. This is suitable for a study on heat stress in dairy cattle where cows are in different days in milk (DIM) by heat load combinations. Implementing a FA would accommodate a covariance structure between the two covariates. It is from this background that we modeled the effects of heat stress under the tropical conditions of Kenya.

Studies analyzing the effects of heat stress on dairy cattle performance generally merge animal performance data with public weather station information. The environmental factors including air temperature, relative humidity, air movement (wind), solar radiation, and precipitation are applied either individually or augmented into an index and merged with production records. Data from a number of meteorological stations can be interpolated onto a two-dimensional grid to provide a “best estimate” of hourly, daily or monthly weather variables at locations away from the observing stations (Haylock *et al.*, 2008). Gridded data are primarily aimed at regional climate model evaluation, therefore producing area average rather than point estimates (Hofstra *et al.*, 2008). This has an advantage of filling weather data gaps especially for regions with few weather stations. Statistically derived gridded datasets for different climate variables and covering areas ranging from global to largescale regions are available from the world’s largest meteorological centres including the ERA-40, European Centre for Medium-Range Weather Forecasts (ECMWF), the Modern-Era Retrospective Analysis for Research and, Applications (MERRA) (Rienecker *et al.*, 2011), National Aeronautics and Space Administration Prediction of Worldwide Energy Resources (NASA/POWER) (Stackhouse Jr *et al.*, 2018) among others. However, such models are not appropriate for application at smaller scale. As a result, reanalysis systems have been developed to achieve much higher spatial and temporal resolutions that account for local events. An example of a regional reanalysis is the COnsortium

for Small-Scale MOdelling (COSMO) limited-area model (Schättler *et al.*, 2011). Higher resolution is needed in climate monitoring. This is because the impact of climate change on regional and local scales are better estimated at higher resolution and the smaller resolved scales can support the understanding of mechanisms responsible for local climate features, feedbacks or regimes (Bollmeyer *et al.*, 2015).

Studies comparing weather station data (WSD) and gridded numerical weather prediction (NWP) data are scarce. Neither have grid-interpolated meteorological data and milk yield records been used in population studies on thermal sensitivity in cattle. Analysis was carried out with the aim of combining test-day milk performance records with high resolution WSD and NWP model data to allow for precise estimation of heat stress effects on milk production traits. Milk performance records were from 12,606 farms with over 16 million test-day records for the years 2010 to 2019 from the southern region of Bavaria, Germany.

The effects of global warming in the coming decades will cause relevant impacts in the livestock production sector. Indicators for which climate-related impacts are likely to increase, and the magnitude by which they will increase, are important for effective management strategies for future climate risks (Spinoni *et al.*, 2018). Degree-days offer a good indicator of changes in global temperature. Degree-days have been an important indicator for energy weather-related demand for buildings. Heating degree day (HDD) and cooling degree day (CDD) are measurement indices designed to quantify the demand for energy to heat or cool a building respectively (Matzarakis and Thomsen, 2009). The simplest form of degree-day is the averaging method based on daily maximum and minimum air temperature (Fraisie *et al.*, 2007, Matzarakis and Thomsen, 2009). CDD and HDD are calculated using the commonly used American Society for Heating, Refrigeration, and Air Conditioning (ASHRAE) method (ASHRAE, 2009) and the formula for CDD is defined as:

$$CDD = \sum_1^n (T_d - T_b)$$

where  $T_d$  and  $T_b$  are daily mean outdoor air and threshold (base) temperatures respectively,  $n$  is the chosen time period e.g. day, months, season, year.

In agronomy, the heat experienced by crops is expressed as growing degree-days (GDD) or heat units to describe the timing of biological processes (McMaster and Wilhelm, 1997). The idea behind GDD is that development of a plant will occur when the temperature exceeds a specific base temperature for a given period of time (Fraisie *et al.*, 2007). The concept of degree-day

has been applied to monitor and predict fish growth and development (Neuheimer and Taggart, 2007, Chezik *et al.*, 2014). This is based on the fact that, when maintained within limits, higher temperatures result in high enzymatic reactions that regulate growth (Neuheimer and Taggart, 2007). This concept was applied to develop cooling THI-days and study the effects of heat load on milk production traits. It was expected that the heat load accumulating when the temperature or THI exceeds the threshold for a certain time period would have an effect on the estimated production loss or increase for a given trait.

### **Structure of the thesis**

**Chapter one** of the thesis presents a review of the trends and recent developments in temporal resolution of performance records as well as spatio-temporal resolution of weather data necessary for improvement of evaluation of heat stress effects on milk production traits in dairy cattle.

In **Chapter two**, the milk yield loss due to heat stress in a tropical environment was investigated. By applying random regression models on test-day records, the milk yield loss and heat stress thresholds for different heat stress indicators were determined. Average daily temperature and THI had similar milk yield loss estimation capabilities. The covariances between days in milk (DIM) and thermal load associated with increased vulnerability to heat stress with DIM could not be modeled with the unstructured general covariance matrix. An extended factor analytic variance structure for the random cow effects was efficient in estimating all (co)variances between DIM and thermal load with a reduced number of factors. Individual animal variations and estimated proportion of the total variance of test-day milk yield which was due to individual animals (short-term repeatability) were estimated.

**Chapter three** compared high resolution spatio-temporal observational WSD and gridded NWP model data. The datasets were then applied as thermal stress indicators for milk production traits in a temperate region of southern Bavaria, Germany. The validation results indicated good agreement between WSD and NWP daily average temperature, relative humidity and THI. However, positive and negative biases were observed in the pre-Alpine regions. The general herd reaction norms for the milk production traits followed similar trends when estimated by WSD and NWP weather data. The heat stress thresholds were clearly defined for both average daily temperature and THI for milk yield and protein yield. Fat yield, fat content and protein content decreased linearly with increasing heat stress with no definite thresholds. Milk urea increased in a non-linear accelerating pattern with increasing heat stress. There was no undesired heat stress effect on somatic cell score.

**Chapter four** applied the concept of degree-days to develop THI-days. Degree-days are heat units used to relate energy demand for buildings, crop phenology and insect and fish development to air temperature among other applications. We defined the cooling THI-day as the total THI units that accumulated above the heat stress threshold for milk production traits over the entire day. Cooling THI-day was used as heat load indicator for milk production traits. Reaction norms for cooling THI-day with and without conventional THI were analysed. Results indicated that cooling THI-day provided additional significant ( $p < 0.001$ ) information to the conventional THI models. Consequently, conventional THI reaction norm models of average herd milk yield tend to underestimate the effects of heat stress on milk production traits by failing to account for the cumulative heat load.

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# CHAPTER ONE

## **A review of methods for improving resolution of milk production data and weather information for measuring heat stress in dairy cattle**

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**A review of methods for improving resolution of milk production data and weather information for measuring heat stress in dairy cattle production**

**ABSTRACT**

Genetic selection for heat tolerance is a sustainable strategy to supplement housing and feeding modification to alleviate the adverse effects of heat stress in livestock. To identify and select heat tolerant animals, it is necessary to have high temporal resolution animal performance records and high resolution spatio-temporal weather information. The temporal resolution being the amount of time detail in an observation and the spatial resolution the amount of space area i.e. what area and how detailed an observation is. This review highlights some approaches to improve the resolution of data necessary for evaluating heat stress in dairy cattle. Because heat stress occurs only periodically, mainly during summer, information from test-day records only is limited in phenotypic data. The use of only test-day records over time captures a small fraction of the response due to heat stress. It is expected that daily production records would allow for better estimation of heat stress effects. In regions where data from weather stations are scarce or lacking, grid interpolated weather data may improve accuracy of prediction. This is because grid interpolation enables creation of reliable virtual weather stations that can provide complete and long-term weather data. However, studies are lacking to compare the agreement of measured weather data and grid interpolated data to assess the effects of heat stress in livestock. To evaluate the performance of animals under various climatic conditions, environmental parameters are applied either individually or augmented into an index and merged with production records. Although there are many indices defined in literature, most have not been applied in genetic evaluation studies and grid interpolated indices have not been developed for use in livestock studies. This review highlights approaches to contribute to improved temporal resolution of performance records as well as spatial and temporal resolutions of weather data necessary for improvement of heat stress evaluation in dairy cattle.

**Keywords:** Grid interpolated weather, heat stress indicator, milk production traits, spatio-temporal resolution

## INTRODUCTION

It is expected that climate change will affect the production, health, welfare and survival of farm animals. In the temperate regions of Europe, climate change is expected to bring drier hotter summers and increased frequency of extreme weather events (Hill and Wall, 2015). In the tropical and subtropical regions, climate change projections are higher temperatures, changes in frequency, intensity and duration of extreme weather events and intensified water problems with increased frequency and extent of droughts (Henry *et al.*, 2012). These changes will occur in livestock production systems where animals are continuously selected and bred for increased production and reproduction. The impacts of climate on livestock production, reproduction and welfare have been reviewed by several authors including; Nardone *et al.* (2010), Sejian (2013) and Rojas-Downing *et al.* (2017). It is clear that heat stress will be the most important climatic factor that adversely affects livestock. In this regard, several authors have highlighted the effects of heat stress on dairy cattle production, reproduction, health and welfare (Rensis and Scaramuzzi, 2003, West, 2003, Das *et al.*, 2016, Polsky and von Keyserlingk, 2017). Exposure to heat stress may cause a 15-25% reduction in milk yield (Dikmen *et al.*, 2013). In addition to milk yield loss, there are the associated changes in milk composition, milk somatic cell score and mastitis occurrence.

The main intervention strategies to address heat stress in dairy cattle include; management (heat abatement), nutrition, and genetic selection. Genetic selection for heat tolerance could be a sustainable strategy to support mitigations achieved through housing and feeding modification (Boonkum *et al.*, 2011). To achieve this, it is necessary to have high temporal resolution animal performance records and high resolution spatio-temporal weather information. The temporal resolution being the amount of time detail in an observation and the spatial resolution the amount of space area i.e. what area and how detailed an observation is. Traits associated with thermal tolerance need to be identified and included in breeding goals and genotype by environment interactions be considered to identify animals most adapted to specific conditions (Hoffmann, 2010). Animal performance data are generally merged with public weather information to study genetic aspects of dairy cattle performance under heat stress. Among the performance traits, evaluation of milk yield loss is advantageous in that the change is highly detectable, easy to measure without additional equipment and does not unduly affect animal welfare. After exposure to heat stress, milk yield loss can be detected after a few days. Advances in milking automation have made it possible to do either frequent or real-time milk components

analysis. Traits related to physiological temperature can be precisely captured via sensor-derived recording gadgets such as transponders, boluses and non-invasive infrared thermography (McManus *et al.*, 2016, Seidel *et al.*, 2020).

The effort to reduce the occurrence of heat stress among dairy cows involves the search for new environmental methods of predicting heat stress (Herbut *et al.*, 2018). The temperature humidity index (THI) is the most commonly used indicator for heat stress in dairy cattle. However, it only accounts for ambient temperature and relative humidity. There are also variations in its formulation. For different dairy traits, differences occur in thresholds of heat tolerance among indices and between regions depending on the ability of animals to adapt to environmental conditions (Bohmanova *et al.*, 2007, Dikmen and Hansen, 2009, Hammami *et al.*, 2013). Technological advances have facilitated the collection of large-scale and precise environmental parameters such as solar radiation and wind speed and also animal physiological parameters such as respiration rate, core body temperature, rectal and vaginal temperature and panting (Hammami *et al.*, 2013). As a result, new heat stress indicators have been developed, some in consideration of animal physiology (Gaughan *et al.*, 2008, Mader *et al.*, 2010).

This review accentuates the trends and recent developments in temporal resolution of performance records as well as spatial and temporal resolution of weather data necessary for improvement of heat stress evaluation in dairy cattle. Developments in climatic indices as heat stress indicators have also been highlighted.

### **Animal performance data**

There exist thermoneutral zones for farm animals within which animals achieve maximum productivity and physiological costs are minimum including energy of maintenance, water metabolism and metabolic responses among others (Kadzere *et al.*, 2002). For farm animals, this varies based on animal-level factors such as species, breed and physiological differences (age, feed intake, production level, insulation level and behavior of the animal) and external factors such as temperature, relative humidity, wind speed, solar radiation and specific housing conditions (Kadzere *et al.*, 2002, Berman *et al.*, 2016). When exposed to heat stress, the animal responds physiologically towards maintaining or restoring thermal balance. This leads to significant effects on physiology, metabolism, production and reproduction of the animal. After exposure to heat stress conditions, there is an increase in respiration rate and rectal temperature and a reduction in dry matter intake and consequently milk yield (Spiers *et al.*, 2004). To breed

animals that have effective thermoregulatory mechanisms, it is important to overcome the problems related to measuring the relevant phenotypes (Hoffmann, 2010).

Heat tolerance in cattle has been shown to have a genetic component and therefore, animal breeding values can be estimated and genetic selection be applied to increase heat tolerance (Ravagnolo and Misztal, 2000, Nguyen *et al.*, 2016). Because heat stress occurs only periodically, mainly during summer, information from test days only is limited in phenotypic data. This calls for use of high temporal resolution performance records such as daily milk yield in evaluation of heat stress. Productivity related studies for lactating cows focus on milk yield loss. In the literature, the common animal-based indicators are internal temperature with rectal and vaginal temperatures being prominent (Galán *et al.*, 2018). The main focus of this review were on indicators that have recently advanced in automation including decline in milk yield, changes in body temperature and potential biomarkers for heat stress.

### **Decline in milk yield as a phenotype for heat tolerance**

Directly, heat stress affects production by reducing feed intake and ultimately synthesis of milk (see reviews by West (2003) and Das *et al.* (2016)). Dairy cows exposed to a constant ambient temperature of 29°C have been reported to reduce milk yield by 23% (Spiers *et al.*, 2004). According to Bernabucci *et al.* (2010), heat stress induced reduction in milk production may be up to about 14% in early lactation and 35% in mid-lactation. Table 1 presents some examples of the quantity of milk yield loss per unit of heat stress indicator per day in different regions. From the analogy, even with a similar indicator applied in a different region, different heat stress thresholds have been obtained. These differences could arise from climatic differences but may also be associated with average environment such as housing conditions and differences in the statistical models and methods used in each study (Carabaño *et al.*, 2016).

**Table 1.** The level of milk yield loss due to heat stress may differ based on the evaluation indicator in different regions

<b>Milk yield loss</b>	<b>Heat stress indicator</b>	<b>Threshold</b>	<b>Breed</b>	<b>Region/country</b>	<b>Reference</b>
-0.38 kg/°C	Average temperature (°C)	-	Israeli Holstein	Mediterranean – Israel	Barash <i>et al.</i> , 2001
-0.17 kg/°C	Average temperature (°C)	34	Holstein-Friesian	Tropical – Southern Spain	Carabaño <i>et al.</i> , 2014
-0.20 kg/THI	THI <sup>a</sup>	72	Holstein	Subtropical – Georgia/USA	Ravagnolo <i>et al.</i> , 2000
-0.16 to -0.47 kg/THI	THI <sup>a</sup>	70	Holstein	Temperate – Germany	Brügemann <i>et al.</i> , 2012
-0.91 to -1.27 kg/THI	THI <sup>a</sup>	73-76	Italian Holstein	Mediterranean Basin – Italy	Bernabucci <i>et al.</i> , 2014
-0.41 kg/day	THI <sup>b</sup>	69	Friesian-Holstein	Mediterranean – Tunisia	Bouraoui <i>et al.</i> , 2002
-0.22 to -1.12 kg/THI	THI <sup>c</sup>	20-22	Holstein	Subtropical – Georgia/USA	Freitas <i>et al.</i> , 2006
-0.57 kg/THI	THI <sup>d</sup>	73	Holstein	Subtropical – Phoenix/USA	Bohmanova <i>et al.</i> , 2007
-0.29 kg/THI	THI <sup>d</sup>	68	Holstein	Subtropical – Athens/USA	Bohmanova <i>et al.</i> , 2007
-0.26 kg/THI	THI <sup>e</sup>	74	Holstein	Subtropical – Phoenix/USA	Bohmanova <i>et al.</i> , 2007
-0.27 kg/THI	THI <sup>e</sup>	69	Holstein	Subtropical – Athens/USA	Bohmanova <i>et al.</i> , 2007
-0.37 kg/THI	THI <sup>f</sup>	78	Holstein	Subtropical – Athens/USA	Bohmanova <i>et al.</i> , 2007
-0.28 kg/THI	THI <sup>f</sup>	83	Holstein	Subtropical – Phoenix/USA	Bohmanova <i>et al.</i> , 2007
-2.2 kg/day	THI <sup>f</sup>	68	Holstein	Subtropical – Arizona/USA	Zimelman <i>et al.</i> , 2009
-0.37 kg/THI	THI <sup>g</sup>	79	Holstein	Subtropical – Athens/USA	Bohmanova <i>et al.</i> , 2007
-0.27 kg/THI	THI <sup>g</sup>	82	Holstein	Subtropical – Phoenix/USA	Bohmanova <i>et al.</i> , 2007
-0.39 kg/THI	THI <sup>h</sup>	72	Holstein	Subtropical – Athens/USA	Bohmanova <i>et al.</i> , 2007
-0.30 kg/THI	THI <sup>h</sup>	74	Holstein	Subtropical – Phoenix/USA	Bohmanova <i>et al.</i> , 2007

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-0.08 to -0.26 kg/THI	THI <sup>h</sup>	60	Holstein	Temperate – Germany	Brügemann <i>et al.</i> , 2012
-0.164 kg/THI	THI <sup>h</sup>	62	Holstein	Temperate – Luxembourg	Hammami <i>et al.</i> , 2013
-0.094 kg/THI	THI <sup>h</sup>	69	Brazilian Gir	Tropical – Brazil	Santana Jr <i>et al.</i> , 2015
-0.23 kg/day	THI <sup>h</sup>	66	Brazilian Holstein	Tropical – Brazil	Santana <i>et al.</i> , 2017
-0.39 kg/THI	THI <sup>i</sup>	72	Holstein	Subtropical – Athens/USA	Bohmanova <i>et al.</i> , 2007
-0.22 kg/THI	THI <sup>i</sup>	75	Holstein	Subtropical – Phoenix/USA	Bohmanova <i>et al.</i> , 2007
-0.37 kg/THI	THI <sup>j</sup>	71	Holstein	Subtropical – Athens/USA	Bohmanova <i>et al.</i> , 2007
-0.27 kg/THI	THI <sup>j</sup>	74	Holstein	Subtropical – Phoenix/USA	Bohmanova <i>et al.</i> , 2007
-0.152 kg/THI	THI <sub>adj</sub>	64	Holstein	Temperate – Luxembourg	Hammami <i>et al.</i> , 2013
-0.154 kg/CCI	CCI	20	Holstein	Temperate – Luxembourg	Hammami <i>et al.</i> , 2013
-0.109 kg/ESI	ESI	16	Holstein	Temperate – Luxembourg	Hammami <i>et al.</i> , 2013
-0.146 kg/ETI	ETI	18	Holstein	Temperate – Luxembourg	Hammami <i>et al.</i> , 2013
-0.123 kg/HLI	HLI <sup>+</sup>	80	Holstein	Temperate – Luxembourg	Hammami <i>et al.</i> , 2013
-1.0 l/day	HLI <sup>+</sup>	65	Holstein	Temperate – Belgium	Van laer <i>et al.</i> , 2015

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Formulae with similar superscript indicate a similar THI. Further information on the indices is provided on the section on heat stress indicators.

Genetic evaluation studies have advanced from lactation milk yield to test-day performance records (Meyer *et al.*, 1989, Visscher and Goddard, 1995). In studies on heat stress, this enables matching the test-day record to the weather of that particular day or several days prior. Test-day milk yield permits the removal of short-term environmental variation in phenotypic data because it considers the specific environmental effects for each production record.

Basically, genetic studies on heat stress aim to determine the tipping point after which a linear decline of production occurs and regression of phenotypic performance after the threshold, called a broken-line approach, to model the additive genetic variance of heat tolerance (Bernabucci *et al.*, 2014, Carabaño *et al.*, 2014). The upper critical point for milk decline, however, varies as reported by different authors in different regions as presented in table 1. More recently, reaction norm models using polynomial functions have been applied to determine the THI value at which performance begin to drop and also the degree to which performance decline after some unknown THI threshold (Sánchez *et al.*, 2009, Carabaño *et al.*, 2016). Such models have been found to provide more flexible patterns in fitting milk production response to increasing heat loads. In general, the response of milk yield to heat stress varies between regions and environments and cattle genotypes differ in their adaptation to thermal environments. There is no univocal threshold for the beginning and extent of milk yield decline due to heat stress in dairy cattle. Sensitivity of dairy cows to heat stress is however, higher in temperate countries than in tropical and subtropical countries.

### **Temporal resolution of performance data**

Heat stress effect estimated from test-day records may be underestimated. According to Freitas *et al.* (2006), the use of only test-day records over time captures a small fraction of the response due to heat stress compared with daily records. For example, if there are 4 months in a year with substantial heat stress, only four records per animal will be input in the analysis for that year. In addition, this combines with only four days weather information and probably a few days prior to the test day. Daily milk recording is becoming more commercially available because of implementation of automatic milking systems (AMS) and milking robots. Daily milk yield combined with average daily temperature from weather stations modeled in a dynamic time series analysis is valuable in detecting heat stress in addition to monitoring herd trends in feeding (André *et al.*, 2011). Data from AMS could also provide additional information necessary for evaluating heat stress. It has been shown that in AMS, milking frequency decreases during the hot season (Speroni *et al.*, 2006). Nixon *et al.* (2009) reported that cows

that visit the AMS more often have greater genetic merit for milk production. Given that high yielding cows are likely to be more susceptible to heat stress, it would therefore be prudent to include milking frequency in data obtained from AMS.

Advances in milking automation have made it possible to do either frequent or real-time milk components analysis. Higher heritability estimates have been reported for milk yield and components from daily records compared to monthly records primarily because more frequent records yield more representative measure of a cow's longer-term milk composition (Weller and Ezra, 2016). It is expected that daily production records will allow a better estimation of heat stress effects.

### **Potential milk profile biomarkers for heat stress in dairy cattle**

Application of mid-infrared spectroscopy as a tool to collect data for phenotypic and genetic purposes is an evolving area for milk profile traits (De Marchi *et al.*, 2014, Seidel *et al.*, 2020). Milk provides information on the changes in the lactation mechanisms of the cow and can be used as an ideal biological sample for monitoring physiological alterations (Tian *et al.*, 2016).

Heat stress alters the metabolic composition of milk and blood plasma in lactating dairy cows (Tian *et al.*, 2016, Yue *et al.*, 2020). The metabolites are mainly involved in gluconeogenesis, proteolysis and fatty acid synthesis making them potential biomarkers for heat stress. Test-day fatty acid profiling and recording are becoming readily available (Hammami *et al.*, 2015). Milk fatty acids vary phenotypically and genotypically across the lactation and have moderate to high heritabilities which indicate that they can be changed by genetic selection (Hammami *et al.*, 2015). The milk fatty acid C18:1 *cis*-9 was reported as the most sensitive to heat stress (Hammami *et al.*, 2015). Different biomarkers are anticipated in different regions because of differences in breed and feeding management. There are potentially more biomarkers to be discovered as analytical techniques improve.

### **Physiological heat stress indicators: changes in body temperature**

Development of sensing technologies that automatically collect data has been enabled by advances in engineering coupled with decreasing cost of electronic technologies (Seidel *et al.*, 2020). Sensor-derived trait recording gadgets such as transponders, boluses and camera systems can obtain precise and comprehensive information that support precision farming. Body temperature is a sensitive indicator of physiological response to heat stress because it is nearly

constant under normal conditions. Changes in body temperature can be recorded using body temperature probes either rectally, intravaginally, reticularly using rumen temperature boluses, in the ear (tympanic) or through surface body temperature (Ammer *et al.*, 2016). Wheelock *et al.* (2010) reported an increase in rectal temperature from 38.6 to 40.4°C for Holstein cows exposed to temperatures ranging from 29.4 to 38.9°C. A THI threshold of 65 was reported to maintain reticular temperature constant at 39.2 °C for Holstein-Friesian in Germany (Ammer *et al.*, 2016). When the THI increased above 70, the rectal temperature increased to 39.4°C. Rectal temperature has been reported to increase by 0.02 and 0.03°C for shaded and non-shaded Holstein cows respectively, per unit increase in HLI during summer (Van laer *et al.*, 2015). In a study by Kaufman *et al.* (2018), the relationships among THI with rectal, vaginal and udder surface temperatures are evaluated in dairy cows experiencing heat stress.

Variations in body temperature due to heat stress is heritable, therefore, it is possible to improve thermotolerance by selection for the trait (Dikmen *et al.*, 2012). The correlation between body temperature and THI would make body temperature an indicator trait for heat stress. Dikmen *et al.* (2012) reported a correlation of 0.5 between rectal temperature and THI and estimated the heritability of rectal temperature to be  $0.17 \pm 0.13$  for dairy cows in freestall barns under heat stress conditions. However, rectal and vaginal temperatures differ significantly between breeds, their production levels, and time of day (Dikmen and Hansen, 2009). Rectal temperature is also prone to error in variability of depth and duration of probe insertion and variation in animal handling (West *et al.*, 2003).

Reticular temperature has been used as an indicator trait for heat stress (Ammer *et al.*, 2016). Positive correlation coefficient between daily median reticular temperature and THI have been reported ( $r=0.22$ ) for  $\text{THI} \geq 70$ . The limitations of reticular temperature is that it is generally greater compared to other body temperature measurements because of heat produced during fermentation and would therefore affect measurement interpretation. In addition, it may be influenced by water intake. Metabolic heat also increases with milk production and therefore animal performance might be a main factor that determines it (Ammer *et al.*, 2016).

Through infrared thermography, thermographic images are developed that may indicate circulatory changes induced by increased body temperature related to environmental heat stress (Cardoso *et al.*, 2016, McManus *et al.*, 2016). Temperature variations for skin surface and specific organs such as the eye, ear and feet can be monitored reliably by thermography (Church *et al.*, 2014, Cardoso *et al.*, 2016). The temperatures are digitized and processed by computer

and displayed in the form of a thermal map over the animal, which provides a detailed analysis of the temperature profile (McManus *et al.*, 2016). The main benefit of thermography is improving animal welfare during the evaluation by reducing handling stress and gaining a larger number of evaluations in a shorter time without animal restraint. However, technical issues such as camera setting and distance to object, and environmental variables such as solar radiation and wind speed influencing body surface temperature can affect interpretation of thermal images in measuring thermal stress (Church *et al.*, 2014). Despite the sensitivity of infrared thermography to changes in thermal patterns of animals, the methodology may not be sufficient to identify its cause (McManus *et al.*, 2016).

Limitations of core body temperature as a phenotype from the genetics perspective include the fact that it has to be maintained within a narrow range that cannot be exceeded under normal physiological conditions. The body temperature is also prone to fluctuations due to normal physiological processes like digestion, exercise, drinking water, and diurnal changes in the environment. The inheritance of physiological traits such as rectal temperature, vaginal temperature, reticular temperature and pulse rate is not well understood primarily because of the difficulty and cost of routine measurement of these traits (Hammami *et al.*, 2015). Unless advanced mechanisms are in place to frequently measure such physiological traits, selection for heat tolerance based on them may not be practical.

## **Weather data**

### **Climate data from public weather stations**

Public weather stations contain useful information for studies on heat stress in dairy cattle (Ravagnolo and Misztal, 2000, Freitas *et al.*, 2006). Animal performance data is basically merged with public weather information to study genetic aspects of dairy cattle performance under heat stress (Ravagnolo and Misztal, 2002). The problem with weather stations is their sparse distribution and may therefore not reflect the weather conditions on the farm and the effect of heat stress may be masked by heat abatement measures that are not routinely recorded (Ravagnolo and Misztal, 2000, Shock *et al.*, 2016).

It has been observed that ambient temperature, relative humidity and resulting THI values are usually significantly higher within the barn than those of the closest official meteorological station (Shock *et al.*, 2016). Despite this, on-farm weather recording can be complex and controlled experiments are scarce rendering weather station data more useful especially for

large-scale evaluations. In addition, weather stations apply standardized protocols and definitions.

### **Satellite weather data**

Low spatial density of meteorological stations may result in relatively large errors during data interpolation making it difficult to retrieve the spatial pattern of the weather variables (Vogt *et al.*, 1997). Weather satellites offer the possibility of mapping and monitoring the spatial distribution of weather variables such as air temperature with the help of time series images. In contrast to weather stations which are sparsely distributed, satellites see entire landscape and are able to provide precise measurements at every location (Mendelsohn *et al.*, 2007). They carry instruments that detect visible, infrared or microwave radiation which are then digitized and made available in the form of images. For instance, the launch of Moderate Resolution Imaging Spectroradiometer (MODIS) made it possible to get spatial estimates of land surface temperature at high temporal (daily) and spatial resolution (1 km) across the world (Zhu *et al.*, 2013). Temperature measurements from satellites have even been reported to slightly outperform weather station interpolations in some instances (Mendelsohn *et al.*, 2007).

### **Temporal resolution of weather data in heat stress studies**

The temporal resolution of weather data is expected to have an effect on determining the threshold for animal comfort zone. Most studies, however, consider only the THI of a particular day considered significant to production. This might lead to biased estimates of the lag effect on production. This is because a delayed response of test-day milk yield is expected after the onset of heat stress. The weighted average of the statistically significant lag days (between 8 and 12 days) before the test day has been analyzed for milk yield and components (Bernabucci *et al.*, 2014). The most significant effects were observed 3 to 4 days before the test day. In some studies the THI is averaged over the 3 days before the test day for milk yield traits and SCS (Bohmanova *et al.*, 2008, Hammami *et al.*, 2015) or on the test day and two days before (Santana *et al.*, 2017). Weather parameters summarized across a week's timescale from the test day have been reported to explain milk yield and content better than a shorter scale such as 1 or 3 day in a temperate climate (Hill and Wall, 2015). The moving average over three days explain more variability in milk production than just one or two days before the test day (Santana *et al.*, 2017).

The inclusion of weather variables into an index (e.g. THI) considers either average daily maximum temperature and daily minimum humidity or hourly-recorded temperature and humidity (Ravagnolo and Misztal, 2000, Brügemann *et al.*, 2012). Brügemann *et al.* (2012) found a better goodness of fit based on average of hourly THI than on THI calculated with daily maximum temperature and daily minimum humidity. This is probably because cool night temperatures may counteract the effects of high values during the day and offer relief of gained heat stress (Carabaño *et al.*, 2014). Availability of hourly meteorological data from weather stations is expected to provide useful information that may improve evaluation of heat stress.

### **Grid interpolated weather data**

Data from a number of meteorological stations, satellite data and prediction models can be interpolated onto a two-dimensional grid to provide a “best estimate” of hourly, daily or monthly weather variables. At locations where observing stations are not available, information is provided by surrounding stations. In cases where neighboring stations are scarce, it is possible to improve the estimates of specific variables by using “hybrid” interpolation techniques (Krähenmann *et al.*, 2018). Gridded data are primarily aimed at regional climate model evaluation, therefore producing area average rather than point estimates (Hofstra *et al.*, 2008).

From the global and regional climate databases, localized data can be obtained at required spatial and temporal resolutions. Common applications in climate impact research, hazard management and planning include modelling, reanalysis, monitoring, forecasting of drought, and crop growth among others (Hiebl and Frei, 2016). Statistically derived grids for daily climate variables have been published including wind maps (Walter *et al.*, 2006), gridded daily air temperature and relative humidity (Frick *et al.*, 2014), daily maximum and minimum temperatures (Frei, 2014, Hiebl and Frei, 2016) precipitation and evapotranspiration (Rauthe *et al.*, 2013, Xavier *et al.*, 2016). The gridding method depends on the climate variable. These include regression models, splines (e.g. Thin Plate Splines), inverse distance weighting (IDW), angular distance weighting (ADW) and ordinary point kriging (Jeffrey *et al.*, 2001, Hofstra *et al.*, 2008). A detailed comparison of some interpolation methods is presented by Hofstra *et al.* (2008).

## **Spatial resolution of gridded data**

There are many statistically derived grids of daily climate variables published in the recent decades covering areas from global to local regions. Earlier datasets had coarser spatial resolution than present. The gridding of high-resolution daily datasets is needed to monitor extreme weather situations (Krähenmann *et al.*, 2018). By applying different gridding methods, several climate variables can be created in the same dataset as present in table 2. This implies that climatic indices based on gridded data can be formulated to evaluate heat stress in dairy cattle.

**Table 2.** Examples of global and regional grid interpolation models with varieties of weather parameters that can be combined into an index

<b>Model</b>	<b>Climate variables</b>	<b>Spatial Resolution</b>	<b>Temporal resolution</b>	<b>Region</b>	<b>Source</b>
-	rainfall, maximum and minimum temperatures, evaporation, solar radiation and vapor pressure	0.05° × 0.05°	Daily	Australia	Jeffrey <i>et al.</i> , 2001
E-OBS	maximum and minimum temperatures and precipitation	25 × 25 km	Daily	Europe	Haylock <i>et al.</i> , 2008
HYRAS	Daily mean temperature and relative humidity	5 × 5 km	Daily	Central Europe	Frick <i>et al.</i> , 2014
WorldClim 2	temperature (minimum, maximum and average), precipitation, solar radiation, vapour pressure and wind speed	1 × 1 km	Monthly	Global land areas	Fick and Hijmans, 2017
Daymet	minimum temperature, maximum temperature and daily total precipitation	1 × 1 km	Daily	USA, Canada, Mexico and Puerto Rico	Thornton <i>et al.</i> , 2017
-	temperature, dew point, cloud cover, wind speed and direction, global and direct shortwave radiation, down- and up-welling longwave radiation, sea level pressure, relative humidity and vapour pressure	1 × 1 km	Hourly	Germany	Krähenmann <i>et al.</i> , 2018

## **Studies that used gridded data to evaluate heat stress in dairy cattle**

In livestock studies, grid interpolated data (5 km<sup>2</sup> grid) have been used to assess the magnitude of genotype × environment interactions on milk production and also to carry out a genome wide association study for sensitivity of milk production for different environmental descriptors (Hayes *et al.*, 2003, Hayes *et al.*, 2009). Gridded data have also been used to analyze historical heat stress trends and predict likely future changes in heat stress incidence and severity for a dairy region in Australia (Nidumolu *et al.*, 2014). In the United Kingdom (UK), grid cells of 25 km<sup>2</sup> spatial resolution have been applied in estimation and projection of milk yield loss and the economic consequence due to heat stress in dairy cows (Fodor *et al.*, 2018). In a case of big dairy data analysis, Duruz *et al.* (2020), applied data interpolated from weather stations to test the influence of among others, environmental factors on dairy cattle productivity during transhumance in the Swiss Alps. In the absence of adequate weather station data Carrara *et al.* (2021), used gridded data from the NASA/POWER database to estimate genetic parameters for milk yield and quality as a function of heat stress in Brazil. Gridded climate variables, though not extensively applied, have the potential to assess weather effects, and in particular, heat stress in the livestock industry.

## **Advantages of gridded over single-station data**

Gridded weather data (GWD) obtained from a combination of interpolated ground weather stations and satellite data have the advantage of filling weather data gaps especially for regions with few weather stations. Gridding enables creation of reliable virtual weather stations that can provide complete and long-term weather inputs for assessment of climate impacts on agricultural yield and variability (Monteiro *et al.*, 2018). Studies are available that have compared measured weather data (MWD) and GWD in agricultural applications to estimate crop phenology and yields (Mourtzinis *et al.*, 2017, Battisti *et al.*, 2019) and how dense a weather network needs to be so that resulting interpolation can be considered robust (Mourtzinis *et al.*, 2017). Comparisons of minimum and maximum air temperature show a good agreement between MWD and GWD. The GWD also appear robust for applications that require temperature for prediction of crop stages. The quality of interpolated weather data ultimately depends on the density and distribution of weather station network (Mourtzinis *et al.*, 2017). Given that GWD are useful for applications in agricultural crop phenology and crop yields indicates that they have the potential of estimating

effects of heat stress in dairy cattle more precisely. However, studies are lacking that addresses the suitable grid size and grid temperature humidity indices to evaluate heat stress in livestock.

### **Problems in grid interpolation**

In some circumstances, gridded datasets may not capture all the variations that may occur in a given area at the required spatial resolution. This occurs in areas of complex terrain like mountains and urban areas. In mountainous areas, the terrain creates spatially and temporally complex climatic patterns that may be difficult to extrapolate. The study by Frei (2014) explores some of the interpolation challenges of high-mountain regions such as the European Alps and constructs high-resolution regional temperature datasets for such a terrain. Urban areas are characterized by artificial surface material and higher blockage effects by adjacent buildings which capture and emit a large proportion of the anthropogenic heat and solar radiation (Hsu *et al.*, 2017). As such, sophisticated localized urban climate models are required for comprehensive modelling of urban microclimates.

### **Heat stress indicators**

To evaluate the performance of the animal under various climatic conditions, environmental factors including air temperature, relative humidity, air movement, solar radiation, and precipitation are applied either individually or augmented into an index and merged with animal production records. These weather variables are available from meteorological weather station databases. Ambient temperature is the commonly used individual weather variable in heat stress studies especially in experimental studies. Large scale, non-experimental studies on individual cow response to heat stress in dairy cattle apply either average daily temperature or maximum daily temperature (André *et al.*, 2011, Carabaño *et al.*, 2014, Yano *et al.*, 2014).

An index is a combination of several parameters into a single value by a mathematical formula. For example, THI is a single value representing the combined effects of air temperature and humidity associated with the level of thermal stress (Bohmanova *et al.*, 2007). The THI is the commonly used bioclimatic index for evaluation of heat stress in cattle. Over the years, the formulae for calculating THI have been modified and corrected numerous times by various authors some of which are presented in Table 3.

**Table 3.** Formulae developed to calculate temperature humidity index (THI)

Author	Formula
Thom, 1959	$0.4 \times (T_{db} + T_{wb}) + 15^*$
Bianca, 1962	$0.35 \times T_{db} + 0.65 \times T_{wb}$
Bianca, 1962	$0.15 \times T_{db} + 0.85 \times T_{wb}$
Kibler, 1964	$1.8 \times T_{db} - (1 - RH)(T_{db} - 14.3) + 32$
Cargill and Stewart, 1966	$(0.55 \times T_{db} + 0.2 \times T_{dp}) + 15^*$
NRC, 1971	$(1.8 \times T_{db} + 32) - (0.55 - 0.0055 \times RH) \times (1.8 \times T_{db} - 26)$
NRC, 1971	$(0.55 \times T_{db} + 0.2 \times T_{dp}) \times 1.8 + 32 + 17.5$
Maust <i>et al.</i> , 1972	$0.72 \times (T_{db} + T_{wb}) + 40$
Oliveira and Esmay, 1982	$T_{db} + 0.36 \times T_{bp} + 41.2$
Yousef, 1985	$T_{db} + (0.36 \times T_{bp}) + 41.5$
Ravagnolo and Misztal, 2000	$(1.8 \times T_{db-max} + 32) - (0.55 - 0.55 \times RH_{min}) \times (T_{db-max} - 26)$
Mader <i>et al.</i> , 2006	$(0.8 \times T_{db}) + (RH/100) \times (T_{db} - 14.4) + 46.4$
Hahn <i>et al.</i> , 2009	$0.8 \times T_{db} + RH \times (T_{db} - 14.4) + 46.4$
Berman <i>et al.</i> , 2016	$3.43 + 1.058 \times T_{db} - 0.293 \times RH + 0.0164 \times T_{db} \times RH + 35.7$

RH is relative air humidity in %,  $RH_{min}$  is minimum daily humidity in %,  $T_{db}$  dry bulb air temperature,  $T_{db-max}$  is maximum daily dry bulb air temperature,  $T_{wb}$  wet bulb air temperature,  $T_{dp}$  dew point temperature. Temperatures are in °C, those marked with \* are in °F. Studies where data is in °F apply the term  $T_{bd} \times 1.8 + 32$  to convert temperature from °C to °F.

The equivalence of these indices has not been established. The most common indicator in genetic evaluation studies is the formula of NRC (1971) e.g. Ravagnolo and Misztal (2000), Bohmanova *et al.* (2007), Brügemann *et al.* (2012). Bohmanova *et al.* (2007) reported that THI formulae with higher weight on humidity work better for humid climates whereas those with more weight on temperature work better for drier climates. Despite its wide application on heat stress studies in dairy cattle, the THI has limitations of not including important climatic factors such as solar load and wind speed. As a result, other indices have been developed which include the ones presented in Table 4.

**Table 4.** Indices developed incorporating several weather parameters as heat stress indicators for cattle

<b>Index</b>	<b>Weather parameters</b>	<b>Authors</b>
Black globe-humidity index (BGHI)	Temperature, humidity, solar radiation	Buffington <i>et al.</i> , 1981
Equivalent temperature index (ETI)	Temperature, humidity, wind velocity	Baeta <i>et al.</i> , 1987
Respiratory rate predictor index (RRP)	Temperature, humidity, wind speed and solar radiation	Eigenberg <i>et al.</i> , 2005
Adjusted temperature humidity index (THIadj)	Temperature, relative humidity, wind speed and solar radiation	Mader <i>et al.</i> , 2006
Heat load index (HLI) - for feedlot cattle	Temperature, humidity, wind speed and solar radiation	Gaughan <i>et al.</i> , 2008
Comprehensive climate index (CCI)	Temperature, humidity, wind speed and solar radiation	Mader <i>et al.</i> , 2010
Index of thermal stress for cows (ITSC)	Temperature, wind velocity, humidity, and solar radiation	Da Silva <i>et al.</i> , 2015
Equivalent temperature index for cattle (ETIC)	Air temperature, relative humidity, air velocity, and solar radiation and their interactions	Wang <i>et al.</i> , 2018b
Dairy heat load index (DHLI)	Black globe temperature and relative humidity	Lees <i>et al.</i> , 2018

A comprehensive review with comparison of the most popular indices evaluating their suitability for forecasting heat stress in dairy cattle is presented by Herbut *et al.* (2018). In another systematic review, Wang *et al.* (2018a), described 16 of the most commonly used cattle-related thermal indices, how they were developed and their relevance to the heat transfer characteristics. Although these and many other indices have been defined in literature, they have not been widely applied in genetic evaluation studies. In all the different indices reported, there is usually a higher correlation between the index and ambient temperature compared to other meteorological parameters such as relative humidity, wind speed, and solar radiation (Bohmanova *et al.*, 2007, Dikmen and Hansen, 2009, Hammami *et al.*, 2013). It has also been argued that solar radiation and wind speed are of minor importance for cows kept in insulated barns (Brügemann *et al.*, 2012).

From the foregoing, it is clear that numerous indices have been developed and applied in modeling heat stress. Most of these indices simply incorporate weather data readily available from weather

stations. Studies on heat stress mostly adopt these indices as originally developed or with adjustments. This is without due consideration of the conditions under which they were developed. Some were developed for young heifers or steers, cattle breeds were either dairy, beef or even crossbred and physiological responses may cause variations in rectal temperature, respiration rate, dry matter intake and milk yield (Wang *et al.*, 2018a). It is also not obvious THI are better predictors of body temperature in heat-stressed cows than other measurements of environmental conditions (Dikmen and Hansen, 2009). Although gridded weather parameters are available, gridded index parameters e.g., gridded THI are not readily available and their application in heat stress in livestock is scarce. Therefore, more research is needed to identify improved comprehensive heat stress indicators that can be incorporated into heat tolerance selection decisions.

## CONCLUSION

There have been remarkable advances in the main components necessary for evaluating heat stress in dairy cattle. Performance records evaluation has seen advancement from lactation milk yield to test-day milk yield and use of daily milk records is becoming prevalent.

High temporal resolution weather data such as hourly meteorological data from weather stations that are readily available can provide useful information that will improve evaluation of heat stress. Studies have shown that gridded weather data are useful for applications in agricultural crop phenology and crop yields and as such, they may have the potential of improving the evaluation of heat stress effects in dairy cattle.

With climate change, there is need for routine genetic evaluation in dairy cattle populations. It is anticipated that identification and genetic selection of heat tolerant cattle will result in animals with minimum decline in production under conditions of heat stress. So far, there is no univocal threshold for the beginning and extent of production loss due to heat stress in dairy cattle. Selection for increased milk in the face of heat stress will therefore require robust prediction models combining high temporal resolution performance records and high spatial and temporal resolution weather information.

## **RECOMMENDATIONS**

Use of high temporal resolution animal production data to analyze effects of heat stress in dairy cattle. In this regard, daily milk yield data are more precise than test-day records. Test-day records are scattered and may not capture the precise weather changes occurring between test days. It is expected that daily production records combined with high-resolution weather data will allow for precise estimation of heat stress effects. So far, no such studies have been carried out.

Use of high spatial resolution weather data to analyze effects of heat stress in dairy cattle. This may include studies to evaluate how weather station data compares with grid-interpolated data. It may also be important to establish the grid size of interpolated data that would improve precision of heat stress detection. Although gridded weather parameters are available, gridded index parameters e.g., gridded THI have not been made readily available and their application in heat stress in livestock is relatively unexploited.

Development of more robust statistical models taking into account the production systems incorporating high spatial and temporal resolution weather data and high temporal resolution animal production records. Accurate prediction models will precisely rank animals on their breeding values and heat tolerant animals identified and selected.

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## CHAPTER TWO

### **Modeling heat stress effects on milk production in a tropical environment using test-day records and random regression models**

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## **Modeling heat stress effects on dairy cattle milk production in a tropical environment using test-day records and random regression models**

### **Abstract**

In tropical environments, dairy cattle production is constrained by several factors, including climate. The seasonal loss of milk due to heat stress is a recurring challenge for many dairy producers. The objective of this study was to detect heat stress thresholds, milk yield loss and individual animal variations using random regression models for dairy cattle from test-day milk records. Data were obtained from the Kenya Livestock Breeders Organization for the years 2000–2017 and merged with weather data. The weather parameters were grid-interpolated solar and meteorological data obtained from the National Aeronautics and Space Administration/Prediction Of Worldwide Energy Resources (NASA/POWER). After editing, the records comprised 49 993, 45 251 and 36 136 test-day records for first, second, and third lactations, respectively, for the four main dairy breeds; Friesian (68.0%), Ayrshire (21.1%), Jersey (7.6%) and Guernsey (3.3%). Variance components were estimated using Restricted Maximum Likelihood in ASReml software. Random regression models with third-order Legendre polynomials were fitted to the average and individual lactation curves and the reaction norms. An extended factor analytic variance structure for the random cow effects was used to estimate (co)variances between days in milk and thermal load. The daily average temperature (TA) and temperature humidity index (THI) were identified as the most suitable thermal load indicators for assessing milk yield losses. Considering a one day lag, the estimated heat stress thresholds were about 22°C and 69 index units for TA and THI respectively. Almost no differences were observed for estimated residual variances between the thermal load indicators, indicating there was no better model fit by TA or THI. The heat stress thresholds and milk loss patterns are important for management of dairy production systems in the tropics with climatic conditions similar to this study. Data recording should be improved as a tool to monitor the expected impacts of climate change and mitigation measures.

**Keywords:** Dairy breeds, extended factor analysis, heat stress threshold, milk yield loss, thermal load indicators,

## Implications

Environmental stress is a major limiting factor to dairy production in the tropics. This is expected to be aggravated by the impact of climate change, and therefore, the need for quantifying the effects of heat stress on milk production. To quantify heat stress effects on milk yield loss, random regression models were applied on test-day milk yield records and weather data. Dairy cattle producers should expect milk yield loss associated with heat stress when daily average temperature and temperature humidity index are above 22°C and 69 index units, respectively, if no heat abatement measures are available.

## Introduction

Heat stress is important for dairy production because of its negative effects on productivity and ultimate profitability. The seasonal loss of milk yield and changes in milk composition due to heat stress during hot weather are recurring challenges for many dairy producers. The factors that influence heat stress in lactating dairy cows and how this affects milk production have been presented in a comprehensive review by Kadzere *et al.* (2002). The continuous selection of dairy cows for elevated milk production may have inadvertently resulted in a correlated decrease in heat tolerance (Ravagnolo and Misztal, 2000). This is because maintaining body temperature relatively constant in an environment of increasing heat stress is complicated by higher metabolic heat production. Such effects are expected to become even more exaggerated with the impact of anticipated climate changes. Against this background, there is growing interest in modeling and quantifying the effects of heat stress on dairy production.

To quantify heat stress effects on milk yield loss, Ravagnolo and Misztal (2000), proposed using test-day milk yield records and weather data from public weather stations. Test-day milk yield permits the removal of short-term environmental variation in phenotypic data because it considers the specific environmental effects for each production record, which is not possible for 305-day data (Visscher and Goddard, 1995). Weather data (e.g., temperature and humidity) can be included in a Best Linear Unbiased Prediction model both as a fixed or a random effect. Moreover, reaction to temperature may have a genetic component. For this purpose, reaction norm models implemented using random regression test-day models have been shown to be effective in

evaluation of heat stress effects on production traits in different geographical regions (Brügemann *et al.*, 2012, Carabaño *et al.*, 2016). Previous studies have been carried out in subtropical regions of the United States (Bohmanova *et al.*, 2007), in hot and dry conditions of Spain (Carabaño *et al.*, 2014), in tropical and subtropical climates of Brazil (Santana *et al.*, 2017) and also under temperate conditions of Europe (Hammami *et al.*, 2013, Carabaño *et al.*, 2016). However, data from tropical regions, including East Africa, have been little investigated.

As in other tropical environments, dairy production in Kenya is constrained by several factors, including small herd size, lack of systematic identification, inadequate animal performance and pedigree recording, lack of industry organization (Kahi *et al.*, 2004) and climate. Dairy production is largely undertaken in the highlands where the climate, infrastructure and market access are favorable (Kosgey *et al.*, 2011). The production systems include: large-scale commercial dairy, mixed crop-livestock and intensive smallholder farms where the main genotypes are purebreds, crossbreds and purebred/crossbred combinations, respectively (Ojango *et al.*, 2016 and references therein). The largest proportion of milk is produced by smallholder farmers who keep animals of mixed breed-types (Marshall *et al.*, 2019; Ojango *et al.*, 2019). In these systems, there is no systematic breeding of crossbred cattle and pedigree and/or performance records are rare (Marshall *et al.*, 2019). The trends in the performance traits of dairy cattle seem to vary from breed to breed and from herd to herd due to non-genetic differences such as locality, management and selection objectives (Amimo *et al.*, 2007). Furthermore, The patterns of animal response to meteorological parameters may differ across climatic conditions, production systems, or both (Carabaño *et al.*, 2016). Previous studies in Kenyan highlands reported that dairy cows do not suffer undue heat stress to affect milk yield, with the heat stress being only transient (Ikiror, 2001; King *et al.*, 2006). These studies were, however, localized and based on a limited number of observations. No study has yet evaluated the effect of heat stress at national level with a large population and different production environments, which is essential for breeding and management programs.

Global climate change is projected to increase temperatures by 1.5°C above pre-industrial levels by between 2030 and 2052 if it continues at the current rate, with the number of hot days projected to increase in most land regions, with highest increases in the tropics (IPCC, 2018). Increase of temperatures will affect livestock more in higher rather than lower altitudes, because in the latter they are better adapted to high temperatures and drought (Thornton *et al.*, 2009). Management of

production losses may therefore demand the utilization of more heat-tolerant breeds and selection within breeds in high altitude regions.

More recently, reaction norm models using polynomial functions have been applied to determine the temperature humidity index (THI) value at which performance begins to drop and also the degree to which performance declines after some unknown THI threshold (Sánchez *et al.*, 2009, Carabaño *et al.*, 2016). Such models provide more flexible patterns in fitting milk production response to increasing heat loads (Brügemann *et al.*, 2012, Carabaño *et al.*, 2016). In this way, individual variation in the onset and persistence of heat stress can be determined and heat-tolerant animals can be identified and selected. So far, little research has been carried out to evaluate the milk yield response to heat stress in dairy cattle under tropical conditions. The objective of this study was, therefore, to implement reaction norm models using random regression to detect heat stress thresholds and individual animal variations from test-day milk yield records.

## **Materials and methods**

### **Data source and description**

Data on milk performance were obtained from the Kenya Livestock Breeders Organization (KLBO) and Livestock Recording Centre (LRC). The KLBO is a farmers' organization with functions of livestock registration and milk recording, whereas LRC is a government department under the Ministry of Agriculture and has the responsibility of genetic evaluation of recorded cows, estimating the breeding values of A.I. bulls and progeny testing.

For the years 2000–2017, the test-day records were used from the first three lactations of the main dairy cattle breeds: Friesian, Ayrshire, Jersey and Guernsey. Although the database contains crossbreds, they were not included in the current study. It is important to note that animal registration and milk recording are voluntary and it is the producer who sends the milk records to KLBO. Test-day records are normally sent in once every month preferably on the 15<sup>th</sup> day. However, some farmers send in records on any other day within the month. For our study, a cow's record made on any day other than the 15<sup>th</sup> was retained in the database if there were more than two records for that herd-test-day (HTD). The data were edited to include days in milk (DIM) 5–305 of lactation. To reduce the milk yield outliers, records in the upper and lower 1% quartile were

pruned. It was realized that these records contained numerous typological errors. As a result, test-day records below 3 kg and above 30, 34 and 36 kg in the first, second and third lactations respectively were excluded. Cows with missing calving dates were also excluded because their DIM could not be determined. This led to a reduction in the total number of test-day records by 41.2%, 39.7% and 38.5% for the first, second and third lactations, respectively. After editing, the average number of records per cow was  $7.6 \pm 3.3$ ,  $7.9 \pm 3.3$ , and  $8.1 \pm 3.1$  for first, second and third lactations, respectively, with some cows having more than one record in a given test-month.

Calving seasons were categorized into four: January–March for the dry season, April–June for the main wet season, July–September as the intermediate rains and October–December as the second wet season. Dairy cattle management systems are mainly free-stall housing without environmental modification.

### **Weather information**

Weather information was obtained from the NASA Langley Research Center Prediction Of Worldwide Energy Resource (POWER) Project (<https://power.larc.nasa.gov/data-access-viewer/>). The POWER Data Access Viewer contains solar and meteorological datasets for support of agricultural needs, renewable energy and building efficiency needs. The weather parameters are based upon solar radiation derived from satellite observations and meteorological data from assimilation models. The parameters are provided on a global grid with a spatial resolution of  $0.5^\circ$  latitude by  $0.5^\circ$  longitude. The tool has Geographic Information System (GIS) functionality as an option to the data ordering/access option (Stackhouse Jr *et al.*, 2018). Each herd has information of its location by postal address. However, the coordinates for exact location of the farms are not provided. When the coordinates of the farm location or nearest town were input, weather data from nearby bi-linearly interpolated grid were obtained in a time series for the specified period. Climate data obtained were relative humidity (RH, %), wind speed (WS, m/s), daily minimum, average and maximum air temperature (Tmin, TA, Tmax, °C) all at 2 m above the surface of the earth and solar radiation (SRAD, MJ/m<sup>2</sup>/day) as All Sky Insolation Incident on a Horizontal Surface. The herds' production information was then matched with the grid-interpolated climate data. This resulted in data comprising of individual cow's records and the corresponding weather information for the test day.

### Climatic indices evaluated

Several thermal load (TL) indicators have been applied in the evaluation of heat stress in dairy cattle. The dry-bulb temperature is generally considered to be the principal thermal measure. The effect of high temperature is exacerbated by high humidity or solar radiation whereas wind reduces its adverse effects. These have been combined into indices that assess heat stress in dairy cattle. Four different kinds of TL indicators relevant for a tropical climate were employed successively in order to evaluate the heat load for each test day. First, the effect of ambient temperature as a TL indicator was evaluated for both daily TA and Tmax. Next, THI and heat load index (HLI) indices were calculated from the weather parameters and their effects evaluated.

From the TA and RH for the test day, THI was calculated following National Research Council (NRC, 1971), as follows:

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

where, TA is the average daily temperature in °C and RH is relative humidity in %.

This THI was selected because it has been associated with thermal stress in dairy cattle and is designed to indicate the level of heat stress for outdoor cattle (Bohmanova *et al.*, 2007).

The other index evaluated was HLI, which has been found to be a suitable predictor of heat stress in dairy cattle in the tropics because of its favorable correlation with rectal temperature and respiration rate. The HLI was calculated following Silva *et al.* (2007) as:

$$HLI = 33.2 + 0.2RH + 1.2T_g^* - (0.82WS)^{0.1} - \log(0.4WS^2 + 0.0001)$$

$T_g^* = 1.33TA - 2.56TA^{0.5} + 3.21\log(SRAD + 1) + 3.5$  is the predicted globe temperature (°C)

where, TA is average daily ambient temperature in °C and RH is in %, WS is average daily wind speed (m/s) and SRAD is total daily solar radiation (MJ/m<sup>2</sup>/day).

For all the TL indicators, a timelag of up to three days prior to test day was evaluated. The objective of this was to identify the day on which the weather had the largest influence on milk yield in

relation to the test day. For this study, Tmax failed to depict a heat stress threshold and HLI failed to give consistent results with regard to the shape of the breed specific reaction norms and therefore further results are sparingly discussed.

### Statistical models

First, a mixed model was applied comprising random additive genetic effects as well as permanent environmental effects (see supplementary material S1). From this analysis, it was implausible to disentangle the additive and permanent environmental variance components. The estimated additive variance unrealistically tended towards zero. However, total phenotypic variances were plausible and within the expected range. Therefore, these results are not presented.

The second set of analysis was a random regression test-day model whereby genetic variance components were not further considered and a cow model was used (model 2). The reaction norm model used time dependent (DIM) and TL dependent covariates. The model accounted for the population level response and individual cow's variation along the DIM and TL gradients. In this model the individual cow effect was fitted as a random effect. This was to account for the fact that response to increasing heat load may vary across individuals. Cows were assumed to be genetically unrelated. The random regressions were modeled with third-order Legendre polynomials (intercept, linear, quadratic and cubic terms) for DIM and (linear, quadratic and cubic terms) for TL indicators. Cow variances were assumed to be the same for all breeds, but differing from lactation to another. Data were analyzed using a univariate model in which the three lactations were treated as separate but correlated traits. Different residual variances were applied for the different lactations. The model was fitted as follows:

$$Y_{ijknop} = \mu + L_i + B_j + LB_{ij} + CS_k + \sum_{l=0}^3 \phi_{ijkl} d_{ijkl} + \sum_{m=1}^3 \tau_{im} r_{im} + HTD_n + \sum_{l=0}^3 \phi_{iol} c_{iol} + \sum_{m=1}^3 \tau_{iom} p_{iom} + e_{ijknop}$$

where  $Y_{ijknop}$  is milk yield (kg) of cow  $o$  within lactation  $i$  of breed  $j$  in calving season  $k$ ,  $L_i$  is lactation number,  $B_j$  is the fixed effect of breed  $j$ ,  $CS_k$  is the fixed effect of  $k^{th}$  calving season,  $d_{ijkl}$  is the  $l^{th}$  fixed regression coefficient on DIM of breed  $j$  in lactation  $i$  and calving season  $k$ ,  $\phi_{ijkl}$  is the corresponding  $l^{th}$  covariate describing the shape of the fixed lactation curve,  $r_{im}$  is the  $m^{th}$

regression coefficient on the TL indicator of lactation  $i$ ,  $\tau_{im}$  is the corresponding  $m^{th}$  covariate describing the population response to a given TL indicator,  $HTD_n$  is the random effect of herd-test-day  $n$ ,  $C_{iol}$  is the  $l^{th}$  random regression coefficient on DIM of cow  $o$  in lactation  $i$  for individual cow effect,  $\phi_{iol}$  is the corresponding  $l^{th}$  covariate describing the shape of the lactation curve of cow  $o$  in lactation  $i$ ,  $p_{iom}$  is the  $m^{th}$  random regression coefficient on TL indicator of cow  $o$  in lactation  $i$ ,  $\tau_{iom}$  is the corresponding  $m^{th}$  covariate describing the response to a given TL indicator by individual cow  $o$  in lactation  $i$  and  $e_{ijknop}$  is the residual term.

To evaluate the heat stress effects for the different breeds, model 2 was modified to model 3 as follows:

$$Y_{ijknop} = \mu + L_i + B_j + LB_{ij} + CS_k + \sum_{l=0}^3 \phi_{ijkl} d_{ijkl} + \sum_{m=1}^3 \tau_{ijm} r_{ijm} + HTD_n + \sum_{l=0}^3 \phi_{iol} c_{iol} + \sum_{m=1}^3 \tau_{iom} p_{iom} + e_{ijknop}$$

where  $r_{ijm}$  is the  $m^{th}$  regression coefficient on thermal load indicator of lactation  $i$  for breed  $j$ ;  $\tau_{ijm}$  is the corresponding  $m^{th}$  covariate describing the breed specific response to a given thermal load indicator, other parameters are as described in model 2.

For the breed effect, the hypothesis that there was no difference on milk yield at all test days between breeds was tested. Significance of differences was tested by Wald F statistics. The assumed (co)variance structure of random effects was:

$$Var \begin{bmatrix} \phi \\ \tau \\ \mathbf{e} \end{bmatrix} = \begin{bmatrix} \mathbf{I}_n \otimes \mathbf{C}_\phi & \mathbf{I}_n \otimes \mathbf{C}_{\tau\phi} & \mathbf{0} \\ \mathbf{I}_n \otimes \mathbf{C}'_{\tau\phi} & \mathbf{I}_n \otimes \mathbf{C}_\tau & \mathbf{0} \\ \mathbf{0} & \mathbf{0} & \mathbf{I}_n \sigma_e^2 \end{bmatrix}$$

where  $\mathbf{C}_\phi$  and  $\mathbf{C}_\tau$  are covariance matrices of random regression coefficients for cow effects by DIM and by TL respectively,  $\mathbf{C}'_{\tau\phi}$  is covariance matrix for cow effects for DIM and TL combination and  $\sigma_e^2$  is the residual variance.  $\mathbf{C}_\phi$  is a  $12 \times 12$ ,  $\mathbf{C}_\tau$  a  $9 \times 9$  and  $\mathbf{C}'_{\tau\phi}$  a  $12 \times 9$  (co)variance matrix,  $\mathbf{I}_n$  is identity matrix of size  $n \times n$  ( $n$  is the number of cows with records).

The expected susceptibility to heat stress across the range of DIM allowed estimation of covariance components between DIM and TL. An Extended Factor Analytic (XFA) variance structure for the random cow effects was used to estimate (co)variances between DIM and TL. The XFA model was fitted as factor loadings, on DIM and TL with a single intercept but different slopes. Analyses were carried out by fitting Restricted Maximum Likelihood (REML) method using the ASReml 4.1 program (Gilmour *et al.*, 2015).

$$\mathbf{V} = \begin{bmatrix} \mathbf{C}_\phi & \mathbf{C}_{\tau\phi} \\ \mathbf{C}'_{\tau\phi} & \mathbf{C}_\tau \end{bmatrix}$$

was approximated by an XFA model (Meyer, 2009) where:

$$\mathbf{V} = \mathbf{\Gamma}\mathbf{\Gamma}' + \mathbf{\Psi}$$

where  $\mathbf{\Gamma}$  is a  $21 \times 3$  matrix of factor loadings and  $\mathbf{\Psi}$  is a diagonal matrix.

For each lactation ( $l$ ), a matrix  $\mathbf{V}_l$  of variances and covariances of individual cow effects was estimated as:

$$\mathbf{V}_l = \mathbf{\Phi}_l \mathbf{C}_l \mathbf{\Phi}_l'$$

where  $\mathbf{\Phi}_l$  is a matrix of Legendre polynomial functions of DIM and TL for lactation  $l$  and  $\mathbf{C}_l$  is a  $7 \times 7$  covariance matrix for the same lactation. Trajectories of individual cow variance due to DIM ( $\sigma_{c(d)}^2$ ) or to TL ( $\sigma_{p(t)}^2$ ) were obtained by holding TL or DIM constant.

Short-term repeatability at a certain DIM and TL point was estimated as the proportion of individual cow variance due to DIM and TL to the total variance as follows:

$$r_{d,t} = \frac{\sigma_{c(d)}^2 + \sigma_{p(t)}^2 + 2\sigma_{cp(dt)}}{\sigma_{c(d)}^2 + \sigma_{p(t)}^2 + 2\sigma_{cp(dt)} + \sigma_e^2}$$

where  $2\sigma_{cp(dt)}$  are covariances of the  $d^{th}$  DIM and  $t^{th}$  level of TL, other model terms are as previously described.

This can be interpreted as short-term repeatability because milk yield is measured at consecutive days whereby correlation is expected to be close to unity, as usually assumed in a repeatability model. In the long run correlations tend to be lower. By varying both the DIM and TL, a repeatability surface was plotted.

## **Results**

### **Data summary statistics**

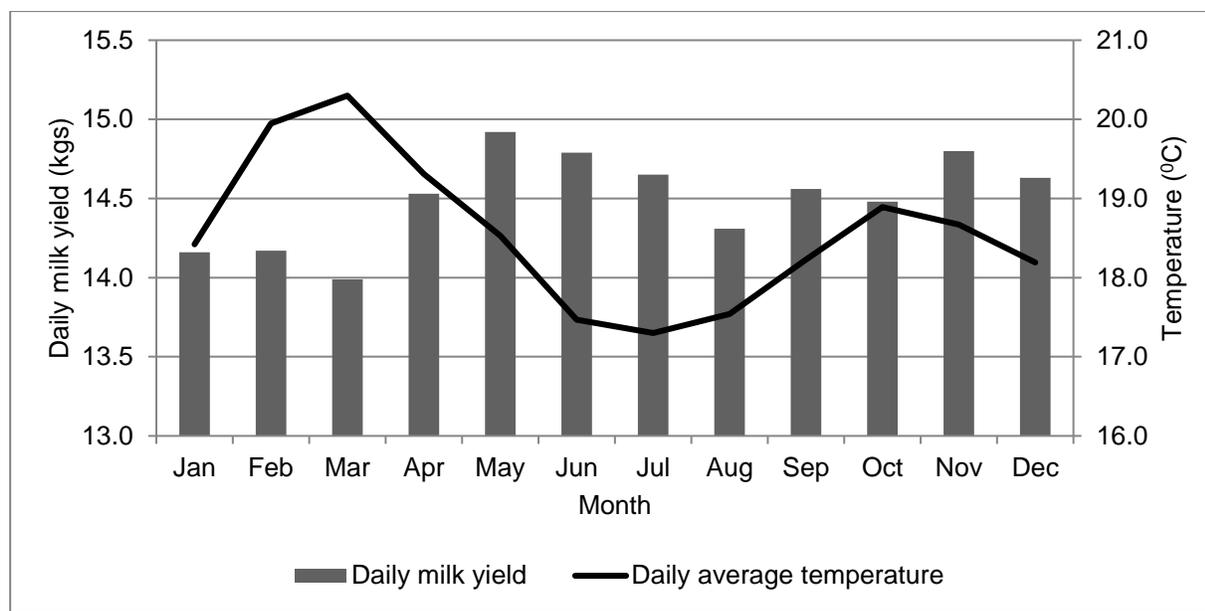
Table 1 presents the summary statistics for the cows with records in the first three lactations after data editing. Friesian was the most popular breed among the dairy cattle in the country. The distribution of calving season was 24.0, 26.0, 25.2 and 24.8% respectively for the four seasons. The distribution of breeds within herds was 45.2, 15.6, 4.6 and 1.7% with Friesian, Ayrshire, Jersey and Guernsey breeds, respectively, while 32.9% had mixed breeds.

**Table 1.** Description of the test-day milk yield data used to model heat stress effects in the first three lactations of dairy cattle in Kenya.

	Lactation 1	Lactation 2	Lactation 3
Mean daily milk $\pm$ SD (kg)	13.7 $\pm$ 5.4	14.7 $\pm$ 6.4	15.4 $\pm$ 6.8
Total number of test-day records	49 993	45 251	36 136
Number of cows by breed			
Friesian	4 469	3 872	2 938
Ayrshire	1 441	1 304	1 094
Jersey	396	344	258
Guernsey	203	184	149

### Weather information

The average daily Tmin, TA and Tmax were  $13.0 \pm 2.0^{\circ}\text{C}$ ,  $18.6 \pm 1.9^{\circ}\text{C}$  and  $25.4 \pm 2.7^{\circ}\text{C}$ , respectively. Calculated THI and HLI were on average  $63.9 \pm 2.5$  and  $77.0 \pm 2.6$ , respectively. Figure 1 presents the distribution of average milk yield and TA across months. The general pattern was a seasonal fluctuation of milk yield following weather variation, particularly temperature. The lowest milk yield was in February and March which also had the highest average daily temperatures of  $20.0^{\circ}\text{C}$  (range  $16.0\text{--}25.3^{\circ}\text{C}$ ) and  $20.3^{\circ}\text{C}$  (range  $16.5\text{--}26.0^{\circ}\text{C}$ ), respectively. These months had the lowest RH at 53.7% and 54.7%, respectively.



**Figure 1.** Distribution of average test-day milk yield for dairy cattle and average temperature across months in Kenya.

### Statistical models

Fitting HTD as a fixed effect was faced with model convergence issues probably because of small herd sizes and was, therefore, fitted as a random effect. With regards to the timelag in relation to test day, the Log-likelihood for the different TL indicators are presented in Table 2. The values were highest for 1 day lag for TA and THI, and 2 days for HLI. All proceeding results are based on these timelags.

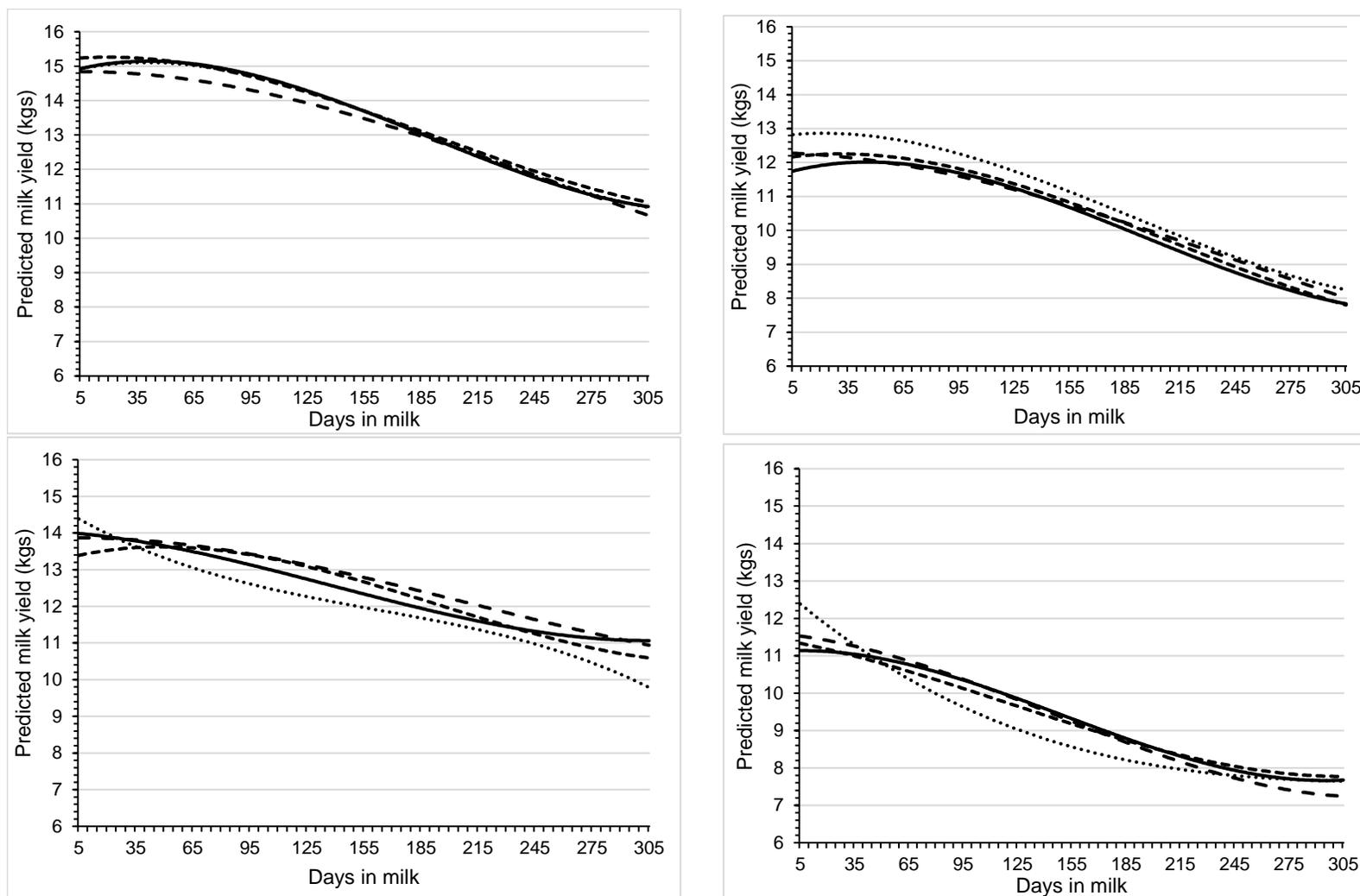
**Table 2.** Restricted Maximum Likelihood (REML) Log-likelihoods for the different thermal load indicators, for the three days prior to milk production test-day for dairy cattle in Kenya.

Thermal load indicator	Day 1	Day 2	Day 3
Average Temperature	-7319.83	-7320.93	-7320.43
Temperature Humidity Index	-7331.40	-7323.68	-7328.58
Heat Load Index	-7336.91	-7323.84	-7336.12
Maximum temperature	-7315.68	-7309.87	-7337.17

## Lactation curves

The average lactation curves for the different breeds by calving season are presented in Figure 2. There were significant differences in milk yield between breeds ( $p < 0.001$ ) for all TL indicator models. Milk yield was lower in the first lactation and increased in subsequent lactations. In comparison to the Friesian, milk yields for the Jersey, Ayrshire and Guernsey breeds were lower by  $-1.4 \pm 0.37$ ,  $-2.3 \pm 0.22$  and  $-4.3 \pm 0.5$  kg, respectively.

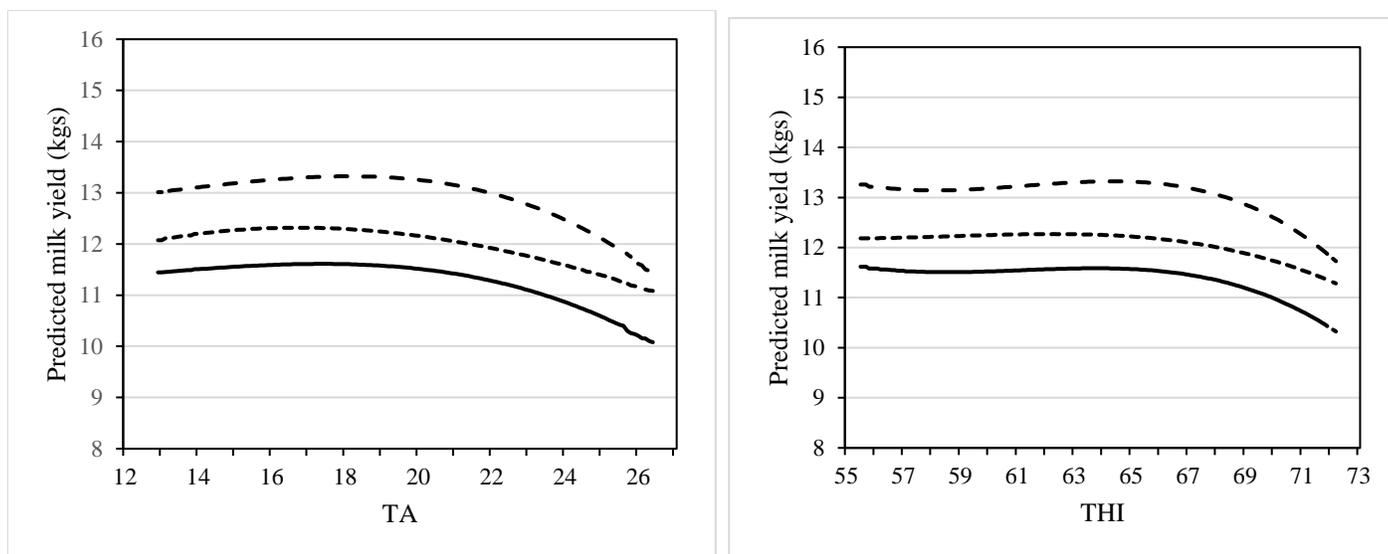
The Friesian and Ayrshire breeds, as the breeds with the most numerous records, exhibited typical lactation curves in all calving seasons. This is whereby the shapes of the lactation curves started with a slightly ascending phase from parturition to a non-distinct peak, followed by a declining phase until drying off. In contrast, continuously decreasing curves were estimated for Jersey and Guernsey breeds.



**Figure 2.** Average lactation curves for the different dairy cattle breeds, Friesian upper left, Ayrshire upper right, Jersey lower left and Guernsey lower right by calving season ( — CS1 ..... CS2 --- CS3 -.- CS4).

### Population level reaction norms of milk production to increasing heat load

The trends of daily test-day milk yield response to TA and THI TL indicators for the first three lactations are presented in Figure 3. These average curves represent least square means for distinct values of a particular TL indicator and lactation number.



**Figure 3.** Reaction of test-day milk yield to average temperature (TA °C) and temperature humidity index (THI units) thermal load indicators for the first three lactations ( — Lactation 1 ..... Lactation 2 --- Lactation 3) of dairy cattle in Kenya.

The comfort zone for TA was about below 22°C after which a declining trend was observed. An average TA of 22°C on a given test day could be realized from different combinations of Tmax and Tmin. Extreme examples in the data were Tmax of 31.2°C with Tmin 14.1°C and Tmax 26.5°C with Tmin 18.1°C, reflecting different daily temperature variations.

The THI comfort zone was below 69, above which there was a declining trend in milk yield. The average milk yield loss was -0.29, -0.19 and -0.37 kg/THI unit per day for the first, second and third lactation respectively. A THI of 69 could be obtained from different combinations between TA and RH, for example, TA of 21.4°C and RH of 78.9%, and TA of 23.4°C and RH of 43.1%.

Although not consistent, a threshold of about 81 index units from the average curve can be assumed for HLI. The HLI of 81 could be obtained at the lowest TA of 17.2°C with the following combination: 83.4% RH, 23.2 MJ/m<sup>2</sup>/day SRAD (with a range 4.2–30.1) and 0.8 m/s WS (with a range of 0.3–5.4). In such a situation, despite the low temperature, the low wind speed and high RH make it difficult for the animals to lose heat through evaporative cooling. A similar HLI could be obtained from a combination of highest TA of 24.2 °C in combination with 58.1% RH, 16.0 MJ/m<sup>2</sup>/day SRAD and 2.4 m/s WS.

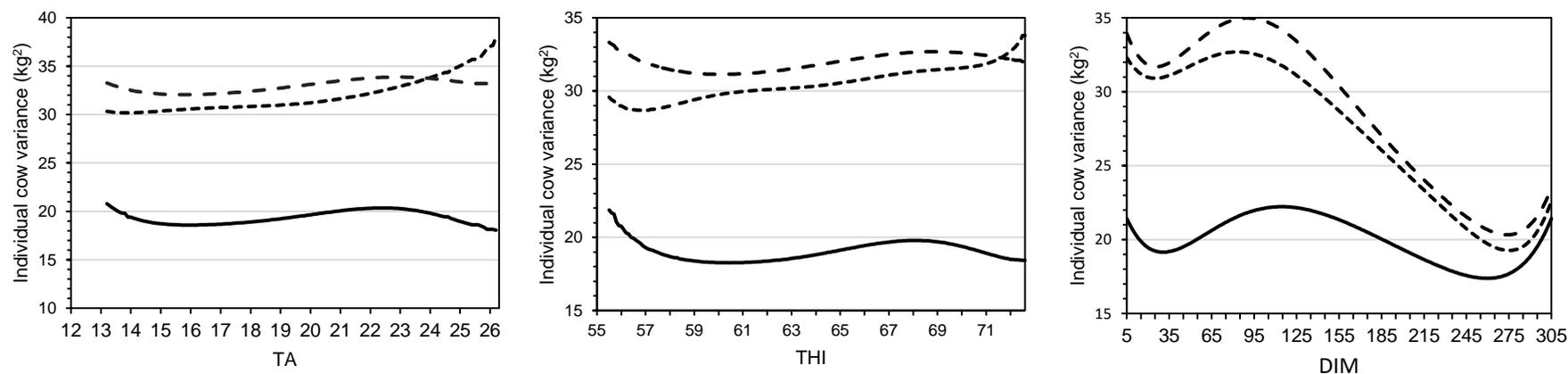
**Table 3.** Milk yield loss (kg/day) per unit increase in average temperature and temperature humidity index thermal load indicators for the first three lactations of dairy cattle in Kenya.

Thermal load indicator	Thermal load indicator range	Lactation 1	Lactation 2	Lactation 3
Average Temperature	22–23	-0.18	-0.16	-0.23
	23–24	-0.24	-0.18	-0.29
	24–25	-0.28	-0.19	-0.37
	25–26	-0.38	-0.24	-0.49
	26-26.4 (maximum TA)	-0.12	-0.07	-0.17
Temperature Humidity Index	69–70	-0.20	-0.15	-0.26
	70–71	-0.27	-0.18	-0.35
	71–72	-0.32	-0.22	-0.43
	72-72.6 (maximum THI)	-0.24	-0.15	-0.30

The reactions of test-day milk yield response to TA, THI, Tmax and HLI for the individual dairy cattle breeds are presented in Supplementary Figures S1, S2, S3 and S4 respectively. For TA and THI indicators, with exception of the Guernsey, the other breeds had a trend similar to the average curve in the first and third lactations. There were inconsistencies across breeds in the second lactation, but generally, the Friesian and Guernsey showed a more stable reaction with minimal milk yield loss. In all lactations, milk yield was more stable in the Guernsey. Inconsistent trends with regard to the shape of the reaction norm were observed for HLI whereby increasing milk yield with increasing heat load was observed.

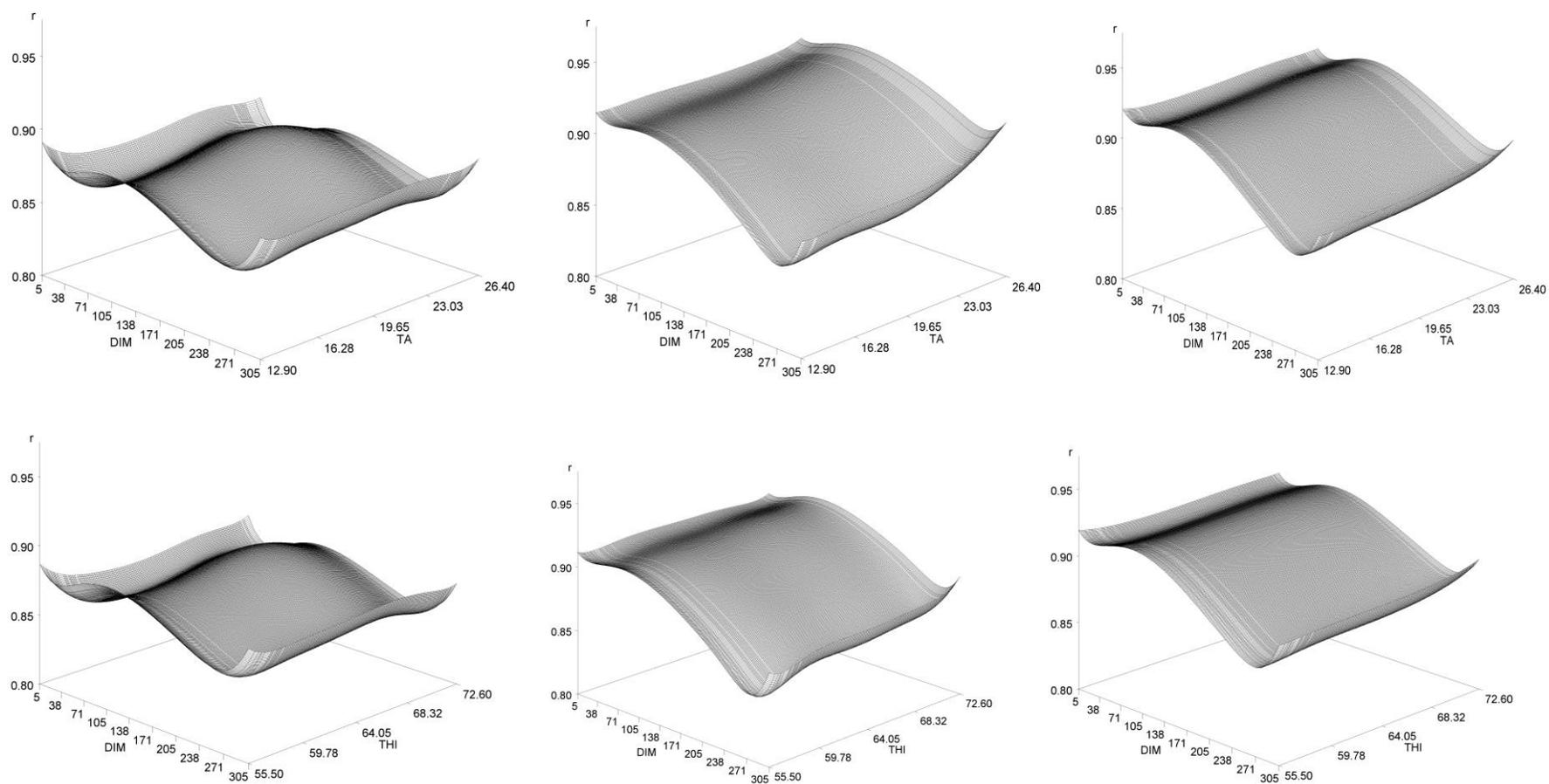
**(Co)variances between cow effects along the thermal load trajectories**

Estimates of all (co)variance parameters between DIM and TL was obtained by an XFA model. The XFA model approximates the (co)variances with a reduced number of factors which was three in this study. No convergence to a positive definite covariance matrix was obtained with more factors. Two random effects were fitted for the individual cow effect but with only a single intercept. As a result, the XFA analysis was achieved with 21 loading parameters per factor. The estimated loading parameters per factor and the variances for the XFA model for TA and THI with a one day lag are presented in Supplementary Tables S1 and S2 respectively. Almost no differences were observed between the estimated residual variances for both TA and THI indicators. So, no better model was fitted using TA or THI. However, there was a consistent increase in the estimated residual variation with lactation number. For parities one to three, the respective residuals variances were 3.0, 3.8 and 4.5. Estimated cow variances along trajectories of TA and THI at a given DIM (e.g. 60) and for DIM at a given temperature (e.g. 22°C) follow the trends presented in Figure 4. The trend for THI along DIM was similar to that of TA and therefore not presented. Above the heat stress threshold, there was a decline in variability between cows in the first and third lactations and an increase in the second lactation. The individual cow variation due to DIM increased with parity. Within parity, the variances started by decreasing in early lactation followed by an increase to peak in mid lactation (about 70–120 days) from where they decrease towards late lactation and then slightly increases towards drying off.



**Figure 4** Individual cow variances (kg<sup>2</sup>) attributed to average temperature (TA °C, left) and temperature humidity index (THI units, middle) thermal load indicators at DIM 60 and to days in milk (DIM days, right) at 22°C for the first three lactations ( — Lactation 1 ..... Lactation 2 — - Lactation 3) of dairy cattle in Kenya.

The estimated short-term repeatability, presented as a three-dimensional function of DIM at different levels of TA and THI are presented in Figure 5. The figures follow the cross-sectional trends presented in Figure 4. Short-term repeatability estimates ranged from 0.84–0.89, 0.85–0.93 and 0.86–0.92 for first to third lactation, respectively, for TA indicator. In general, short-term repeatability estimates were high at combinations of lower TL and early- mid- and late lactations. There was always a decline during the first month of lactation followed by increase in mid-lactation and then a decline comparable to the shape of the lactation curve.



**Figure 5.** Repeatability surfaces for heat load in a given day in milk (DIM) and average temperature (TA °C, upper panel) and temperature humidity index (THI units, lower panel) for first (left), second (middle) and third (right) lactations of dairy cattle in Kenya.

## Discussion

The seasonal variation in milk production can be attributed to environmental changes across months/seasons (Figure 1). This primarily affects milk production both directly through decreased dry matter intake and indirectly through fluctuation in the quantity and quality of feeds and other environmental variations including heat stress. The seasonal variability of milk yield is characterized by high milk during wet seasons and low milk in dry seasons. This is primarily due to pasture availability. With significantly high moisture content during the wet season, pasture growth improves and strongly influences the milk yield. During the dry season, the quality and quantity of forages drastically decline. Any seasonal variation of feed quality should be captured by the sequence of HTD effects, which should capture additional variation that is not related to thermal load. Such meteorological variations have been cited as a major nuisance in the estimation of genetic and environmental trends for cattle under tropical conditions (Mayer and Musani, 2002).

For the current study, daily TA was  $18.6 \pm 1.9^{\circ}\text{C}$  with a range of  $13.0\text{--}26.2^{\circ}\text{C}$ , which largely falls within the thermal-comfort zone for dairy production. Performance records were from four main dairy cattle breeds in Kenya with the Friesian contributing the highest number of test-day records and also having the highest milk yield. In agreement with Bebe *et al.* (2003), the Friesian breed is given top priority by farmers because of the commercial objective to produce surplus milk for income generation. Jersey and Guernsey breeds are considered lower milk producers and are, therefore, less preferred. There are a large number of crossbreeds between the “exotic” dairy breeds and indigenous Zebu cattle utilized as dairy cattle, but these were not included in our analysis. This is because the nature of crossbreeding is generally unstructured resulting in farmers rearing a wide range of crossbred genotypes (Ojango *et al.*, 2019). It is expected that crossbreds would be more heat stress resilient. A methodology of estimating genomic EBV ranking animals with different proportions of exotic dairy breeds like the one implemented by Ojango *et al.* (2019) could incorporate a TL indicator covariate to model heat stress tolerance in crossbreds.

The number of test-day records followed a declining trend with increasing parity. Generally, animal registration and production recording remain relatively low in Kenya. This is probably because livestock recording is done voluntarily by producers to meet their individual farm needs. It is common practice for farmers to record only a few animals within a herd to reduce costs associated

with animal registration and recording. In some instances, farmers record only the best cows (high milk yield) which could reduce within-herd variation and create bias during evaluation. It is also clear from this study that a substantial proportion of records are deleted due to incompleteness or other recording related issues. With variation in types of records and scope of recording between farms, some records are incoherent for the purpose of national genetic evaluation (Wasike *et al.*, 2011). This could also bias the modeling of heat tolerance because highly productive cows which are more likely to be recorded suffer more heat stress than low yielding cows (Carabaño *et al.*, 2014). In addition to the few animals recorded, there is also the challenge of few records per cow. Although the records indicate that the average number of records per cow to be above seven per lactation, it is worth noting that some cows had more than one record in a given test-month. The study of Muasya *et al.* (2014) acknowledged such records but used records of the official test day, a record made on any other day was retained only if no record was available on the official test day. For our study, the extra records provided additional performance and related weather information within the test-month.

Studies at population and individual cow response to heat stress apply either daily average or maximum temperature (Carabaño *et al.*, 2014), or combine several weather parameters into an index such as THI or HLI (Bohmanova *et al.*, 2007, Silva *et al.*, 2007). The estimated values for the heat stress threshold affecting milk yield were 22°C and 69 units for TA and THI, respectively, above which milk yield started to decline. The trend in milk yield response was relatively similar for the TL indicators despite having different combinations of climate parameters, and therefore, measurement scales. The TA provided better results than Tmax which is in agreement with (Carabaño *et al.*, 2016). As expected, TA takes into account both Tmax and Tmin and gives a better prediction method because the high heat load during the day may be relieved by low night temperatures. From the diurnal temperature variation in the tropics, Tmax is achieved at around midday and lasts only a few hours and therefore exerts minimum physiological costs on the animal. This is a crucial aspect when studying heat stress in the tropics. Results from Tmax and HLI showed inconsistent reaction curves and were not considered valuable thermal load indicators. The average temperature thresholds in this study were similar to 23°C and RH of 75% reported in Georgia, USA (Bohmanova *et al.*, 2007). However, this is much lower than the popularly established threshold of 26°C (review by Kadzere *et al.*, 2002) and estimated values of 29°C in Holstein cows in southern Spain (Carabaño *et al.*, 2014).

Different authors have proposed THI classes that define the heat stress effect in cattle, for example,  $\text{THI} < 71$  as the comfort zone, 72–79 as mild stress, 80–89 as moderate stress and  $>90$  as severe stress (Armstrong, 1994). Thom (1959) classified  $\text{THI} < 70$  as comfortable, 70–74 as uncomfortable, 75–79 as very uncomfortable and  $\geq 80$  as serious discomfort. Following the observed trends, the conditions for dairy production in the Kenyan highlands can be considered comfortable with seasonal episodes of discomfort or mild stress. However, despite the comfortable production conditions, milk yield loss due to heat stress was evident. The THI threshold obtained in the current study is in agreement with (Hammami *et al.*, 2013) despite the latter featuring Holsteins in Central Europe.

The milk yield loss per THI increase above the threshold was in agreement with corresponding values of  $-0.25$ ,  $-0.45$  and  $-0.47$  kg/day per THI reported for Brazilian Holstein (Santana *et al.*, 2017) but lower than those reported for Italian Holstein cows at  $-0.9$ ,  $-1.16$  and  $-1.27$  kg/day per THI for first, second and third lactations respectively (Bernabucci *et al.*, 2014). From a similar THI formula, the traditionally considered threshold of 72 corresponded to a milk yield loss of  $-0.39$  kg/day per THI unit for first-parity cows in the warm and humid climate of Georgia, USA (Bohmanova *et al.*, 2007). In general, studies in dairy cattle have shown that  $\text{THI} < 68$  does not cause safety problems in healthy animals, with mild discomfort setting in at  $68 \leq \text{THI} < 72$  (Bouraoui *et al.*, 2002). Apart from animal factors, the differences in heat stress thresholds result from differences in climates where the animals are raised, but the variation in magnitude could be associated with the statistical models and methods applied in each study (Carabaño *et al.*, 2014). For example, heterogeneity in THI calculations, combinations of daily maximum or average temperature, minimum or average humidity (Brügemann *et al.*, 2012) and in the timelag in relation to test day (Bernabucci *et al.*, 2014) could result in different upper threshold for comfort zone. From the study of (Bohmanova *et al.*, 2007), it is clear that temperature humidity indices differ in their ability to detect heat stress. This has also been demonstrated by the different TL indicators applied in this study.

The HLI applied here has been used as a TL indicator in Holstein and Jersey cows in Brazil (Silva *et al.*, 2007). It is a suitable index for tropical conditions because of its correlation with physiological response to thermal stress. The index classification for dairy cattle was  $\text{HLI} < 89$  as safe, 89–92 as caution, 92–95 as extreme caution and  $>95$  as danger based on correlation with rectal

temperature and respiration rate (Silva *et al.*, 2007). Based on this classification, dairy cattle production systems in Kenya can be considered as safe from heat stress. The suitability of this indicator for evaluating dairy cattle in the tropics is, however, limited in literature.

Comparison of milk yield loss for individual breeds indicated a more stable reaction for cows in the second lactation for both TA and THI TL indicators (Supplementary Figure S1 and S2 respectively). In all lactations, the milk yield was more stable in Guernsey. However, it is not conclusive that they are more tolerant to heat stress because of the small number of records in this study and their scanty distribution.

In addition to observing the average response to increasing heat load, estimation of individual cow variation was necessary because phenotypic measurement and genetic selection of cows are implemented at individual cow level. Covariance parameters between DIM and TL using the unstructured model had trouble converging because of non-positive definite matrix. To overcome this problem, XFA model was implemented. This created (co)variance components for all available DIM by TL combinations. Since the random regression on DIM and on TL are both cow effects, a single intercept but different slopes were fitted. Fitting two intercepts led to model convergence problems. Furthermore, two random intercepts for the same cow effects would be unidentifiable (anonymous reviewer). Considering the TL variances, there was little individual cow variation at the comfort zone. This implies that detection of differences between animals is enhanced when cows are subjected to extreme environments. The increase in variability out of the comfort zone could be explained by the fact that high yielding cows are affected differently from low yielding cows. At high thermal loads, the expression of full genetic potential of high yielding cows may be impaired and cause the observed variability (Carabaño *et al.*, 2016). Below the comfort zone, more energy is required for homeostasis at the expense of production. The variation trend along DIM also indicate the influence of lactation stage on variability between cows. For cows in the first lactation, the trend is in agreement with Ojango *et al.* (2019), who reported declining genetic variances from the first to second month but then increasing in mid-lactation for smallholder dairy herds in Kenya.

The high repeatability estimates indicate favourable measurement of consistent individual differences. This indicates that cows that maintain their production even with increasing TL i.e. heat tolerant cows can be identified and selected. The estimates for this study were obtained from

a different approach from the classical genetic approach. Because of this, there are no comparable estimates in literature. However, the main highlight from such an approach is that different genes get turned on and off when animals age or when they move between environments (Brügemann *et al.*, 2011). The application of random regression models with time-dependent (DIM) and TL-dependent (THI) covariates allows flexibility in statistical modeling without defining specific heat stress thresholds. This allows for identification of possible genotype by environment interactions for multiple DIM by TL combinations.

One of the limitations of the weather data used in this study is that it was not herd specific. However, on-farm weather recording can be complex and controlled experiments are scarce, rendering weather station and grid interpolated data more useful especially for large-scale evaluations. In regions where the density of ground weather station is sparse, grid-interpolated weather data such as the NASA/POWER satellite-based system used in this study have been reported to be robust source of climate data for agricultural planning (Monteiro *et al.*, 2018).

The TA and THI thresholds and milk yield loss obtained in this study are important in management of dairy production systems in the tropics with similar climatic conditions. On days when the thresholds are exceeded, it is recommended that cows be provided with cooling facilities (e.g., shade, fans or sprinklers) while accordingly modifying the diet to sustain milk production. Long-term sustainable production would be achieved through selection and breeding of heat-tolerant individuals. As such, future studies should aim at estimating heat stress breeding values in addition to developing methods for identifying heat-tolerant animals. Garner *et al.* (2016) demonstrated that breeding for heat tolerance could be accelerated by genomic selection using SNP markers that predict tolerance to heat stress. With availability of resources, such an approach could be explored.

## Conclusion

Heat stress thresholds and milk yield loss were determined for daily average temperature and temperature humidity index thermal load indicators for dairy cattle in Kenya. Average daily temperature and temperature humidity index had similar milk yield loss estimation capabilities. In addition, there was almost no difference in estimated residual variances across between the TL indicators. This means simply using average daily temperature is sufficient to model heat stress under Kenyan conditions. A lag period of one day prior to test day should be considered. More

detailed studies on heat stress, including genetic aspects will require further improvement of dairy data collection. Good quality milk recording data will, in particular offer the opportunity to monitor both the impacts of expected climate changes on milk production and of measures for mitigation.

## **Acknowledgement**

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## Supplementary material S1

Model 1 was as follows;

$$Y_{ijkno} = \mu + HTD_i + B_j + CS_k + \sum_{i=1}^p \phi_{jkl} d_{jkl} + \sum_{m=0}^{q1} \beta_{mn} a_{mn} + \sum_{m=0}^{q2} \beta_{mn} pe_{mn} + e_{ijkno}$$

where,  $Y_{ijkno}$  is the milk yield in kgs from herd-test-day  $i$  of cow  $n$  in breed  $j$  in calving season  $k$ ;  $HTD_i$  is herd-test-day effect fitted as a fixed or random effect;  $B_j$  is the fixed effect of the breed  $j$ ;  $CS_k$  is the fixed effect of  $k^{th}$  calving season;  $d_{jkl}$  is the  $l^{th}$  fixed regression coefficient on days in milk (DIM) of breed  $j$  in calving season  $k$ ;  $\phi_{jkl}$  is the corresponding  $l^{th}$  covariate describing the shape of the fixed lactation curve;  $a_{mn}$  and  $pe_{mn}$  are the  $m^{th}$  random regression coefficients of cow  $n$  for additive genetic and permanent environmental effects respectively;  $\beta_{mn}$  is the  $m^{th}$  term of the  $q1^{th}$  and  $q2^{th}$  order Legendre polynomial covariates for additive genetic and permanent environmental effects, respectively, depending on DIM and  $e_{ijkno}$  is the residual term.

**Supplementary Table S1** Estimated loadings per factor and the variances for the extended factor analytic (XFA) model for average temperature (TA) heat load indicator with a one day lag for dairy cattle in Kenya.

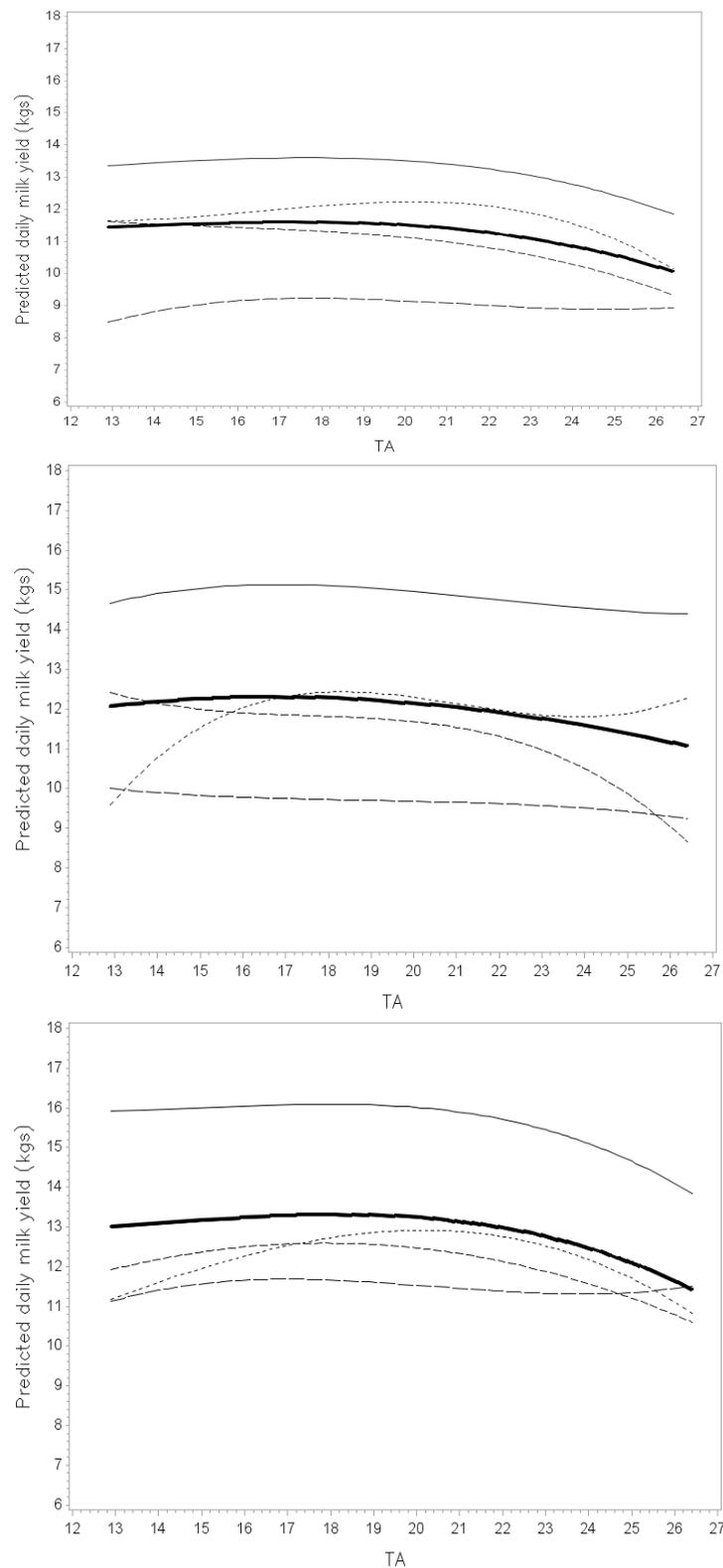
Factor 1	Factor 2	Factor 3	Variances
4.49133	-0.23346	-0.34725	12.62030
-0.04676	0.83002	-0.07307	2.19540
-0.50576	-0.16240	0.53666	0.79012
0.18702	-0.07162	-0.25308	0.37625
6.38976	0.26408	0.84611	3.85326
-0.73595	1.15465	-0.18702	3.11556
-0.58643	-0.19785	0.15531	1.14818
0.18107	-0.19088	-0.07092	0.65514
5.53983	0.17268	-0.48224	15.66780
-0.83910	1.35210	0.22529	3.06685
-0.68751	-0.33288	0.73795	0.97126
0.23917	-0.19239	-0.41701	0.59744
0.04229	0.13015	-0.01765	0.38501
-0.09428	-0.06473	0.13912	0.00000
-0.06968	0.26831	0.10340	0.16568
0.39906	0.17664	0.15863	0.89145
0.06570	0.10131	-0.12083	0.00000
0.08703	0.09395	0.12388	0.13818
0.21007	0.14185	0.00183	0.41262
-0.00557	0.08021	0.62691	0.00000
0.00488	0.20294	0.02588	0.08040

Ordering of effects: Legendre polynomials for days in milk (DIM) for lactations 1, 2 and 3 (12 effects) followed by Legendre polynomials for TA for lactations 1, 2 and 3 (9 effects)

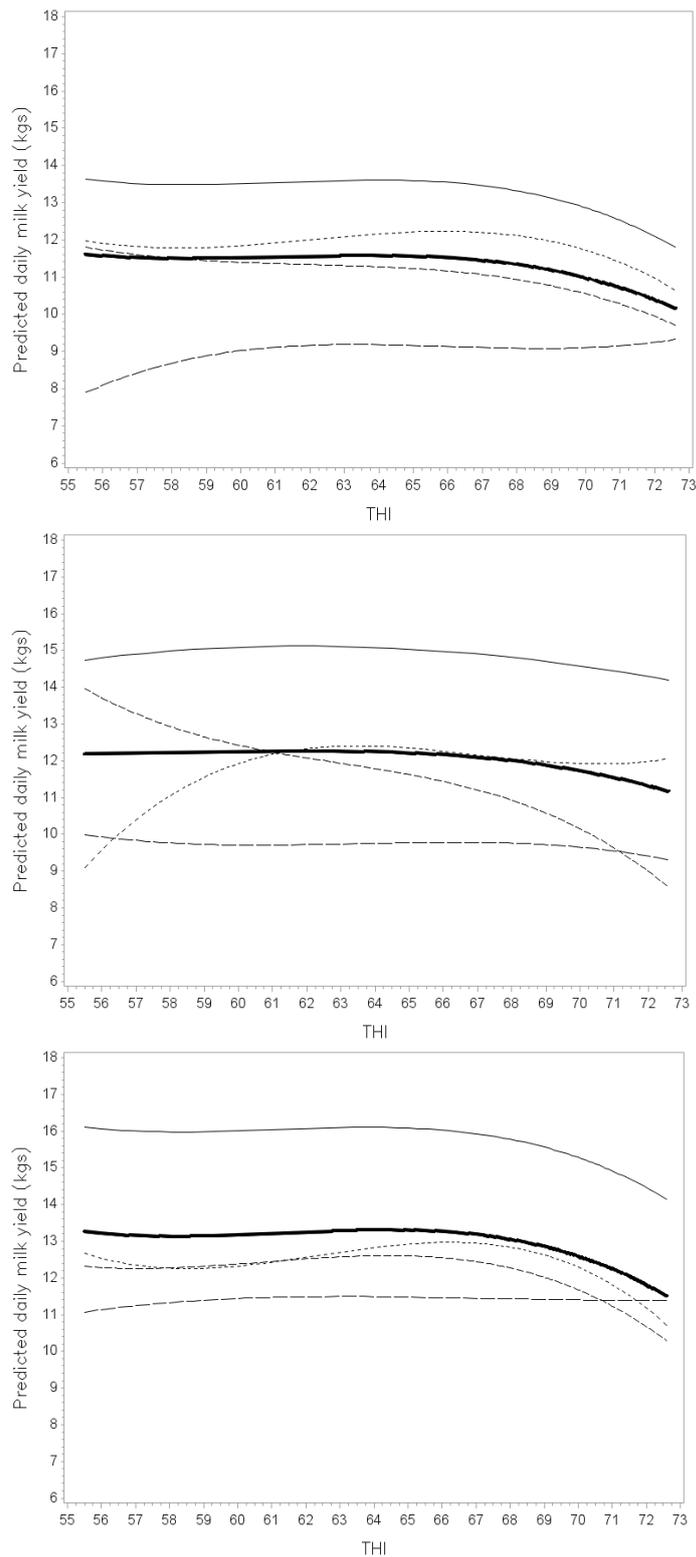
**Supplementary Table S2** Estimated loadings per factor and the variances for the extended factor analytic (XFA) model for temperature humidity index (THI) heat load indicator with a one day lag for dairy cattle in Kenya.

Factor 1	Factor 2	Factor 3	Variances
-4.47100	-0.24711	-0.36539	12.73300
0.05534	0.82781	-0.07361	2.19897
0.50225	-0.16901	0.52855	0.79927
-0.18721	-0.07030	-0.24694	0.38129
-6.25776	0.28686	0.87451	3.51067
0.74794	1.13198	-0.20146	3.15799
0.58297	-0.19927	0.14961	1.15359
-0.18218	-0.18835	-0.06216	0.65965
-5.45942	0.18457	-0.49887	15.77160
0.85315	1.36186	0.21552	3.02447
0.68138	-0.32960	0.75404	0.96177
-0.24117	-0.19557	-0.40487	0.60692
-0.02972	0.07750	0.00015	0.32324
0.05131	-0.21451	0.10688	0.00000
0.08623	0.14373	0.11732	0.17627
-0.22454	0.08217	-0.26903	0.00000
0.03732	0.02254	-0.20627	0.00000
-0.04147	0.11349	0.07535	0.34427
-0.16589	0.13762	0.07083	0.44436
0.01348	0.00334	0.11138	0.00000
-0.01016	0.21565	0.06865	0.00000

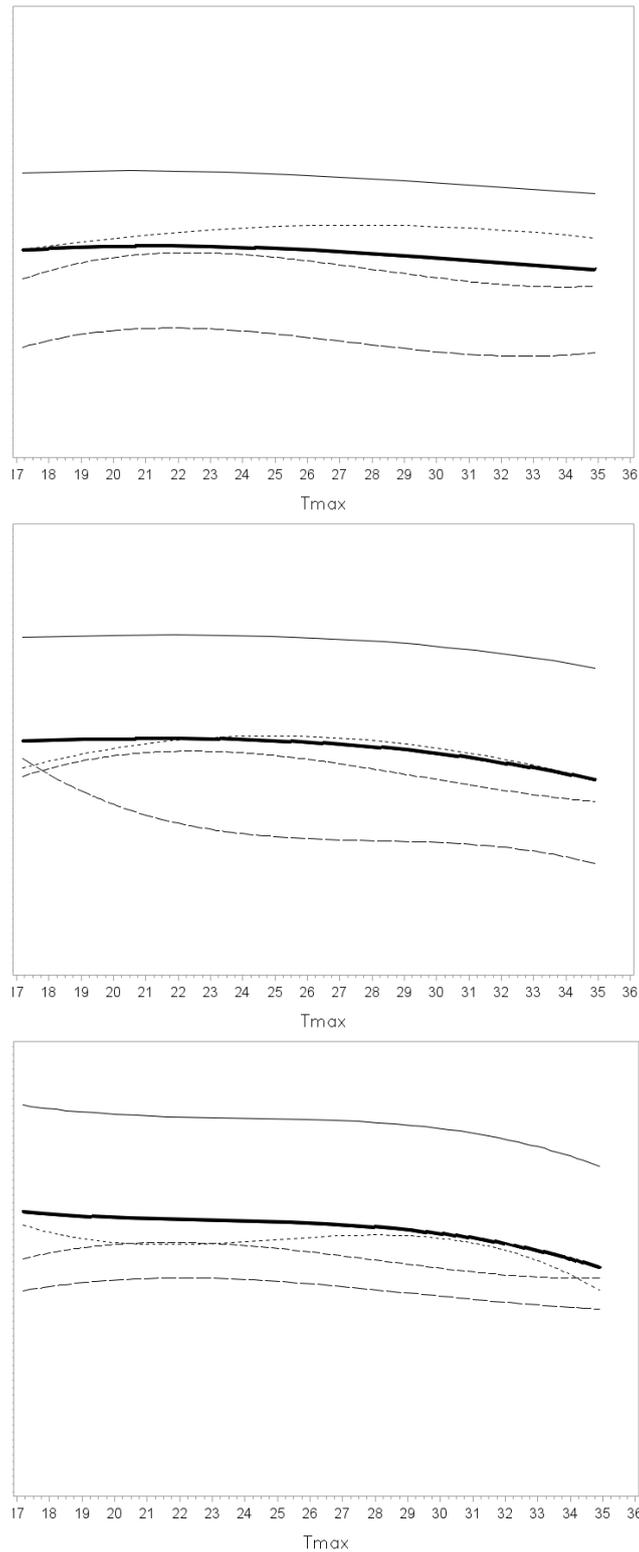
Ordering of effects: Legendre polynomials for days in milk (DIM) for lactations 1, 2 and 3 (12 effects) followed by Legendre polynomials for THI for lactations 1, 2 and 3 (9 effects)



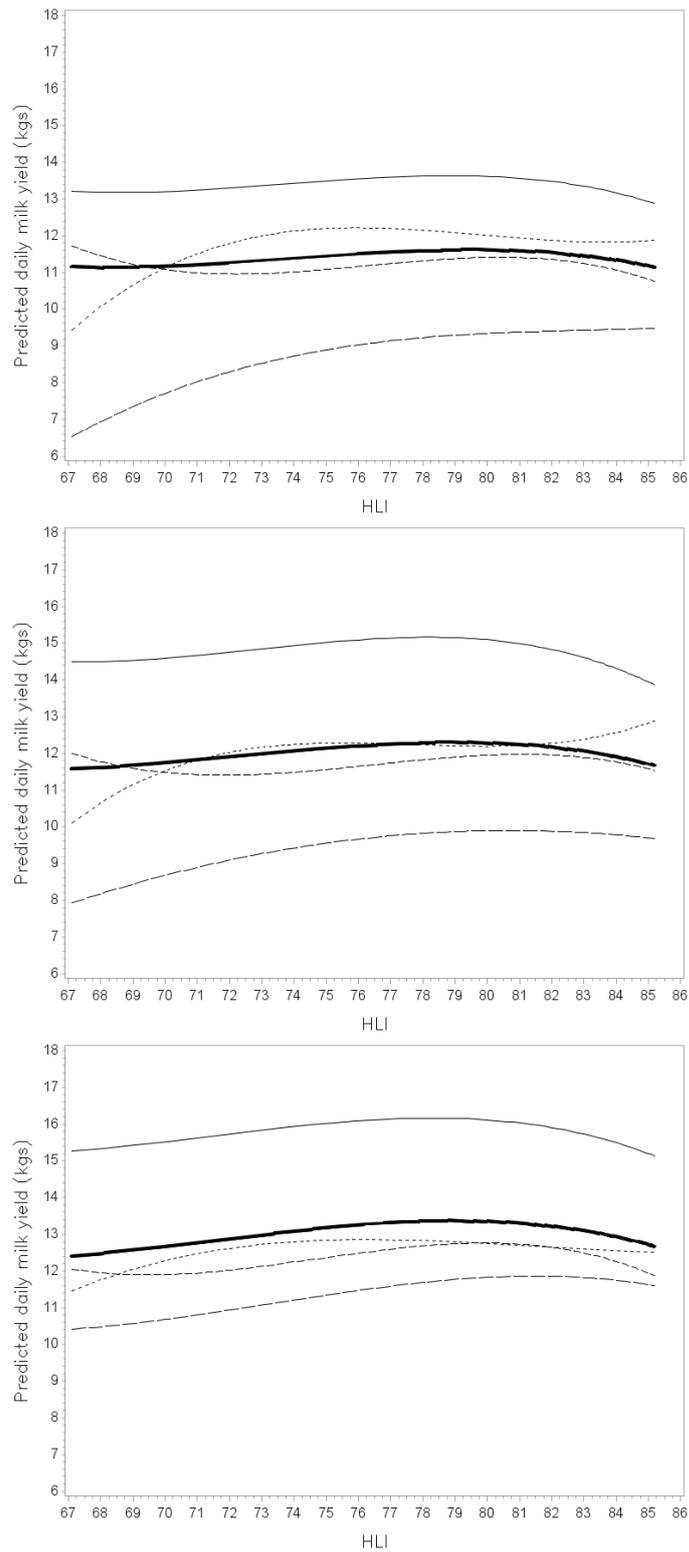
**Supplementary Figure S1** Reaction of test-day milk yield to average temperature (TA) by individual dairy cattle breeds ( — Friesian ····· Jersey - - - Ayrshire - - - Guernsey ——— and average population) in lactations 1–3.



**Supplementary Figure S2** Reaction of test-day milk to Temperature Humidity Index (THI) by individual dairy cattle breeds ( — Friesian ····· Jersey - - - Ayrshire - - - Guernsey — , and average population) in lactations 1–3



**Supplementary Figure S3** Reaction of test-day milk to daily maximum temperature ( $T_{max}$ ) by individual dairy cattle breeds ( — Friesian ..... Jersey --- Ayrshire - - - Guernsey ——— , and average population) in lactations 1–3.



**Supplementary Figure S4** Reaction of test-day milk to Heat Load Index (HLI) by individual dairy cattle breeds ( — Friesian ···· Jersey - - - Ayrshire - · - Guernsey — , and average population) in lactations 1–3.

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## CHAPTER THREE

### **Comparison of high resolution observational station and grid-interpolated weather data and application to thermal stress on herd average milk production traits in a temperate environment**

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## **Comparison of high resolution observational and grid-interpolated weather data and application to thermal stress on herd average milk production traits in a temperate environment**

### **Abstract**

A validation analysis was conducted to compare high temporal resolution observational weather station data (WSD) at hourly intervals from the nearest weather station and matching gridded numerical weather prediction (NWP) model data at a spatial resolution of 6 km. Weather data were from the German meteorological service and reanalysis was based on the Consortium for Small-Scale Modelling (COSMO)-REA6 model. The two datasets were applied on milk production data to analyse the effect of thermal stress in cattle. Animal performance data were from over 16 million monthly test-day records for the period 2010 to 2019 in the southern region of Bavaria, Germany. Individual cow records were transformed to herd averages resulting to 797,455 herd-test-days from 9,726 herds. These were merged with temperature and temperature humidity index (THI) data from 53 weather stations and corresponding gridded data. The traits considered were milk yield, protein and fat content and yield, milk urea and somatic cell score (SCS). There was good agreement between WSD and NWP model data with correlation coefficients of 0.97 for both daily average temperature and THI and 0.84 for relative humidity. However, positive and negative biases were observed in the pre-Alpine regions. Generally, WSD performed better than NWP when models were compared by Wald F statistics. For the milk production traits, the average herd reaction norms were in good agreement and followed similar trends when estimated from WSD and NWP. The general heat stress threshold at which milk yield and protein yield started to decline was 16.0°C for average daily temperature and 60 units for THI. The responses of fat yield, protein content and fat content were generally linear decline with no definite thresholds. Milk urea increased in a non-linear accelerating pattern and there was no undesired effects on SCS. The thresholds obtained in this study may be applied in estimating milk yield loss and implement necessary management measures for mitigation.

**Key words:** Fleckvieh breed, milk production traits, Numerical Weather Prediction, temperate climate, thermal stress

## Introduction

Climate change has an effect on weather variables including air temperature, precipitation, wind and solar radiation. All these have direct and indirect effects on livestock production. In temperate climate zones of Central Europe, predicted climate change conditions including increased frequencies of heat periods and drought (Gauily and Ammer, 2020), are already being experienced. So far, the summers of 2018 and 2019 recorded unusually warm conditions over Europe (Sousa *et al.*, 2019). The year 2018 was the hottest in Europe since 1880, recording a rise in average temperature by more than 1.5°C (Rust, 2019). In Germany, nine out of ten warmest years occurred in the last decade, with 2018, 2020 and 2019 respectively, being the warmest years in history (DWD, 2020). The number of summer days, i.e. days when the maximum temperature is equal to or exceeds 25°C, across Germany increased by 18 days from 1951 to 2015 (Razafimaharo *et al.*, 2020). Future changes for Bavaria predict warmer and drier summer conditions extending from as early as March (Estrella and Menzel, 2013). The high ambient temperatures due to climate change if not mitigated will impose higher heat stress, which will negatively affect milk production, reproduction and health of dairy cows (Silanikove and Koluman, 2015). Some climate change effects on dairy production systems have been discussed in a review by Gauily and Ammer (2020). It is projected that at the end of the century, milk yield decrease due to heat stress may be about 2.8% relative to the current European production, causing financial losses of about 5.4% of monthly income in the summer season (Hempel *et al.*, 2019).

To study the effects of heat stress in dairy cattle, datasets of animal performance records are normally merged with weather data from nearby meteorological stations. Usability of weather observations from stations as far as 318 km from the farm to account for heat stress has been reported (Freitas *et al.*, 2006). However, in that study different thresholds were obtained per weather station and depending on the complication of the terrain by landscapes such as mountains, distant stations could become less useful (Freitas *et al.*, 2006). Typically, a station network comprises unevenly distributed stations. Even in regions with a fairly dense network, some stations may be located at airports and in cities therefore rendering them less reliable for agricultural applications (Mourtzinis *et al.*, 2017). Alternatively, using empirical algorithms, weather information from several meteorological stations can be interpolated to produce gridded estimates of weather parameters at the desired spatial and temporal resolutions (Krähenmann *et al.*, 2016; Mourtzinis *et al.*, 2017). Compared to weather station data, gridded

data are less vulnerable to technical failures because breakdowns at single stations are compensated by surrounding ones (Dalhaus and Finger, 2016). Numerical weather prediction (NWP) local area models obtained by data assimilation technique that combines station observations with model-derived data have the advantage of filling weather data gaps especially for regions with few weather stations. Recently, high resolution NWP in addition to measured weather station data (WSD), have been used to characterize heat wave events in the Iberian Peninsula (Sousa *et al.*, 2019). In agricultural applications, NWP have been reported to be useful in estimating crop phenology and yields (Battisti *et al.*, 2019; Mourtzinis *et al.*, 2017). However, there are no studies to compare the variance of NWP against WSD and their suitability in evaluating heat stress in dairy cattle. For this regional study, we used data generated by the high-resolution reanalysis system COSMO-REA6 with a spatial resolution of  $0.055^\circ$  (6 km). It comprises the assimilation of observational data with the NWP model COSMO by the German Meteorological Service, (Deutscher Wetterdienst, DWD) using a continuous nudging scheme. The reanalysis dataset covers the period 1995 to August 2019 (Bollmeyer *et al.*, 2015).

The objective of this study was to use weather data with high spatial and temporal resolution to identify thermal stress thresholds for milk production traits at herd level in Bavaria. Hourly data from a network of 53 weather stations and gridded data from a weather prediction model were used to compare their suitability. The results from this study will provide useful information to dairy farmers and policy makers on appropriate prediction parameters necessary for herd management of thermal stress in dairy cattle.

## **Material and methods**

### **Study location**

The study location was the Swabia and Upper Bavaria administrative districts (Regierungsbezirke) in the south of the German federal state of Bavaria. Bavaria is classified as warm temperate fully humid in the Köppen-Geiger climate classification (Estrella and Menzel, 2013). Variations within the regions are strong with the north-western part being drier and warmer, the southern region bordering the Alps being cooler and wet and the gradient in precipitation, temperature and latitude being strong from north to south (*ibid.*). The geographical location of the herds was specified by villages, represented by the administrative division “Gemeinde”. A “Gemeinde” may encompass several small villages which are close

together. A map of Bavaria indicating where the herds were located is presented in Supplementary Figure 1.

## **Weather data**

### **Weather station data**

Weather data were obtained from the German meteorological service, DWD. For weather station data, hourly station observations of 2 m air temperature (°C) and 2 m relative humidity (%) were obtained from DWD (Climate Data Center 2018, [https://opendata.dwd.de/climate\\_environment/CDC/observations\\_germany/](https://opendata.dwd.de/climate_environment/CDC/observations_germany/)). In addition, the latitude and longitude coordinates for each weather station were extracted from the database. In total, data were obtained from 109 weather stations across Bavaria but only 53 were distributed within the study location when merged with nearest farms as presented in Supplementary Figure 1.

### **Gridded weather data**

The DWD reanalysis system was based on the Consortium for Small-Scale MOdelling (COSMO) limited-area model (Schättler *et al.*, 2011) which is the operational Numerical Weather Prediction (NWP) model of the DWD. The model is set up for the COordinated Regional climate Downscaling EXperiment (CORDEX) EUR-11 domain (Giorgi *et al.*, 2009) using a ~6 km horizontal resolution and temporal output steps at one hour and below. Variables assimilated included air temperature, wind speed, pressure or relative humidity (analyzed fields), but the NWP model also produces a vast list of parameters that are not directly observed and are just outputs of the NWP model (forecast fields).

The grid interpolated data were obtained from DWD ([https://opendata.dwd.de/climate\\_environment/REA/COSMO\\_REA6/](https://opendata.dwd.de/climate_environment/REA/COSMO_REA6/)). Climate Data Operators (CDO) software was used to process the COSMO-REA data (Schulzweida, 2019). CDO is a collection of many operators for standard processing of climate and forecast model data. This was used to convert data from the rotated COSMO-REA longitude-latitude grid to non-rotated coordinates, computing variables, or to extract data at the position of the irregularly distributed weather stations (observations) by applying a conservative weighted interpolation method (`remapcon`).

### Temperature Humidity Index (THI)

Thermal stress was assessed using a temperature humidity index (THI) calculated using the equation of NRC (1971) expressed as:

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

where  $T$  is the average temperature in °C and  $RH$  is average relative humidity in %.

The average daily THI was obtained by averaging the hourly THI calculated from the hourly temperature and humidity. The THI for each of the 3 days before test-day and the 3-day moving average were used to investigate the length of lag in the response of milk production to thermal stress effects.

### Milk performance records

Milk performance records were provided by the Bavarian dairy recording organization - Landeskuratorium der Erzeugerringe für tierische Veredelung in Bayern e.V. (LKV Bayern). The dominant breed in the region is the Fleckvieh. Data on individual test-day milk performance, calvings, and geographical location of farms were provided in different files. The milk performance file had a total of 746,705 cows in 12,606 farms from the year 2010 to 2019. The total number of individual test-day records for the milk production traits and somatic cell count (SCC) are presented in Table 1. Somatic cell count was log-transformed into somatic cell score (SCS) using the formula:

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

Milk protein and fat yields were calculated from the measured percentage and the daily milk yield.

Prior to analysis, data were edited as follows; records for the year 2010 were not included in the final analysis because records were retrieved with calvings starting on this year and therefore fewer records at the beginning compared to later years. A total of 39,363 cows had moved from one farm to another and were therefore excluded from the analysis because they had records on two different locations. Only days in milk (DIM) between 5 and 305 were retained. In line with national and international rules, ICAR standards for recording and sampling, milk records are taken approximately on a monthly basis, with the first record taken

between the 5<sup>th</sup> and 42<sup>nd</sup> day after calving. The interval between test days was set as not more than 45 days such that if the first test-day was at the beginning of the month, the subsequent test-day should be before the subsequent mid-month. Because of the autocorrelation between test-day records, a farm with an interval of more than one year in between recordings was treated as different farms.

The focus of this study was the response at herd level. After editing, the data were, therefore, transformed such that the herd test-day (HTD) average performance became the observed dependent variable. Test-days per herd were then numbered consecutively. Herds with only one cow on a given test-day were removed. In addition, the average number of cows for the entire study period was calculated and herds with less than three were deleted. A histogram of the distribution of number of cows per herd is presented in Supplementary Figure 2.

The distance between the DWD weather stations and the dairy herds was computed using the haversine formula. Then the DWD station with the smallest distance to the respective herd was chosen. In case of missing data, the second closest station was used to fill the gaps. The weather data were then merged with the corresponding HTD performance records. The average distance between a herd and the nearest weather station was 12 km and a maximum of 47 km where missing data was substituted with next available station. The altitude for both the herds' location and weather station were added from the open data portal of the Bavarian State Government (<https://ldbv.bayern.de/produkte/weitere/opendata.html>).

### **Statistical analysis**

Two sets of analyses were performed. The first was a validation analysis to compare the measured WSD obtained from 53 meteorological weather stations with gridded NWP model data at the respective locations of the stations for the period 2010 - 2019 (the NWP model only runs until 31<sup>st</sup> Aug 2019). From the 53 weather stations used, the hourly temperature and humidity data resulted in 4.20 million observations each. Linear regression of NWP information for temperature, relative humidity and THI were fitted at the coordinates of the 53 weather stations on the respective observed measurements from the same station. Under the assumption of unbiasedness of NWP data, all these linear regression models would result into a zero intercept and a slope of one. Additionally, Pearson correlation coefficients were calculated.

The second analysis was to incorporate WSD and NWP to milk production data. A mixed regression model was fitted to the transformed HTD data. Quartic Legendre polynomial

functions were fitted to determine the pattern of reaction norms to increasing temperature and THI. The general model was:

$$Y_{ijklmnopqs} = H_i + P_j + PLS_{jk} + CT_l + CE_m + mon_n + yr_o + yrs_{op} + \sum_{q=0}^4 \beta_q Z_q(t) + htd_{ir} + e_{ijklmnopqs}$$

where,  $Y_{ijklmnopqs}$  is the herd test-day record for milk yield, protein yield, fat yield, protein content, fat content, milk urea or SCS.  $H_i$  is the herd effect.  $P_j$  is the multiple regression of % cows in different parities (parity 1, 2 and 3) at each herd and test day.  $PLS_{jk}$  is the lactation stage by parity effect; which is a multiple regression on % of cows in different lactation stages (LS1 – LS10) by parity interaction at each herd and test day.  $CT_l$  is the multiple regression of % cows in a given calving type (single or twins) at each herd and test day.  $CE_m$  is the multiple regression of % cows in a given calving ease category (6 calving ease classes; 0 – easy without assistant to 5 – surgical delivery/fetotomy).  $mon_n$  is the month effect.  $yr_o$  is the year effect.  $yrs_{op}$  is the year by season interaction effect. Seasons were defined as follows: winter (December to February), spring (March to May), summer (June to August), and autumn (September to November).  $\beta_q$  are regression coefficients for temperature or THI,  $Z_q$  are the covariates of the qth Legendre polynomial evaluated at temperature or THI point (t).  $htd_{ir}$  is the random short-term test-day effect autocorrelated (AR1) within herd.  $e_{ijklmnopqs}$  is the residual.

From the general reaction norm model results, further analysis were carried out for fat and protein contents. Random slopes were fitted to individual herds using the equation presented in the Supplementary Material.

Analyses were based on herd average information and were, therefore, weighted by the number of cows per herd and test day. To account for repeated measurements over time i.e. for the fact that observations which are proximate are more correlated than measures that are more distant, first-order autocorrelation of test-days within herd was applied. The first-order autocorrelation was defined as the correlation coefficient,  $\text{corr}(htd_{ir}, htd_{ir+1})$ , between consecutive herd-test days. Tests for significance differences of the fixed effects and comparison among models with different thermal stress indicators (single weather station or grid-interpolated) were by Wald F statistics. A model with both WSD and NWP was fitted as the full model and compared to a model fitting only WSD (reduced model 1) or only NWP (reduced model 2). Comparison

between models with different time lags in relation to test day and weather recording was by residual variance. Potential thermal stress thresholds were identified by visual inspection of the trait reaction curves. Animal performance data were processed in SAS (version 9.4; SAS Institute Inc., Cary, NC). Analyses were done using Restricted Maximum Likelihood in ASReml 4.1 program (Gilmour *et al.*, 2015). Weather data handling, maps and plots were carried out in R version 4.0.3 (R CoreTeam, 2020).

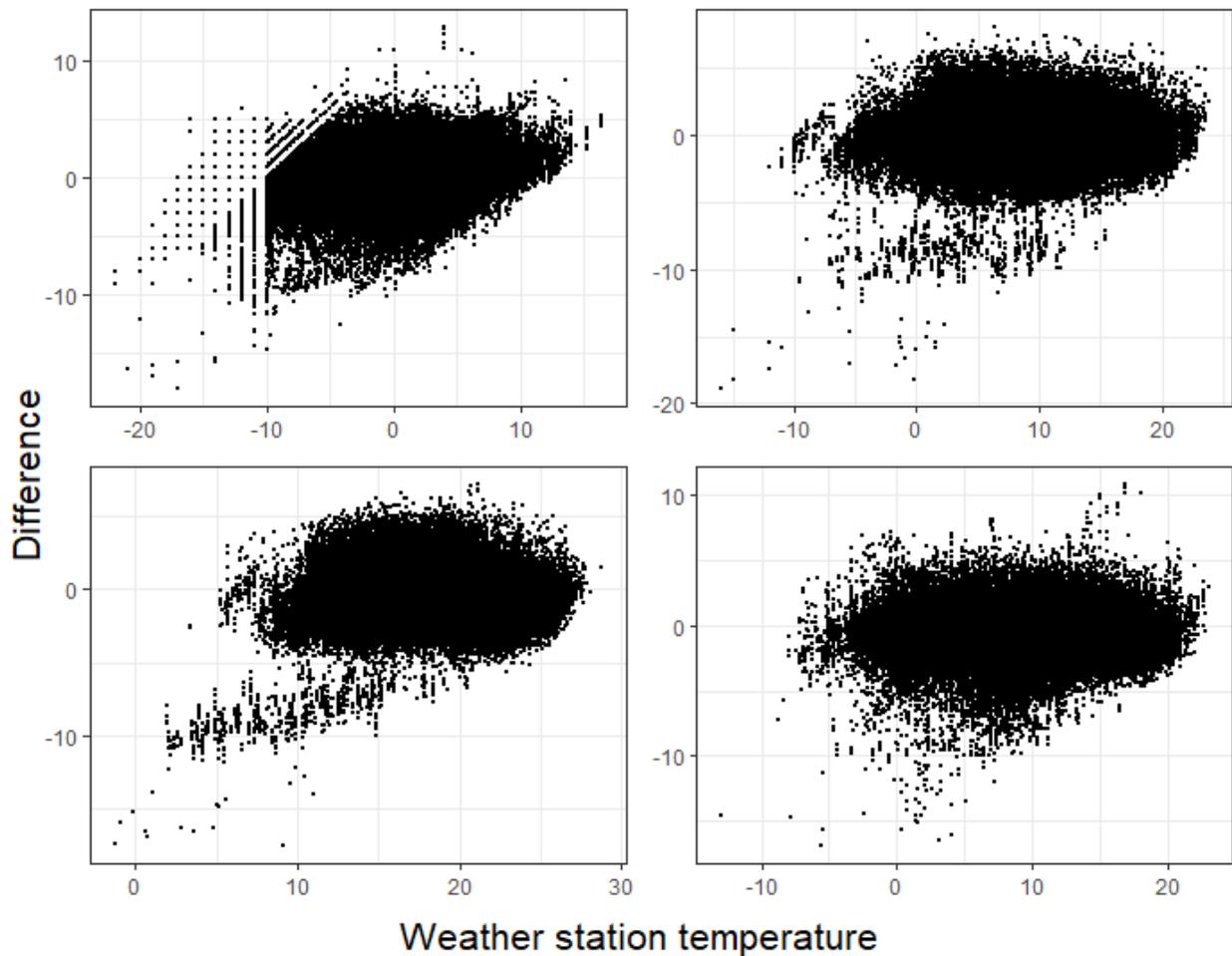
## Results

### Comparison of weather station and gridded weather data

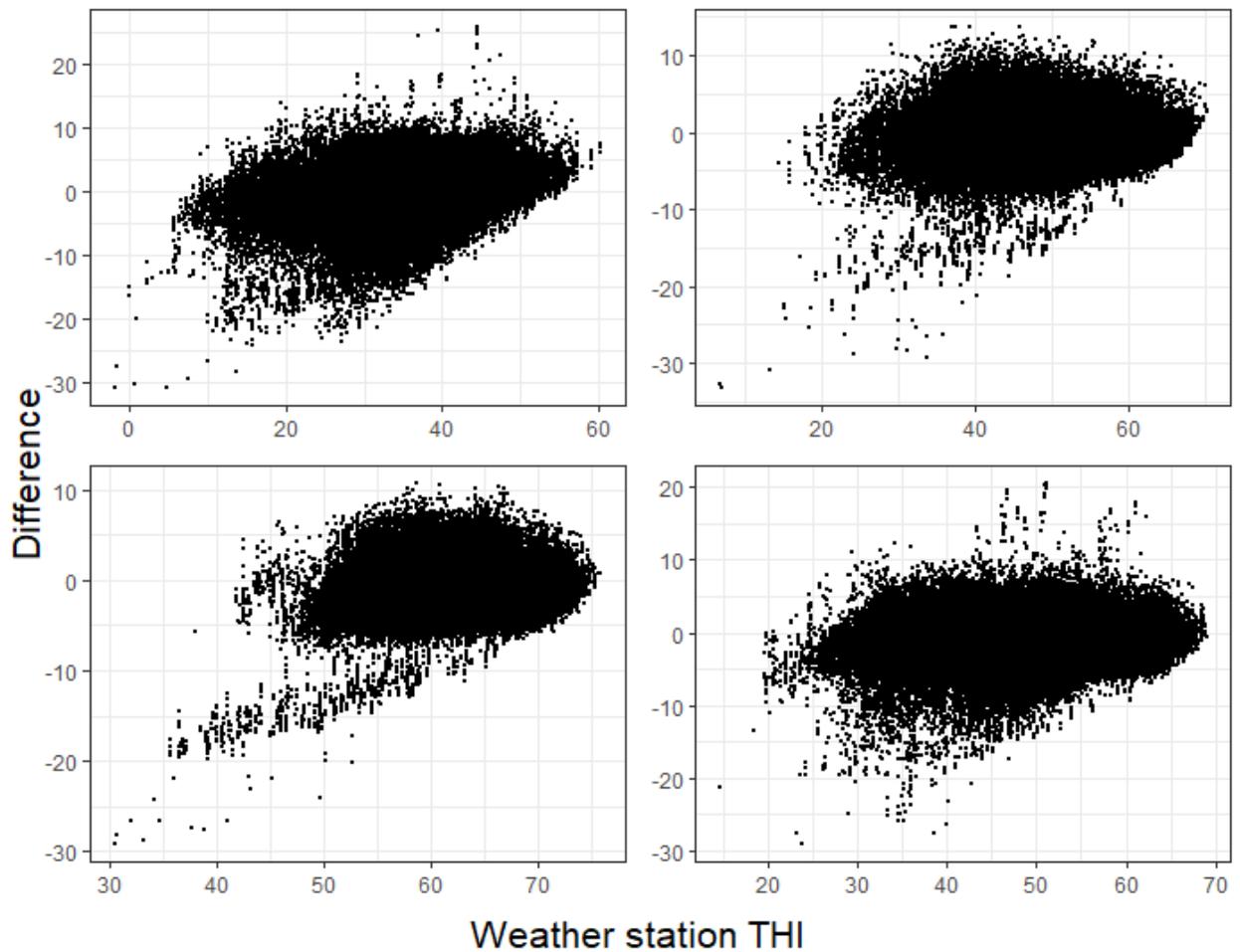
There was good agreement between WSD and NWP with high correlation coefficients ( $r$ ) of 0.97 for both daily average temperature and THI and 0.84 for relative humidity. The slopes were significantly different from zero ( $p < 0.000$ ) but indicating a positive bias (warmer, more humid) of the NWP data especially during the cold season. Comparing the correlation for temperature and THI by season, there was reasonably good agreement in autumn and spring compared to winter and summer. For relative humidity, correlation was higher in spring and summer. The comparisons of the daily average temperature, relative humidity and THI between WSD and NWP are presented in Supplementary Figure 3.

A comparison was carried out for temperature, relative humidity and THI for four different locations within the study area i.e. Harburg and Altomünster-Maisbrunn which are further away from the mountains and Oberstdorf and Zugspitze which are close to the Alps. Correlations were higher for stations further away from the mountains compared to the pre-Alp stations as presented in Supplementary Figure 4.

Despite the high correlation between WSD and NWP average temperature and THI, instances where the difference between the two (weather station – grid) was large were observed as presented in Figure 1 and 2. A negative bias was whereby the weather station temperature or THI was lower than the grid value.



**Figure 1.** Difference between weather station and grid-interpolated average temperature against weather station temperature by season (winter – top left, spring – top right, summer – bottom left and autumn – bottom left). Each data point presents the daily average value corresponding to a herd test-day for the time period 2011 – 2019 from 53 weather stations in southern Bavaria.



**Figure 2.** Difference between weather station and grid-interpolated temperature humidity index (THI) against weather station THI by season (winter – top left, spring – top right, summer – bottom left and autumn – bottom left). Each data point presents the daily average value corresponding to a herd test-day for the time period 2011 – 2019 from 53 weather stations in southern Bavaria.

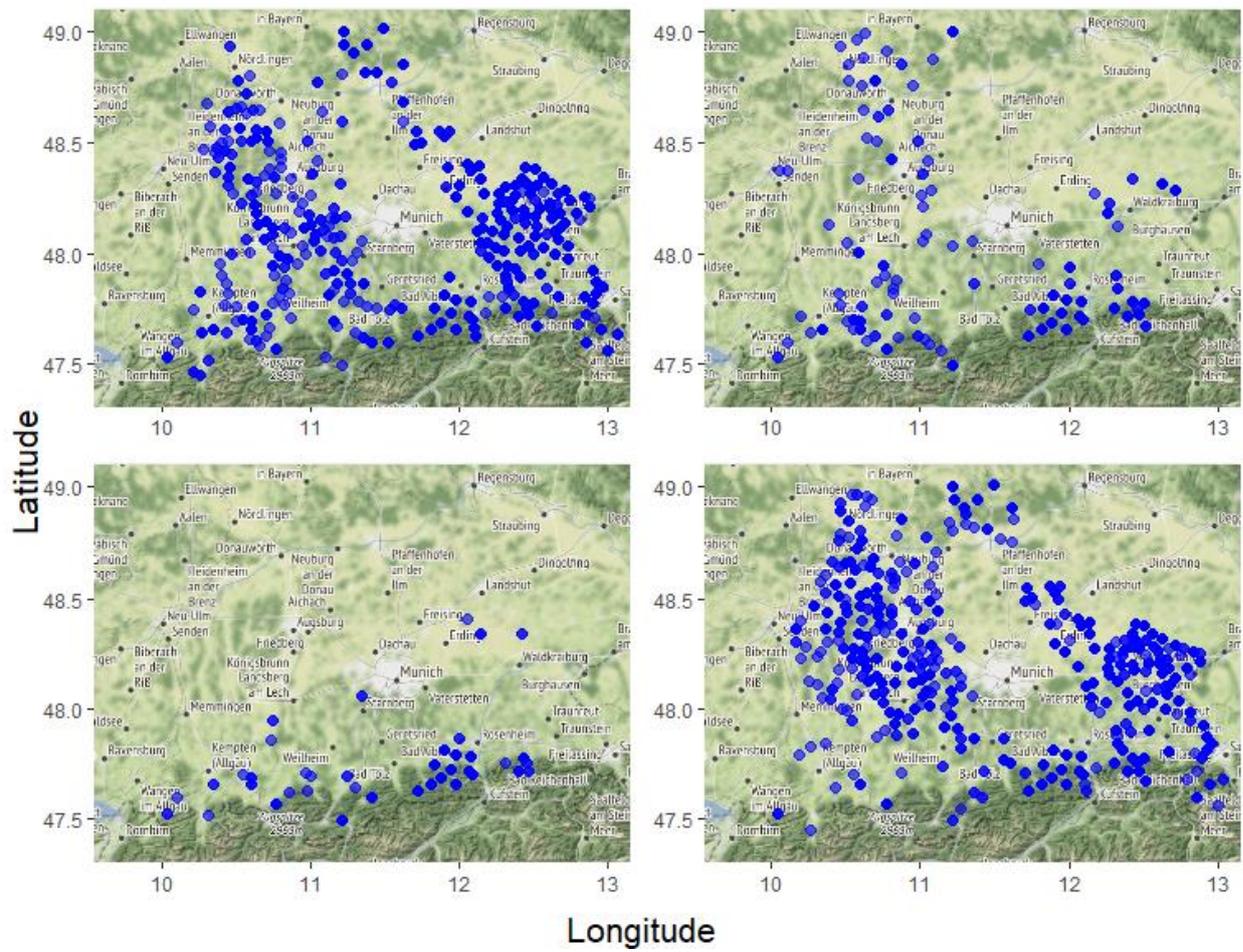
### Animal and herd performance data

The descriptive statistics for the raw and transformed performance data are presented in Table 1. The edited data had 9,726 herds. Average records per herd were  $82 \pm 23$ .

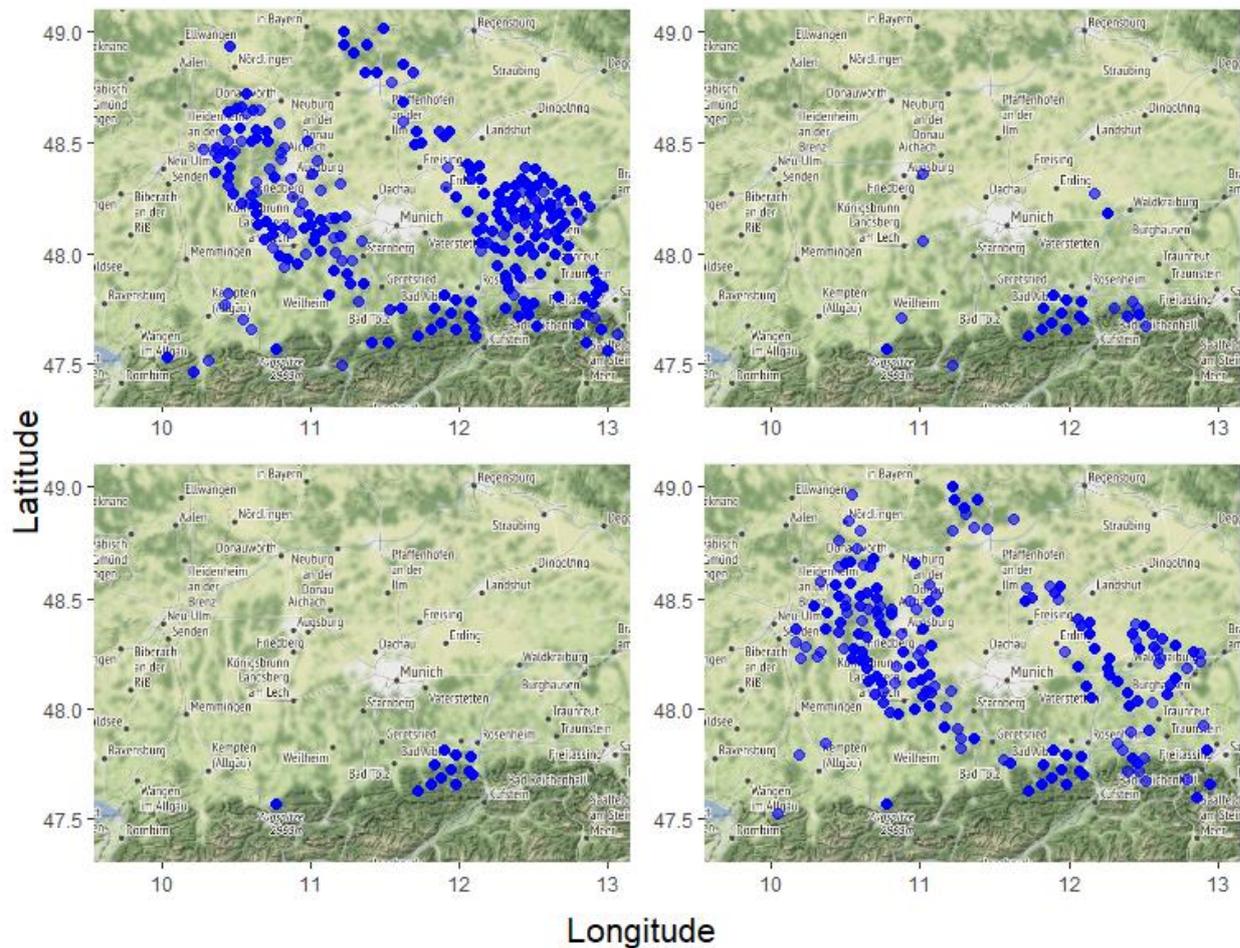
**Table 1.** Summary of individual cow and herd descriptive statistics for different milk production traits and somatic cell score for cattle in southern Bavaria used in the study.

	<b>Milk yield</b>	<b>Protein content</b>	<b>Fat content</b>	<b>Milk urea</b>	<b>Somatic Cell Score</b>
Individual cow test-day records before editing	16,257,769	16,259,531	16,259,499	15,811,551	16,000,139
Individual cow performance	$23.1 \pm 7.44$	$3.6 \pm 0.41$	$4.2 \pm 0.74$	$21.9 \pm 8.41$	$2.4 \pm 1.64$
Herd test-day records	797,455	797,455	797,455	797,455	797,455
Herd performance	$22.0 \pm 4.39$	$3.6 \pm 0.22$	$4.2 \pm 0.37$	$21.3 \pm 7.41$	$3.3 \pm 0.96$

As already mentioned, positive and negative biases were observed in the weather data. When performance records were merged with weather data, a further analysis was carried out for records where negative bias for temperature and THI were less than  $-5^{\circ}\text{C}$  and  $-12$  units respectively. Results indicated that during summer, such differences were recorded for weather stations that were mainly located in the Bavarian pre-Alps as presented in Figure 3 and 4. It follows that these differences were mainly as a result of altitude difference between the farm and the weather stations. If the weather station was located at a higher altitude e.g. on the mountain while the farm was down on the valley, the difference was bigger.



**Figure 3.** Maps of Bavaria indicating the location of farms where the negative bias for temperature between weather station and grid was less than  $-5^{\circ}\text{C}$  by season (winter – upper left, spring – upper right, summer – lower left and autumn – lower right).



**Figure 4.** Maps of Bavaria indicating the location of farms where the negative bias for temperature humidity index (THI) between weather station and grid was less than -12 by season (winter – upper left, spring – upper right, summer – lower left and autumn – lower right).

The response of a milk production trait to thermal load may be immediate or delayed. To compare the length of lag to test-day a trait takes to respond to thermal load, different lag days were considered and compared by the residual variance for each model. Table 2 presents the selected lag periods for the different heat stress indicators for each trait. For these preferred models, WSD performed better than NWP except for milk urea. The test day was preferred for SCS, 3 days lag for milk yield, protein yield, fat content and milk urea and 3-day moving average for fat yield and protein content.

**Table 2.** Residual variances for the most preferred lag day relative to test day comparing weather station data (WSD) with numerical weather prediction (NWP) model data for temperature and temperature humidity index (THI).

Trait	Lag days	Temperature	Temperature	THI	THI
		WSD	NWP	WSD	NWP
Milk yield	3 days	13.8607	13.8612	13.8559	13.8560
Protein yield	3 days	0.02225	0.02224	0.02224	0.02223
Fat yield	3-day moving average	0.04900	0.04898	0.04899	0.04899
Protein content	3-day moving average	0.08300	0.08303	0.08300	0.08302
Fat content	3 days	0.71878	0.71801	0.71875	0.71800
Milk Urea	3 days	78.8326	78.8265	78.8142	78.7759
Somatic cell score	Test-day (0 lag)	4.21538	4.21467	4.21572	4.21471

For each trait, the choice of the lag was unambiguous and not affected by the kind of heat stress indicator (single weather station or gridded data). The complete table of residual variances for the different models to test the most preferred lag day for the different traits is presented in Supplementary Table 1. The model with the smallest residual variance was chosen as the preferred model as summarised in Table 2.

Estimates of residual variance, HTD variance and the autoregressive correlation coefficient ( $\rho$ ) for test-day within herd effects are presented in Table 3. The estimates were for THI thermal load indicator and the best lag day as determined in Table 2. Autoregressive correlations were considerably high in the range of 0.72 and 0.79 except for more volatile milk urea (0.36). Observations were daily herd averages, weighted by the number of animals with records. Therefore, the residual variance is the measurement noise of a single cow, while the HTD variance and  $\rho$  refer to herd averages.

**Table 3.** Estimated components of variance and autoregressive correlations for milk yield traits and somatic cell score for cattle in southern Bavaria.

	Milk yield	Protein yield	Fat yield	Protein content	Fat content	Milk urea	SCS
Residual variance	13.86	0.02	0.05	0.08	0.72	78.89	4.22
HTD variance	4.26	0.01	0.01	0.013	0.024	31.53	0.28
HTD autoregressive correlation	0.79	0.72	0.73	0.77	0.72	0.36	0.75

**Fixed effects**

The fixed effects fitted in the models were mainly effects reported to influence milk production traits. They significantly influenced all the milk traits and SCS across all models as presented in Table 4. However, calving ease did not significantly affect fat content ( $p=0.68$ ) and calving type did not significantly affect milk urea ( $p=0.09$ ). Twinning rate for the study population was 6%.

**Table 4.** Tests of significance for the fixed effects fitted in the model estimated by Wald F statistics.

Fixed effect	p-value
Parity	<0.001
Lactation stage within parity	<0.001
Calving type	<0.001
Calving ease	<0.002
Month	<0.001
Year	<0.001
Reaction norm curves	<0.001

In a follow-up analysis, a simple linear regression model of the temperature or THI difference between weather station and grid-interpolated values on the farm-station-altitude difference, farm-station-distance and their interaction was fitted. Significant differences were observed ( $P<0.001$ ) and altitude difference between farm and weather station explained more variation.

## Reaction norms of milk traits to thermal stress

### Milk yield

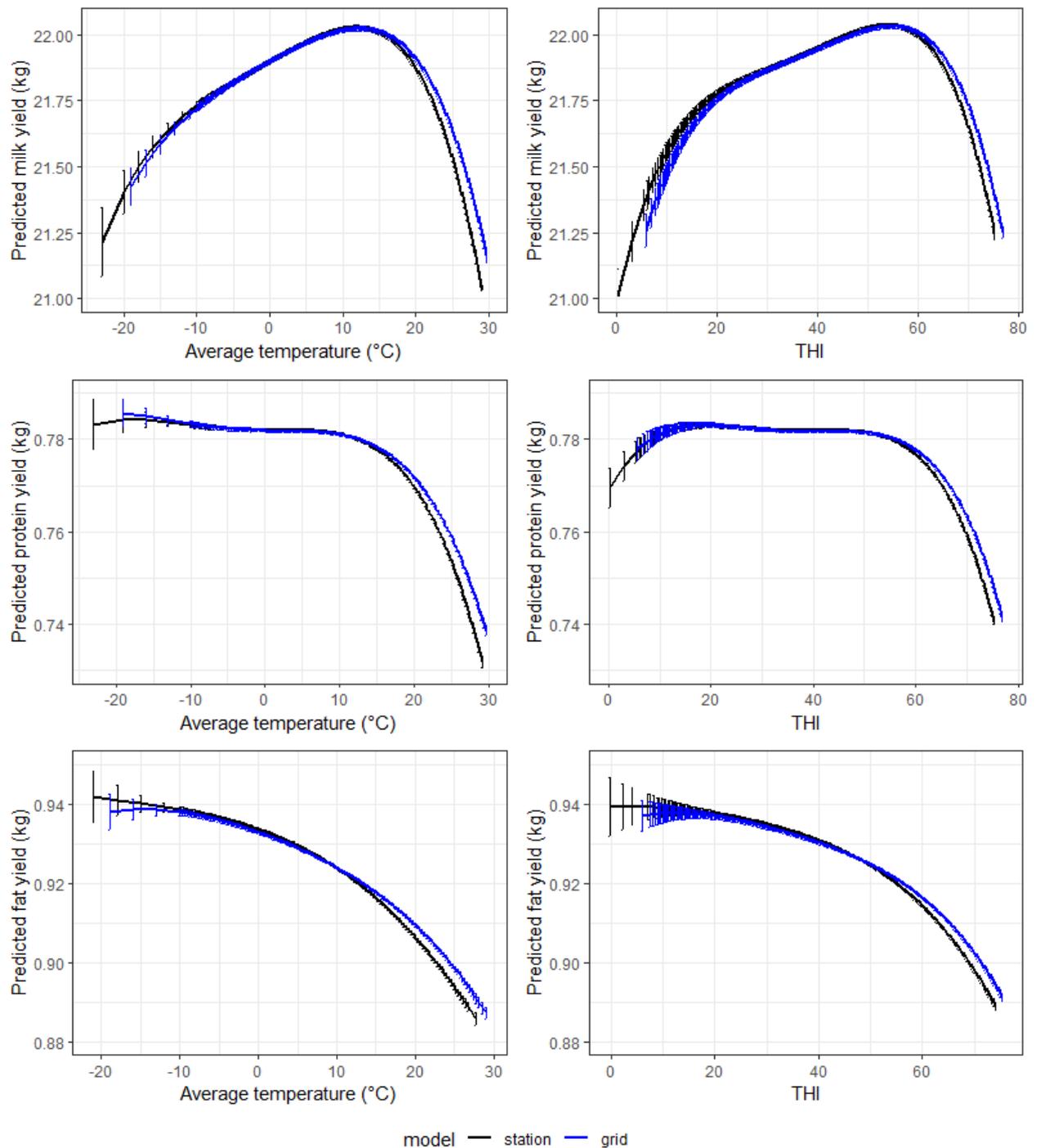
The reactions of average milk protein and fat yields to increasing temperature and THI for both WSD and NWP are presented in Figure 5. For milk yield, the models were able to predict both cold and heat stress regions. The THI below 20, corresponding to an average ambient temperature of  $-8.7^{\circ}\text{C}$ , could be considered as the cold stress region. The region of THI between 20 and 60 could be considered as the thermal comfort zone for milk yield. The optimum region for milk yield was observed at THI 50 – 60 corresponding to WSD temperature of  $10 - 16^{\circ}\text{C}$ . A declining trend in milk yield started at THI 60.

The predicted milk yield at selected average temperature and THI points are presented in Supplementary Table 2. The average milk yield loss due to cold and heat stress was about  $-0.04$  and  $-0.07\text{ kg}/^{\circ}\text{C}$  per cow or  $-0.04$  and  $-0.05\text{ kg}/\text{THI}$  per cow respectively. It is therefore, clear that severe cold stress could have as debilitating effects as heat stress on milk yield. The standard errors were higher at the cold stress region i.e. THI 0 – 20, ranging from  $0.104 - 0.013$ , compared to THI 21 – 60 at  $0.012 - 0.009$  and THI 68 – 75 at  $0.010 - 0.020$ . Estimation errors were larger for low temperatures and THI, for which data were more scarce.

### Protein- and fat yield

The average protein yield was  $0.78 \pm 0.165\text{ kg}$ . The heat stress thresholds for protein yield were commensurate with that of milk yield. Almost no cold stress effects were observed in the reaction norms for protein yield. There was a relatively stable phase at temperature below  $16^{\circ}\text{C}$  and THI range of 15 – 60 which could be considered as the thermal comfort zone for this trait. Beyond THI 60, a steep decline was observed. The average protein yield loss due to heat stress was about  $3.4\text{ g}/^{\circ}\text{C}$  per cow and  $2.4\text{ g}/\text{THI}$  per cow respectively.

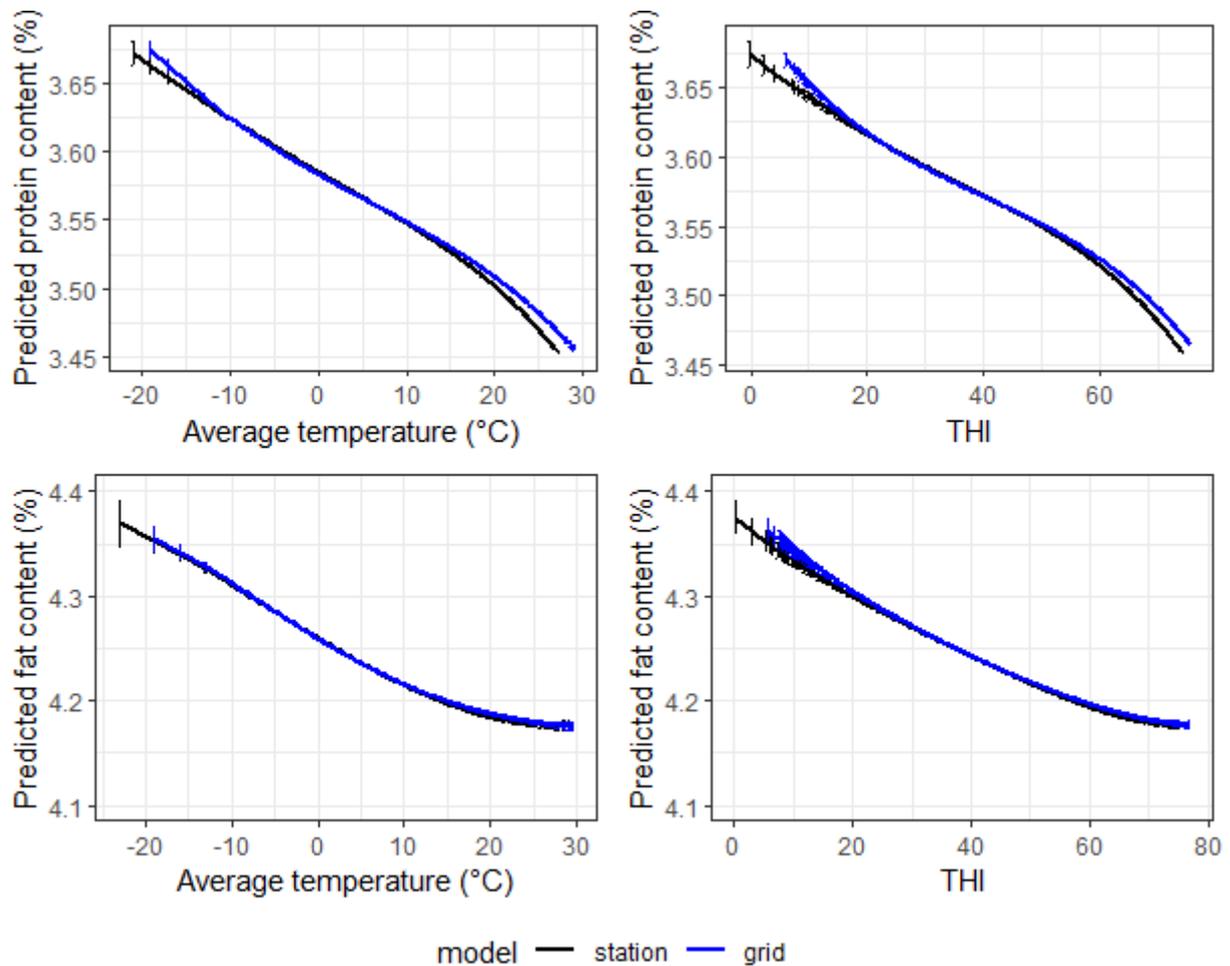
The average fat yield was  $0.92 \pm 0.189\text{ kg}$ . The general reaction was continuous decline with no definite threshold.



**Figure 5.** Reaction of average milk yield (top), protein yield (middle) and fat yield (bottom) to ambient temperature (left) and Temperature Humidity Index (THI, right) measured from weather station (black) and grid interpolation (blue). Standard errors are indicated by vertical error bars.

### Protein- and fat content

Both milk protein and fat contents decreased with increasing temperature and THI at almost linear trends with no observable breakpoints as presented in Figure 6. The total decline for protein and fat contents were, however, small with about 0.2% for each traits.



**Figure 6.** Reaction of protein content (top) and fat content (bottom) to average temperature (left) and Temperature Humidity Index (THI, right) measured from weather station (black) and grid interpolation (blue). Standard errors are indicated by vertical error bars.

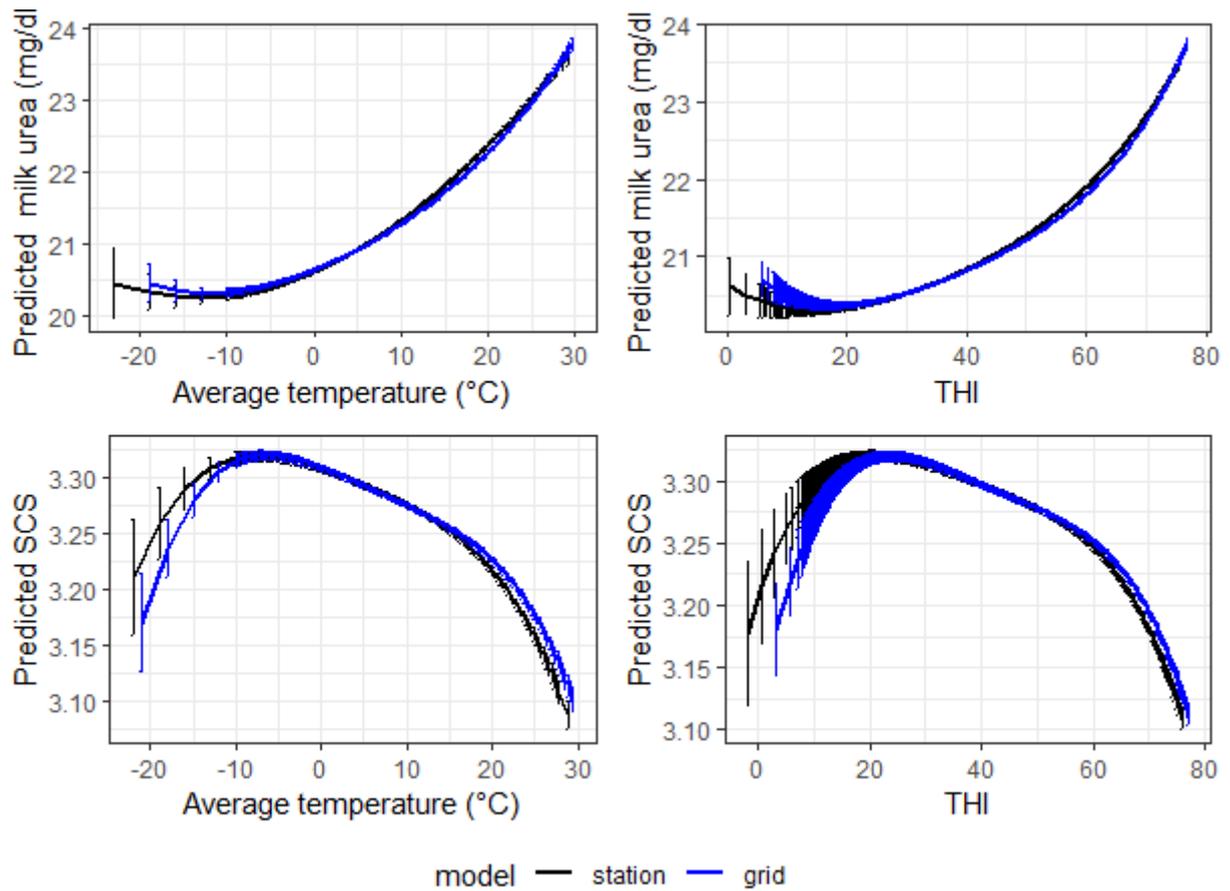
For these traits, a random slope for the individual herd reaction to temperature and THI were fitted (see model in Supplementary Material). By comparing the random slopes of the top 500 herds where NWP THI performed better than WSD THI, results indicated that the herds were not randomly distributed. For both traits, there was significant latitude difference ( $p < 0.05$ ) but no significant longitude difference. There was an increase in LSM means from the south (pre-Alps) towards the north.

**Milk urea**

The reaction of milk urea and SCS to average temperature and THI, obtained from WSD and NWP are presented in Figure 7. For milk urea, the main observation was non-linear accelerating pattern with increasing thermal load with no clearly defined temperature and THI thresholds. The slopes appeared steeper after the points of milk yield breakpoints. In total, there was an increase of about four units. The average milk urea increase above a temperature of 10°C was 0.15 mg/dl per °C per cow and for THI above 50.5 the increase was 0.11 mg/dl per THI per cow.

**Somatic cell score**

The mean SCS was  $3.25 \pm 0.96$  (range 0 to 9). An unexpected reaction to thermal stress was observed whereby SCS decreased with increasing heat stress. At the cold stress region, SCS increased with increasing temperature and concurrently milk yield. It was highest at a daily average temperature of about -8 °C and 20 THI units when animals were considered to enter the thermo-comfort zone. The SCS maintained at slightly above average but declined as thermal load increased only to fall below average when heat stress set in. At extremely low temperatures, the standard errors were highest ranging from 0.0046 – 0.0586 compared to 0.0023 at 10 °C and 55 THI units.



**Figure 7.** Reaction of milk urea (top) and somatic cell score (SCS, bottom) to average daily temperature (left) and Temperature Humidity Index (THI, right) measured from weather station (black) and grid interpolation (blue). Standard errors are indicated by vertical error bars.

## Discussion

### Comparison of weather station to gridded weather data

The hourly weather data used to compare WSD and NWP provided high temporal resolution. In addition, the nearest weather station and the grid interpolation at 6 km provided high spatial resolution weather variables. Different studies have applied different gridding methods to different weather parameters including mean, minimum and maximum temperatures, relative humidity, precipitation and wind at high spatial and temporal resolutions at regional and global scales (Fick and Hijmans, 2017; Frick *et al.*, 2014; Hijmans *et al.*, 2005; Krähenmann *et al.*, 2016). Grid interpolation has also been done for the Alps to model the marked horizontal gradients and non-linear variations (Frei, 2014; Haslinger *et al.*, 2013). The gridded datasets for Germany have shown good agreement with DWD weather station data (Razafimaharo *et al.*, 2020).

Comparison of average temperature indicated a good agreement of MWD and NWP, with a correlation coefficient of 0.97. The agreement of WSD and NWP was moderately good in the Bavarian pre-Alpine region as presented in Supplementary Figure 4. Razafimaharo *et al.* (2020) reported similar observations with good agreement of grid-interpolated and weather station temperatures over flat regions and negative bias in mountainous regions, such as the Harz Mountains, the Thuringian Forest and Black Forest in Germany. This is because of interpolation challenges from weather stations in mountainous terrains and also greater deviations in minimum temperatures such as cold air ponding in valleys not being properly captured by the coarser grid (Frei, 2014; Razafimaharo *et al.*, 2020).

In agreement with our study, WSD relative humidity has been reported to exhibit poor agreement with NWP estimated from two different gridding sources; Daymet (Daily Surface Weather Data on a 1 km Grid for North America) and PRISM (Parameter-elevation Relationships on Independent Slopes Model) in the US corn belt (Mourtzinis *et al.*, 2017). This can be explained by the wide range of average relative humidity across days and seasons. Interpolation of relative humidity is also more uncertain due to its inherent spatial inhomogeneity and indirect derivation, as it depends on moisture content of the atmosphere and on air temperature (Razafimaharo *et al.*, 2020).

There was a good agreement of MWD and NWP THI. This was mainly because the THI formula gives more weight to temperature compared to relative humidity. Although most studies have used weather station data and some gridded weather data, to the best of our knowledge, no previous study compared both sources of weather variables and developed high resolution grid-interpolated THI to study thermal load in dairy cattle. When applied to animal performance data, WSD performed better than NWP.

Razafimaharo *et al.* (2020) evaluated the quality of interpolation by subtracting the grid interpolated values from the weather station measurement to obtain a different error measure. From their study, the bias was always positive i.e. measured temperature was always warmer than grid. The explanation was that the weather stations were at lower elevations and that temperature inversions may have not been fully captured. In our study, some weather stations were at elevations higher than the grid cell which explains the negative bias we obtained. Weather stations and herds' location could have high altitude differences as was the case with Oberstdorf where the weather station was 1500 m above the herds located in the valley below (Supplementary Figure 1). The distribution of surface air temperature in high-mountain regions such as the Alps tend to show considerable horizontal contrasts and deviations from linear vertical dependence (Frei, 2014). In extreme environments e.g. during clear winter nights, temperature inversions of more than 10° over less than 200 m can be observed (Steinacker *et al.*, 2007). During hot summer days, the largest maximum temperatures are often observed in Alpine valleys where there is smaller air volume than the flatland (Frei, 2014). Another phenomenon contributing to the complexity of temperature distribution in the Alpine region is the strong downslope winds called föhn (Frei, 2014). Föhn are associated with marked warm and dry anomalies at the foothills of the Alps (Drobinski *et al.*, 2007). In such locations that contain anomalies that are not representative of the subregion's vertical temperature profile, elevation layers are created and piecewise interpolation functions fitted for each layer (Razafimaharo *et al.*, 2020). In our study, data on different elevation layers were not available in the DWD database.

### **Animal and herd performance**

About a quarter of the German cows are in Bavaria, with a population of about 1.13 million dairy cows as of 2019. The largest cattle population is made up of Fleckvieh (76.9%) and Braunvieh (11.6%) breeds with milk as the main product and beef as a co-product (Bayerisches Staatsministerium für Ernährung, 2020). Analysis of performance records at herd level

substantially reduced computational requirements compared to individual animal repeatability models. The hourly weather station data over a period of 10 years from 109 weather stations across Bavaria resulted to more than 9.2 million observations. To achieve parallel grid-interpolated weather data and at high spatial resolution required substantial computer capacity for data processing.

There was varying lag in response of milk production traits after temperature/THI was recorded. The responses of SCS were instantaneous, milk yield, protein yield, fat content and milk urea had a delayed response of 3 days. The THI of 2 to 3 days before test day have been reported to have the strongest correlation with milk yield (West, 2003). In agreement with our study, Ouellet *et al.* (2019), reported that cows of all parities had to be stressed for at least 3 consecutive days for a significant decrease in protein yield to be observed in cows housed in tiestall barns in Canada. A lag of 3 days and between 7 – 10 days have been reported for milk yield and SCS respectively in Japanese Holstein (Hagiya *et al.*, 2019). The studies of Hammami *et al.* (2015) and Santana *et al.* (2017) applied the THI averaged over 3 days to estimate the effects of heat stress on SCS and identified cold and heat stress thresholds. The lag period seem to vary across traits and regions.

The considerably high autoregressive correlations for milk yield traits, except for milk urea, indicated that the interval between test-day records would have an impact on these traits within the same herd.

### **Fixed effects**

The fixed effects fitted in the models significantly influenced the milk production traits. Generally, as expected parity and lactation stage significantly influenced all the traits. In this study, all multiple births were classified as twinning. The twinning rate was high compared to the ranges of 0.5 to 4% reported in literature for natural twin parturitions (Fitzgerald *et al.*, 2014; Sawa *et al.*, 2015). The economic importance of calving traits include the veterinary costs, calf loss and lower production of cows affected by dystocia (Pausch *et al.*, 2011). There are divergent views regarding the advantages and disadvantages of twinning from fertility and economic perspectives in dairy cattle (Mur-Novales *et al.*, 2018; Sawa *et al.*, 2015). The milk superiority of dams of twins coupled with resulting increase in number of calves born could be desirable for a dual-purpose breed. Calving ease causes stress and determines the course of lactation and subsequent reproductive performance. Calving ease has been reported to have an

effect on milk production and subsequent fertility (Eaglen *et al.*, 2011). Difficult calving associated with dystocia have also been reported to have adverse effects on milk production including decreased milk yield, protein, fat and lactose as well as increased SCC (Antanaitis *et al.*, 2021).

The milk production levels in Bavaria have been increasing over the years. From the period 1988 to 2006, the lactation performance increased by 41%, 36% and 46% in Fleckvieh (Simmental), Braunvieh and Holstein breeds respectively (Knaus, 2009). It is expected that milk yield also increased during the study period because of systematic genetic improvement.

### **Reaction norms of milk traits to thermal stress**

The effect of thermal stress on herd average production was effectively modeled as fixed reaction norm curve with Legendre polynomials of order four. The average reaction norms estimated from WSD and NWP clearly followed similar patterns and were nearly identical.

### **Milk yield**

The thermoneutral zone and heat stress thresholds for dairy cattle vary with cow-specific factors including breed, stage of lactation and productivity, and the local environment in which the cows are adapted (Hempel *et al.*, 2019). Studies have shown that the heat stress thresholds and milk yield decline for each unit increase in THI is dependent on the region (Bohmanova *et al.*, 2007; Brügemann *et al.*, 2012). The overall heat stress threshold identified for milk yield of 60 THI units was in agreement with estimates from a similar THI evaluation method of hourly THI averaged over a day in the lower Saxony region of Germany (Brügemann *et al.*, 2012). From this threshold, also reported as the upper critical THI in the same region by Lambertz *et al.* (2014), dairy cows in the temperate climate of Germany may be exposed to heat stress not only during summer season, but also other days in spring and autumn. The milk yield loss in this study was lower than that reported for Holstein cows in crop production and pasture based systems at -0.08 and -0.16 kg/THI respectively (Brügemann *et al.*, 2012). For a similar THI, Hammami *et al.* (2013), reported a threshold of 62 and milk yield loss of -0.164 kg /THI for Holstein in Luxembourg. The milk yield loss in this study could be attributed to the general production level which is lower, because in Bavaria the cows are mainly dual-purpose and therefore milk yield is lower than for purely dairy breeds like Holstein.

In terms of management environment, the effects of weather conditions on milk yield depend on whether cattle were indoors or outdoors on the test day (Hill and Wall, 2015). It is expected that outside conditions will expose animals to extreme heat load causing a decrease in yield compared to shaded counterparts. However, under pasturing systems animals are able to choose whether to graze or stay in barns and therefore deal with exposure to heat stress more efficiently than cows in constant indoor confinement (Lambertz *et al.*, 2014). Our study did not account for the housing conditions and management systems such as cooling facilities that may affect the thermal stress on cattle.

The cold stress zone for milk yield was much lower in Bavaria (THI=20) than reported for Lower Saxony (THI=30) (Brügemann *et al.*, 2012). Similarly, Gorniak *et al.* (2014), reported decrease in milk yield for THI<40 measured in barns for mid-lactation Holstein dairy cows in the latter region. The impact of cold stress in dairy cattle has been evaluated with the wind chill temperature index (Angrecka and Herbut, 2015). The study reported that, for cows kept in barns with milk yield of 21.6 kg, cold stress developed when air temperature fell below -12.1 °C and wind velocity exceeded 0.7 m/s. Exposure of lactating cows to cold causes increased energy expenditure and feed intake to maintain homeothermy (Collier *et al.*, 2017). In addition to the biological explanations, the cold stress could also be attributed to technical effects of the system such as ability to provide fodder under cold conditions. Furthermore, it depends on if the animals are housed indoors or not. Animals confined indoors are less susceptible to cold stress than those outside on the same day given that temperatures are warmer inside barns (Hill and Wall, 2015).

### **Protein- and fat yield**

The reaction of protein yield somehow followed that of milk yield. This is mainly because it was computed using the average daily milk yield and its relative concentrations in milk, therefore, a correlated response was anticipated. Although the reaction of protein yield to thermal stress was small, it was visible due to the large amount of data over a long period of time. The upper critical THI for protein yield obtained in this study was in agreement with that reported for German Holstein cows (Brugemann *et al.*, 2011). Although from a different THI definition, Gernand *et al.* (2019) also reported a declining trend in protein yield after the THI threshold in Holstein cows in Thuringia state, Germany. Gorniak *et al.* (2014) reported a very weak impact of THI on milk protein content and observed that the decrease in protein yield with increasing THI beyond the threshold was attributed to the decrease in milk yield. In an

experiment on effects of heat stress on multiparous lactating Holstein cows, Gao *et al.* (2017) reported a 19% decrease in both protein and fat yields associated with the overall reduction in milk yield.

The reaction of fat yield followed that of fat content whereby it declined at all temperature/THI ranges. Fat yield has been reported to decline steadily with no definite threshold even when different temperature humidity indices were applied in Holsteins under temperate climate (Hammami *et al.*, 2013). Fat yield was also reported to decrease in all the range of temperatures with no definite threshold for Friesian-Holstein in southern Spain (Carabano *et al.*, 2014). In that study, although milk yield had an upper comfort threshold of 29.2 °C or a corresponding THI of 73, the threshold for fat yield was 15 °C.

### **Protein- and fat content**

This study reported steady declines in milk protein and fat contents with increasing temperature and THI with no definite thresholds which was in agreement with other studies in the region (Brügemann *et al.*, 2012; Gorniak *et al.*, 2014). The trends of decreasing milk protein and fat percentage with increasing THI have been reported in German Holsteins (Lambertz *et al.*, 2014). Bertocchi *et al.* (2014) reported decreasing protein and fat contents and were able to identify THI (maxTHI) thresholds of 50.2 and 65.2 respectively for bulk tank milk in Italian Holsteins. Other studies reported inconsistent response of milk protein and fat contents reaction to heat stress with reports of increasing, decreasing or no change (see review by Becker *et al.*, 2020).

The decline of milk protein and fat contents has been associated with the reduced feed intake due to heat stress. However, in experiments with pair-fed dairy cows, a decrease in protein content attributed to direct effect of heat stress beyond what would be expected of a reduction in feed intake has been reported (Gao *et al.*, 2017; Wheelock *et al.*, 2010). Cowley *et al.* (2015), reported that reduction in milk protein concentration was a result of specific downregulation of mammary protein, rather than a general reduction in milk synthesis.

From the protein content reaction norms, it was clear that heat stress had a negative effect. In addition to decrease in milk protein content, milk protein fractions particularly casein have been reported to be affected by heat stress which in return affect cheese making properties (Bernabucci *et al.*, 2015). Heat stress also reduces the short-chain fatty acids while increasing the long-chain fatty acids in milk (Hammami *et al.*, 2015; Liu *et al.*, 2017). It is thought that

heat stress modifies the triacylglycerol and polar lipids profiles through changing fatty acid pool and animal metabolism (Liu *et al.*, 2017).

### **Milk urea**

Milk urea (MU) is among the traits in routine German milk performance evaluation. From the reaction norms, it was clear that MU increases with increasing heat load. However, given that the average MU was 21.3 mg/dl, the increase was not large. The levels of urea nitrogen in the milk is known as milk urea nitrogen (MUN) and is obtained from MU by the equation  $MU \times 0.47$  (Gävan *et al.*, 2017). Therefore, both basically express the same trait. Heat stress conditions have been reported to increase MUN of lactating cows (Gao *et al.*, 2017; Gernand *et al.*, 2019). A trend similar to our study was reported for MUN with a constant phase at thermoneutral conditions and substantially increased with increase heat stress in Holstein cows (Gernand *et al.*, 2019). Heat stressed cows have been reported to increase MUN by 24.5% compared with pair-fed cows in thermoneutral conditions (Gao *et al.*, 2017).

The high MUN during heat stress conditions have been associated with increased plasma nitrogen levels (Wheelock *et al.*, 2010). Moreover, this may be an indication of energy deficiency due to reduced feed intake during heat stress (Gernand *et al.*, 2019).

### **Somatic cell score**

Studies on the effect of heat stress on SCS have been contradictory. In our study, heat stress did not precipitate undesired effects on SCS which is in agreement with the study of Brügemann *et al.* (2012) in a pasture production system, Lower Saxony, Germany. In contrast, however, the same study reported increasing SCS at both low and high THI in the maritime region. Hammami *et al.* (2013) also reported increasing SCS for both cold and heat stress in Luxembourg. Carabano *et al.* (2014) reported decreasing SCS with increasing THI in Holstein-Friesian cows in Southern Spain. Santana *et al.* (2017) reported that increasing THI, particularly for values  $\geq 76$  favoured reduction of SCS in milk of Brazilian Holstein cows.

In general, the effect of heat stress on SCS depends on the environmental conditions under which the animals are reared (Santana *et al.*, 2017). Carabano *et al.* (2014) also proposed that temperature could be a factor affecting SCS only in the presence of mastitis. It could be that the bacterial load in pastures is not as high as that in barns and therefore the decrease in SCS during summer when animals are on pasture.

The aim of dairy farmers should be to reduce the adverse effect of heat stress on milk production traits and other physiological parameters. Various cooling mechanisms have been reported in literature that would mitigate the adverse effects of heat stress. The benefits of providing shade, which is considered simple and most effective for cooling cows on pasture, have been reported to reduce the negative effects of heat stress in a temperate climate (Van Laer *et al.*, 2015; Veissier *et al.*, 2018). From an average THI reaction norm, there was a loss of about 0.85 kg of milk/cow per day at peak THI compared to the optimum. On the temperature scale this was about 1.0 kg/cow per day. Given the average herd size of 40 cows in Bavaria, that would amount to 34 – 40 kg milk. With the average daily milk yield of 22 kg/cow, that would account for almost 1.5 – 2 cows less in the average herd. By cooling the animals down (compared to current practice) one could have the milk of two additional cows in the barn notably almost without any additional emissions. Thermal optimum is associated with minimum methane emissions in dairy cows (Hempel *et al.*, 2016). On the cold stress slope, the loss was about 0.6 and 0.8 kg milk/cow per day for temperature and THI respectively, which would amount to 24 – 32 kg milk per herd. This means that through heating during cold stress, there could also be room for „an additional cow“. The economics to consider would be the value of milk of an additional cow against the herd cooling or heating cost.

## Conclusion

There was good agreement between WSD and NWP. As a result, missing weather station data can be adequately filled-up by grid-interpolated data. The agreement was better in the relatively flat and further away from the Alps compared to mountainous regions. Both positive and negative biases were observed. Negative bias during summer season when heat stress in dairy cattle is expected were apparent in the Bavarian pre-Alpine regions. In a mountainous region such as the Bavarian pre-Alps, the altitude difference between the farm and weather station should be put into consideration when using gridded data. Care should also be taken to account for both vertical and horizontal topographical profiles.

When applied to the analysis of thermal stress on milk production traits, WSD performed better than NWP data. The overall reaction norms of milk yield traits estimated from WSD and NWP were not very different. The general threshold above which milk yield substantially declined was identified and was in agreement with other studies in the region. Protein content, fat yield and fat content decreased linearly with increasing heat stress with no identifiable thresholds.

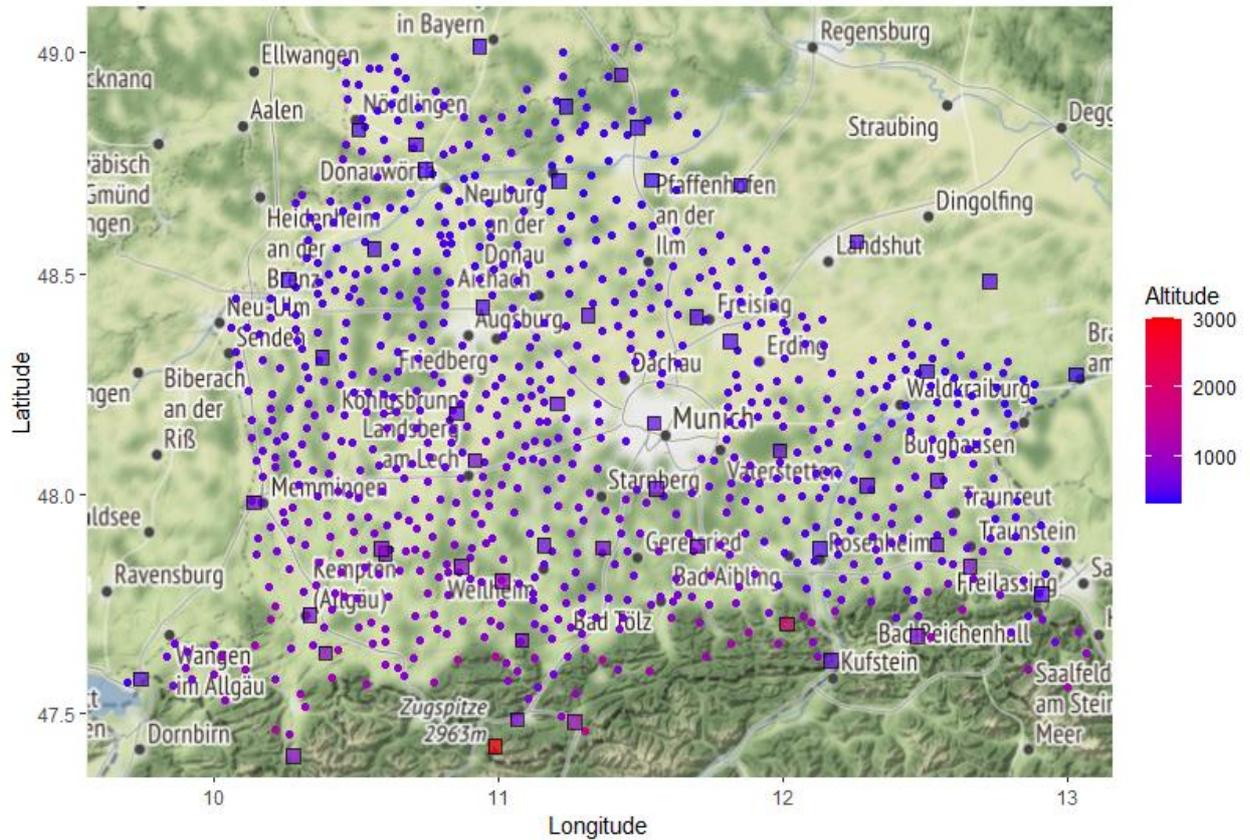
There was no substantive effect of thermal stress on SCS, but, milk urea increased with increasing heat load.

Current frequencies of hot days may be used for estimating how many “optimum” cows can be stocked over the year. The potential milk yield loss may increase in future due to climate change and higher average milk yield due to genetic selection. Further studies could focus on individual cow milk production and other performance traits and develop selection criteria for heat tolerance with a baseline of parameters obtain in this study.

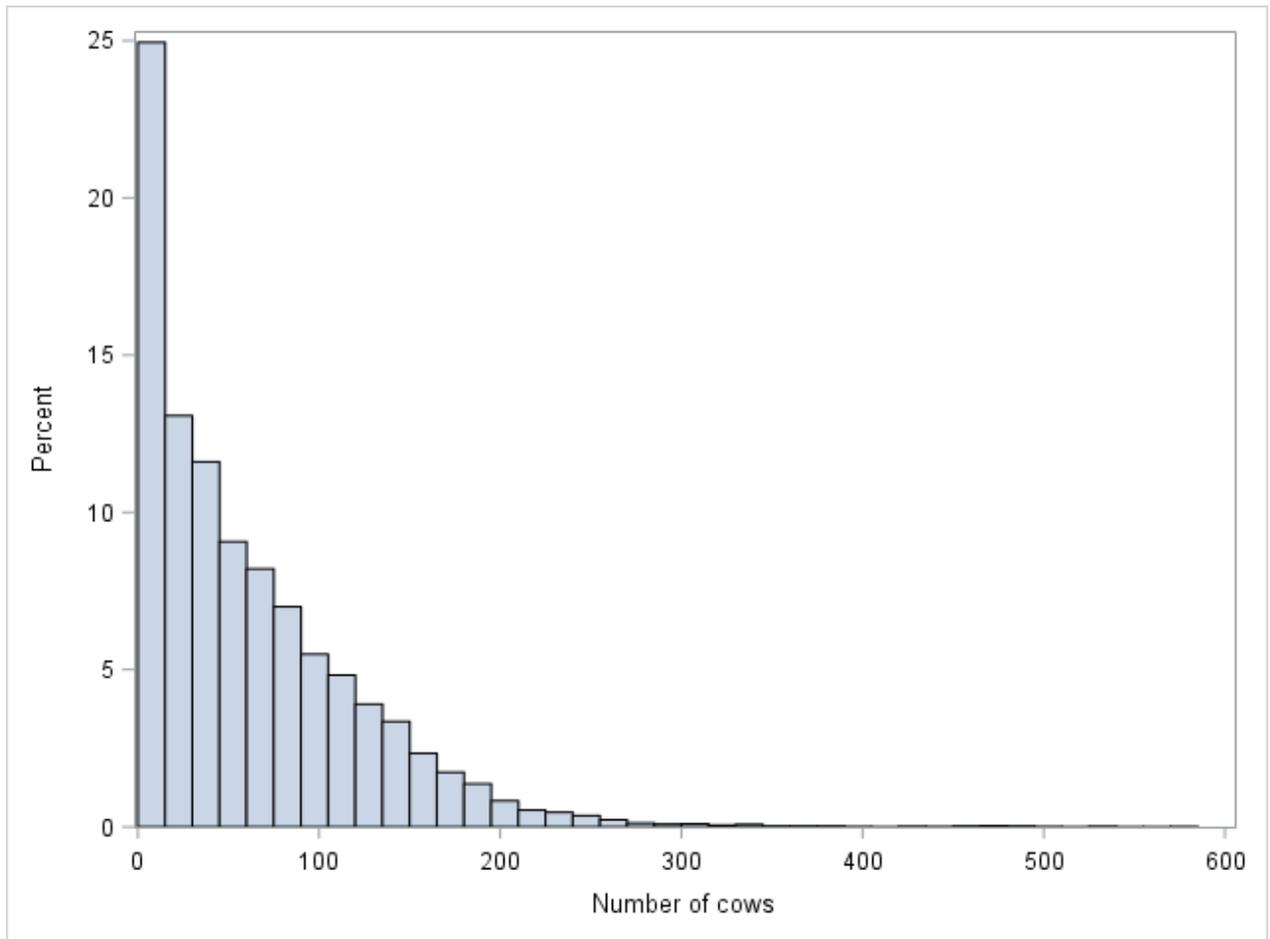
## **Acknowledgements**

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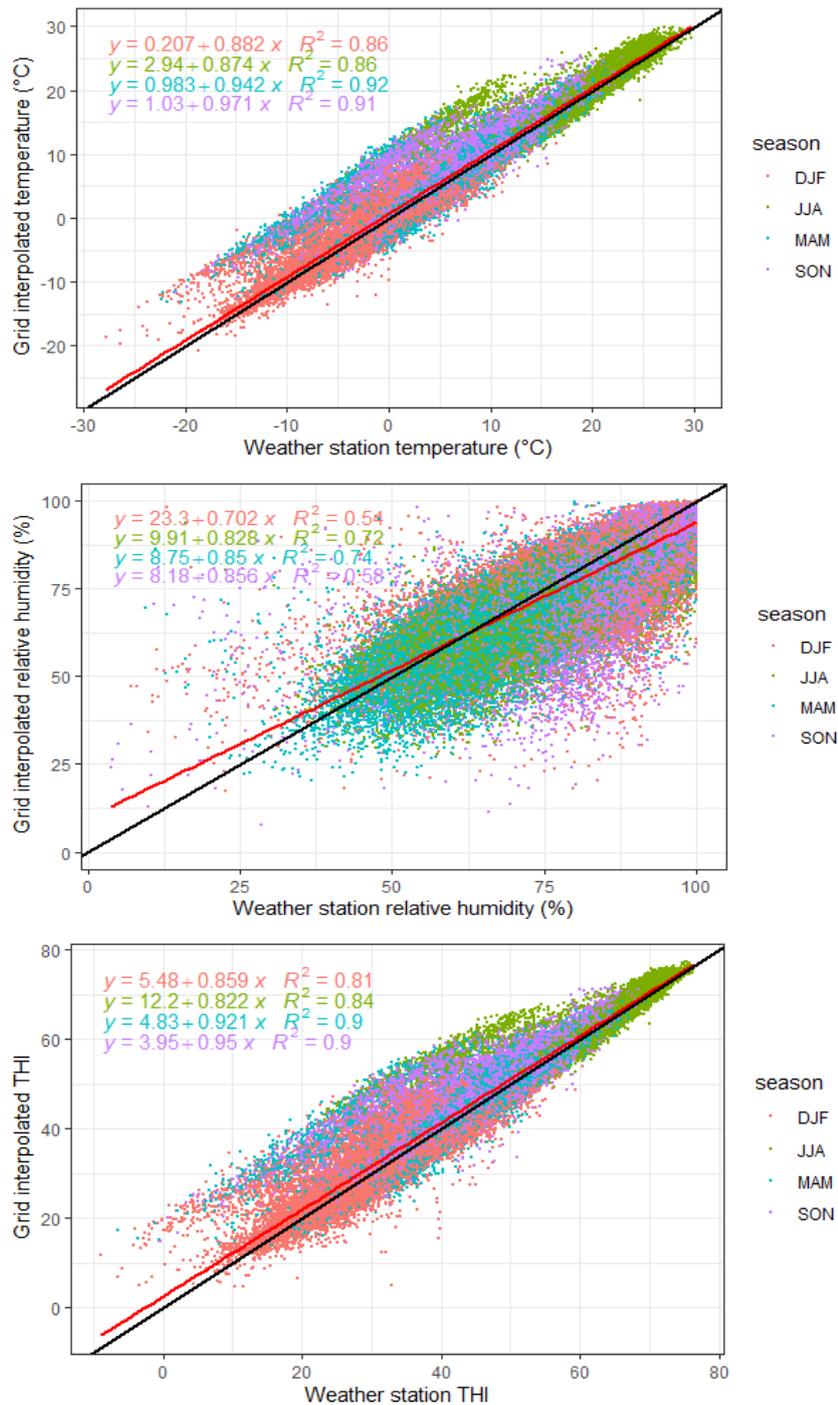
## Supplementary material



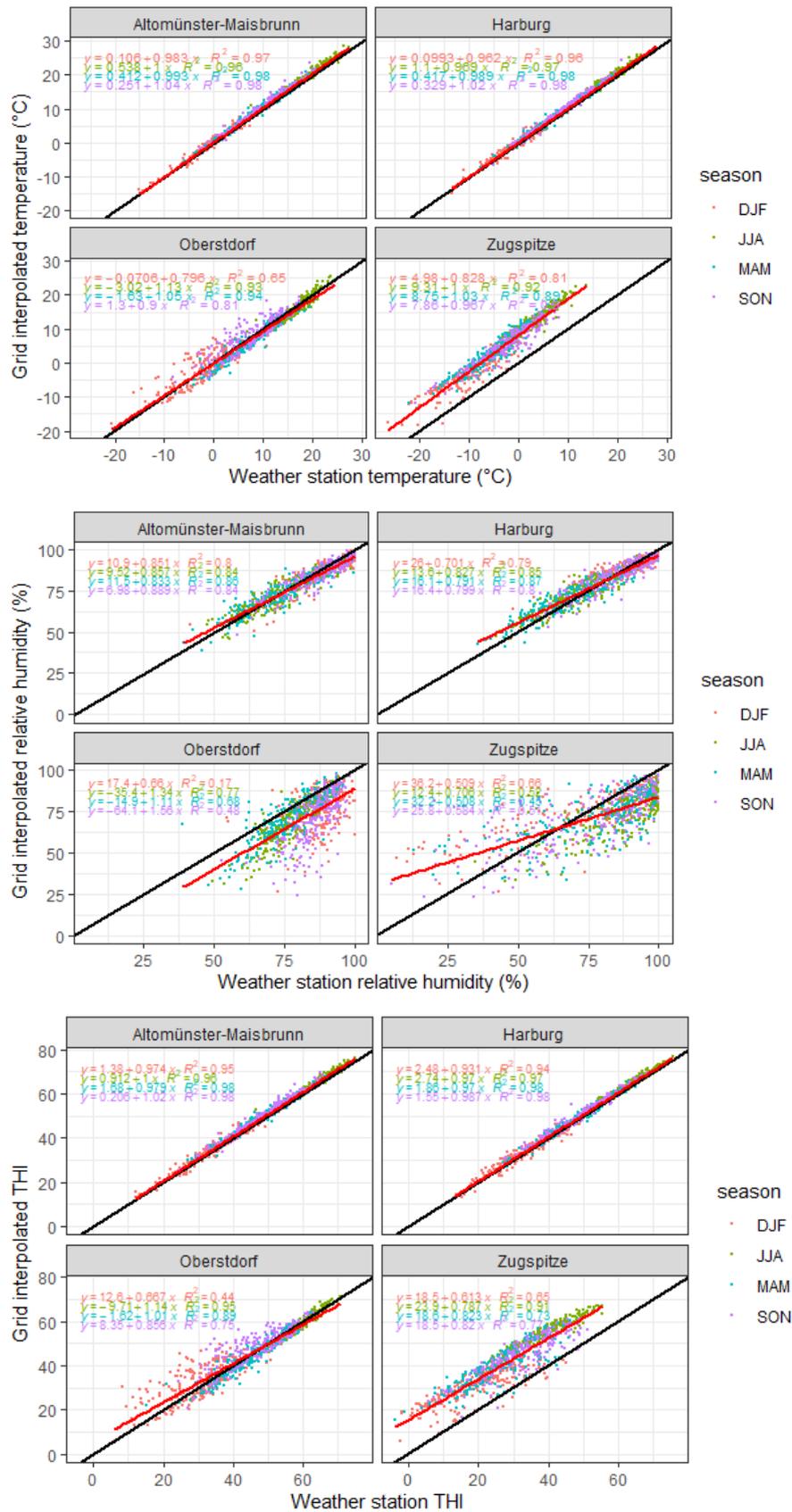
**Supplementary Figure 1.** Map of location of study herds represented by the villages (“Gemeinden”, circles) and 53 Deutscher Wetterdienst (DWD) weather stations (squares) with a colour scale for the altitude in southern Bavaria.



**Supplementary Figure 2.** Distribution of number of cows per herd.



**Supplementary Figure 3.** Correlation between weather station and grid-interpolated temperature, relative humidity and temperature humidity index (THI) by season. Each data point indicates the daily average value for the time period 2010 – 2019 from 53 weather stations in southern Bavaria. The solid black line is  $y=x$  and the solid red line is the fitted linear regression line. The fitted linear regression and correlation are shown.



**Supplementary Figure 4.** Comparison of weather station and grid-interpolated temperature, relative humidity and temperature humidity index (THI) from four locations in southern Bavaria

with varying distance from the Alps. Each data point indicates the daily average value for the time period 2010 – 2019. The solid black line is  $y=x$ . The fitted linear regression and correlation are shown.

**Supplementary Table 1.** Residual variance for the different models to test the most preferred lag day relative to test day for weather station and gridded temperature and temperature humidity index (THI).

	Test-day	1 day lag	2 day lag	3 day lag	3 days moving average
<b>Milk yield</b>					
Station temperature	13.8958	13.8793	13.8717	<b>13.8607</b>	13.8774
Grid temperature	13.8970	13.8823	13.8736	<b>13.8612</b>	13.8800
Station THI	13.8939	13.8771	13.8668	<b>13.8559</b>	13.8745
Grid THI	13.8955	13.8793	13.8700	<b>13.8560</b>	13.8777
<b>Protein yield</b>					
Station temperature	0.0222675	0.0222575	0.0222531	<b>0.0222502</b>	0.0222487
Grid temperature	0.0222671	0.0222596	0.0222509	<b>0.0222442</b>	0.0222474
Station THI	0.0222649	0.0222555	0.0222469	<b>0.0222433</b>	0.0222460
Grid THI	0.0222630	0.0222540	0.0222438	<b>0.0222330</b>	0.0222426
<b>Fat yield</b>					
Station temperature	0.0489671	0.0490076	0.0490393	0.0490234	<b>0.0490007</b>
Grid temperature	0.0489569	0.0489977	0.0490268	0.0490082	<b>0.0489846</b>
Station THI	0.0489615	0.0490019	0.0490306	0.0490203	<b>0.0489932</b>
Grid THI	0.0489469	0.0489876	0.0490182	0.0489990	<b>0.0489990</b>
<b>Protein content</b>					
Station temperature	0.0830180	0.0831942	0.0833960	0.0835730	<b>0.0830035</b>
Grid temperature	0.0830899	0.0831956	0.0833704	0.0835458	<b>0.0830359</b>
Station THI	0.0830235	0.0832089	0.0834022	0.0835700	<b>0.0830066</b>
Grid THI	0.0830749	0.0831843	0.0833592	0.0835253	<b>0.0830217</b>
<b>Fat content</b>					
Station temperature	0.722105	0.721751	0.719583	<b>0.718783</b>	0.723129
Grid temperature	0.719813	0.719977	0.718662	<b>0.718012</b>	0.721029
Station THI	0.721814	0.721403	0.719321	<b>0.718755</b>	0.722805
Grid THI	0.719488	0.719729	0.718548	<b>0.718000</b>	0.720789
<b>Milk urea</b>					
Station temperature	79.4210	79.1566	78.9264	<b>78.8326</b>	79.1054
Grid temperature	79.3852	79.1214	78.9473	<b>78.8265</b>	79.0959
Station THI	79.4042	79.1281	78.8905	<b>78.8142</b>	79.0661
Grid THI	79.3604	79.0722	78.8751	<b>78.7759</b>	79.0385
<b>Somatic cell score</b>					
Station temperature	<b>4.21538</b>	4.21677	4.21654	4.21649	4.21609

Grid temperature	<b>4.21467</b>	4.21612	4.21628	4.21654	4.21568
Station THI	<b>4.21572</b>	4.21688	4.21657	4.21652	4.21652
Grid THI	<b>4.21471</b>	4.21627	4.21653	4.21664	4.21594

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**Supplementary Table 2.** Predicted milk yield at selected average temperature and Temperature Humidity Index (THI) points.

Temperature (°C)	THI (units)	Predicted milk yield (kg)
-20.0	0.4	21.40
-15.0	11.3	21.62
-10.0	17.8	21.74
-5.0	25.3	21.82
0.0	33.2	21.90
5.0	42.5	21.97
10.0	50.5	22.02
12.0	53.7	22.03
15.0	58.4	22.01
16.0	60.0	22.00
18.0	62.9	21.94
20.0	65.7	21.87
22.0	68.1	21.76
23.0	69.5	21.69
24.0	70.6	21.62
25.0	71.7	21.53
26.0	72.7	21.43

For protein and fat contents, the random slope model was fitted as:

$$Y_{ijklmnopqrs} = H_i + P_j + PLS_{jk} + CT_l + CE_m + mon_n + yr_o + yrs_{op} + \sum_{q=0}^4 \beta_q Z_q(t) + htd_{ir} + \beta_q H_i + e_{ijklmnopqrs}$$

where  $\beta_q H_i$  is the random slope for individual herds reaction to temperature or THI. All other model terms are as described in the general model within material and method section in the main text.

The difference in random slope estimates between station and grid was calculated and the top 500 farms with highest negative differences were selected. PROC GLIMMIX was used to test the significance of latitude and longitude between the observed and expected values.

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## CHAPTER FOUR

### **Degree-days as heat load indicator for milk production traits**

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## **Degree-days as heat load indicator for milk production traits**

### **Abstract**

Well defined and accurate climatic indicators are important for evaluating heat stress in dairy cattle. This is imperative for sound management decisions for mitigating production losses. The most popular indicator for heat stress is the temperature humidity index (THI). In this study we apply the degree-day concept to develop THI-day. We defined the cooling THI-day as the total THI units that accumulated above the heat stress threshold for milk production traits over the entire day. We then modeled reaction norms for cooling THI-day to analyse the effect of cumulative heat load expressed by THI-day on milk production traits. Milk performance records were from the southern region of Bavaria Germany for the years 2011 to 2019. Individual cow records were averaged by herd and test-day resulting in 797,455 herd test-day records from 9,726 herds. Weather data for the same period were provided by the German Meteorological Service. Results indicated that cooling THI-day provided additional significant ( $p < 0.001$ ) information to the conventional THI models. We found out that reaction norm models of average herd milk yield that do not account for the cumulative heat load tend to underestimate the effects of heat stress on milk production traits. Reaction norms modeled with cooling THI-day were more plausible for milk production traits that have a defined heat stress threshold including milk yield and protein yield.

### **Keywords**

THI threshold, cumulative heat load, Hourly Average Integration, heat stress indicator

## Introduction

Heat stress in livestock is evaluated by use of different environmental variables, either individually or combined into an index. The temperature humidity index (THI), combines the effect of air temperature and relative humidity and remains the commonly applied indicator for thermal stress in livestock (Bohmanova *et al.*, 2007). The THI is often divided into classes that reflect the severity of heat stress. In dairy cattle for example, Armstrong (1994) define  $THI < 72$  as comfort zone,  $72 > THI < 79$  as mild stress,  $80 > THI < 89$  as moderate stress and  $> 90$  as severe stress. However, different thresholds for the comfort zone have been reported depending on region, production system and model specifications. For milk yield, a THI threshold of 72 has been extensively used (Ravagnolo *et al.*, 2000, Bohmanova *et al.*, 2007, Aguilar *et al.*, 2010). Other THI thresholds have been reported including 69 for a Mediterranean climate (Bouraoui *et al.*, 2002), 74 in semi-arid and 78 in subtropical environments of US (Bohmanova *et al.*, 2007), 62 in temperate Luxembourg (Hammami *et al.*, 2013). In Germany, a THI of 60 has been identified as the threshold above which milk yield starts to decline (Brügemann *et al.*, 2012). Heat stress thresholds for some milk composition traits including protein and fat contents have not been identified (Brügemann *et al.*, 2012), while for other traits, the thresholds have been inconsistent. The focus of most heat stress studies has been the rate of decline per unit increase in THI after the threshold. So far the cumulative heat load above the threshold has not been modeled.

For long, degree-days have been widely used as indicator for energy consumption for cooling and heating buildings (CIBSE, 2006). Cooling degree-days (CDD) and heating degree-days (HDD) are computed based on the cumulated daily deviations above (CDD) or below (HDD) a given temperature threshold (Spinoni *et al.*, 2018). CDD refers to the amount of energy required for a given day or period, to cool the building in a hot climate to a specified base temperature, and the alternative reflects the HDD (Matzarakis and Thomsen, 2009, Spinoni *et al.*, 2018). Another application of degree-days is in crop phenology where the accumulated heat, commonly referred to as growing degree-days (GDD), is used to predict the development and harvest of a variety of crops (McMaster and Wilhelm, 1997). In entomology, degree-day accumulation has been used to model the life-cycle development of insects (Chen *et al.*, 2015). The concept of degree-day has been applied to monitor and predict fish growth and development (Neuheimer and Taggart, 2007, Chezik *et al.*, 2014). However, its application in endotherms is lacking. Applying the approach of temperature degree-days, we calculated THI-

days. We calculated the cooling THI-day in a given day as the total THI units that accumulated above the heat stress threshold over the entire day. We modeled the effect of cumulative heat load expressed by cooling THI-day on herd-average milk production traits. The main objective of this study was to determine the variability of reaction norms of milk production traits to heat stress with or without cooling THI-days.

## Material and methods

### Study location and data

This study builds up on our earlier study where information on milk performance records and weather are described in detail. In summary, the study location was the Swabia and Upper Bavaria administrative districts (Regierungsbezirke) in the south of the German federal state of Bavaria. Milk performance records were provided by the Bavarian dairy recording organization - Landeskuratorium der Erzeugerringe für tierische Veredelung in Bayern e.V. (LKV Bayern) for the period 2011 to 2019. Individual cow records were averaged by herd and test-day. There was a total of 797,455 herd test-day (HTD) records from 9,726 herds. The dominant breed in the region is the Fleckvieh which is a dual-purpose breed of cattle.

Weather data were provided by the German Meteorological Service, (Deutscher Wetterdienst, DWD). Weather station data for hourly observation 2 m air temperature (°C) and 2 m relative humidity (%) were obtained from DWD for the period 2011 – 2019 (Climate Data Center 2018, [https://opendata.dwd.de/climate\\_environment/CDC/observations\\_germany/](https://opendata.dwd.de/climate_environment/CDC/observations_germany/)). For this analysis we used data from 53 weather stations within the study area.

The temperature humidity index (THI) was calculated using the equation of (NRC, 1971) as:

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

where  $T$  is the average temperature in °C and  $RH$  is average relative humidity in %. Hourly THI was calculated from hourly  $T$  and  $RH$  and averaged for the day.

### THI-day

The THI-day was calculated using Hourly Average Integration method (Fraisie *et al.*, 2007). The hourly THI above the threshold was summed up over the day to give the daily cooling THI-

day. The THI threshold (base THI) was set at 60 THI units. This is the threshold above which milk yield in cattle is affected by heat stress in Germany (Brügemann *et al.*, 2012).

$$\text{CoolingTHI - day} = \sum_{i=1}^t (\text{THI}_{a,i} - \text{THI}_b)$$

where  $\text{THI}_a$  is the mean hourly THI,  $\text{THI}_b$  is the THI threshold and  $t$  is the number of hours THI was above the base threshold.

### Statistical models

To determine the effect of THI and cumulative heat load expressed by cooling THI-day on milk production traits, we fitted three models i.e. a full model fitting reaction norms for THI and cooling THI-day (model 1), a reduced model fitting THI only (model 2) and a reduced model fitting cooling THI-day only (model 3). We implemented model 1 to test the significance of cooling THI-day in a standard THI model. We tested the null hypothesis that cooling THI-day does not explain additional variation.

Model 1 was a mixed model fitting quartic Legendre polynomial functions to THI and cooling THI-day. The model was as follows:

$$Y_{ijklmnopqrsu} = H_i + P_j + PLS_{jk} + CT_l + CE_m + mon_n + yr_o + yrs_{op} + \sum_{q=0}^4 \alpha_q Z_q(t) + \sum_{r=0}^4 \beta_r Z_r(d) + htd_{is} + e_{ijklmnopqrsu}$$

where,  $Y_{ijklmnopqrsu}$  is the herd test day record for milk yield, protein yield, fat yield, protein content, fat content, milk urea or SCS.  $H_i$  is the herd effect.  $P_j$  is the multiple regression of % cows in different parities (parity 1, 2 and 3) at each herd and test day.  $PLS_{jk}$  is the lactation stage by parity effect; which is a multiple regression on % of cows in different lactation stages (LS1 – LS10) by parity interaction at each herd and test day.  $CT_l$  is the multiple regression of % cows in a given calving type (single or twins) at each herd and test day.  $CE_m$  is the multiple regression of % cows in a given calving ease category (6 calving ease classes; 0 – easy without assistant to 5 – surgical delivery/fetotomy).  $mon_n$  is the month effect.  $yr_o$  is the year effect.  $yrs_{op}$  is the year by season interaction effect. Seasons were defined as follows: winter (December to February), spring (March to May), summer (June to August), and autumn

(September to November).  $\alpha_q$  are regression coefficients for THI,  $Z_q$  are the covariates of the  $q^{\text{th}}$  Legendre polynomial evaluated at THI point (t).  $\beta_r$  are regression coefficients for cooling THI-day,  $Z_r$  are the covariates of the  $r^{\text{th}}$  Legendre polynomial evaluated at cooling THI-day point (d).  $htd_{is}$  is the random short-term test-day effect auto-correlated (AR1) within herd and  $e_{ijklmnopqsu}$  is the residual.

To determine the reaction of milk production traits to increasing THI, we fitted model 2 as:

$$Y_{ijklmnopqsu} = H_i + P_j + PLS_{jk} + CT_l + CE_m + mon_n + yr_o + yrs_{op} + \sum_{q=0}^4 \alpha_q Z_q(t) + htd_{is} + e_{ijklmnopqsu}$$

the model terms are as previously defined.

We also modeled the reaction norm of the cooling THI-day as the only heat load indicator by fitting model 3 as:

$$Y_{ijklmnoprsu} = H_i + P_j + PLS_{jk} + CT_l + CE_m + mon_n + yr_o + yrs_{op} + \sum_{r=0}^4 \beta_r Z_r(d) + htd_{is} + e_{ijklmnoprsu}$$

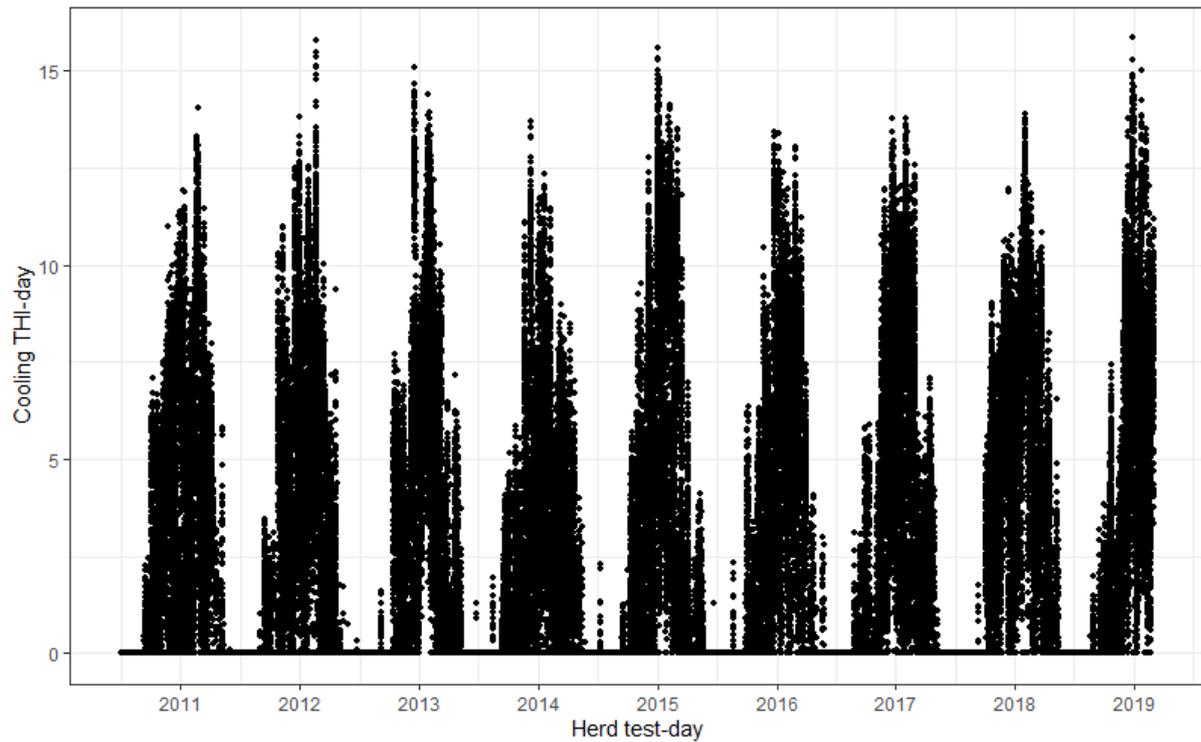
the model terms are as previously defined.

Milk performance records were processed in SAS (version 9.4; SAS Institute Inc., Cary, NC). Mixed models were used to fit the data with variance components estimated by restricted maximum likelihood in ASReml 4.1 program (Gilmour *et al.*, 2015). Test for significance differences of the fixed effects were by Wald F-Test as implemented in ASReml. Weather data handling and figure plots were carried out in R version 4.0.3 (R CoreTeam, 2020).

## Results and discussion

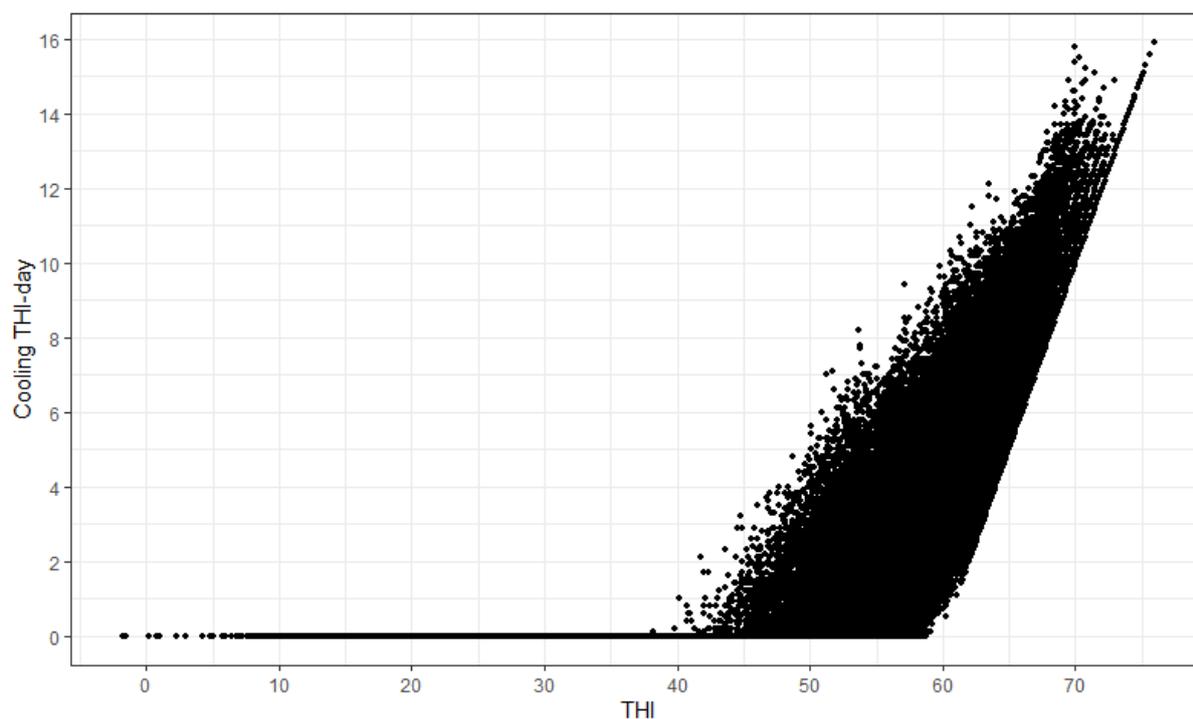
### THI and cooling THI-day

The daily average THI was 48.3 with a range of -1.8 to 75.9. The cooling THI-day above the threshold ranged from 0 to 15.8 units. The distribution of cooling THI-days calculated with a THI threshold of 60 for the study period are presented in Figure 1.



**Figure 1.** Cooling THI-days calculated with a THI threshold of 60. Each data point presents the daily value corresponding to a herd test-day in southern Bavaria.

A scatter plot of cooling THI-day units against daily average THI is presented in Figure 2. Non-linear relationships were observed with no cooling THI-days at lower THI and more cooling THI-days at higher THI.



**Figure 2.** A scatter plot of daily cooling THI-day units against average daily THI. Each data point presents the daily value corresponding to a herd test-day.

The average cooling THI-day duration i.e. the number of hours used to calculate the cooling THI-day was 5.4 hours. The cooling THI-day duration was 12.7, 16.3 and 15.6 hours in the summer months of June, July and August respectively.

### Statistical models

All the fixed effects fitted in the model were statistically significant ( $p < 0.001$ ). The reaction norms for THI and cooling THI-day were significantly different ( $p < 0.001$ ) for all models. The conditional F-statistics indicated that cooling THI-day provided additional significant information to the THI model.

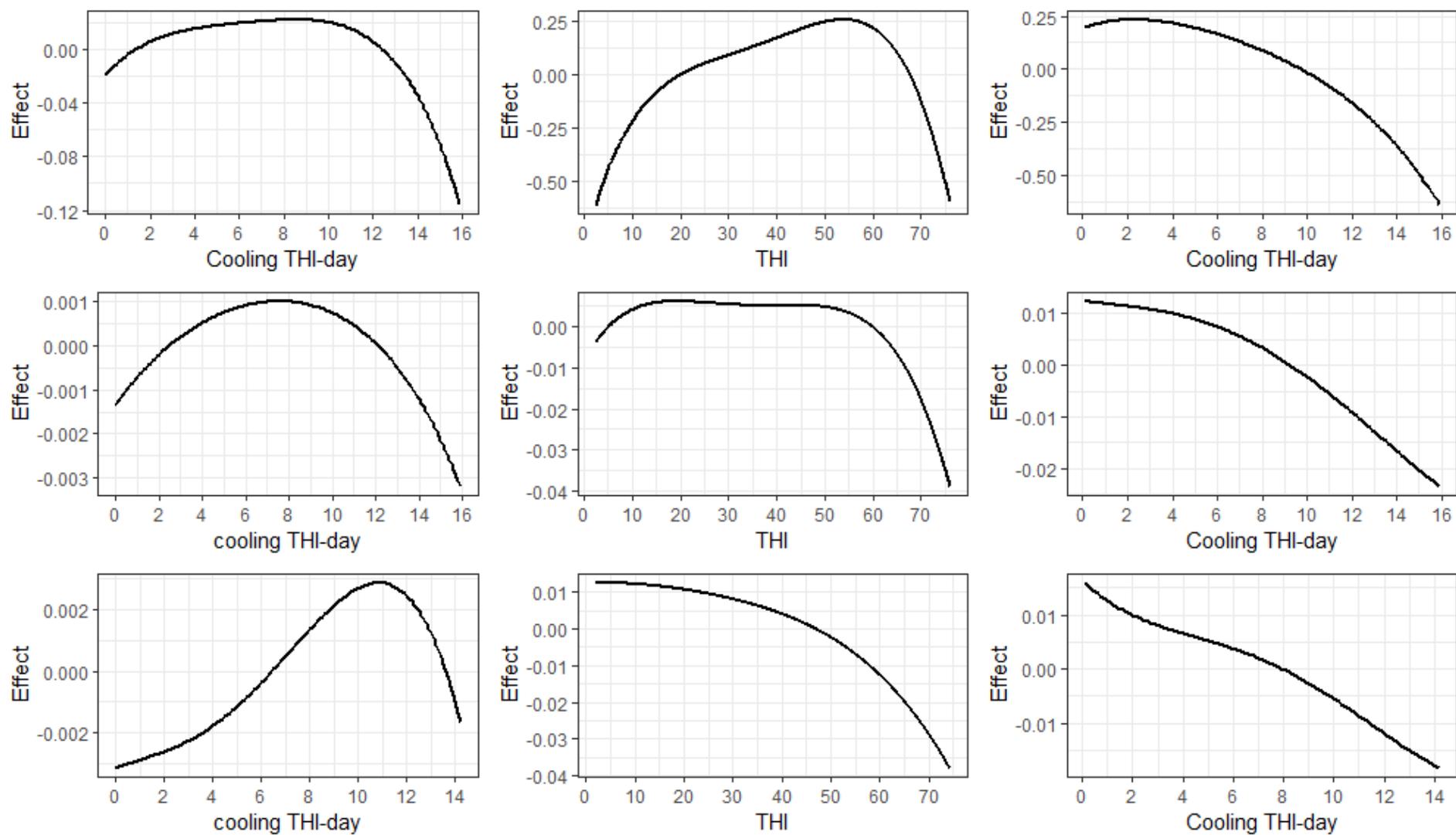
### Reaction norms of milk traits to thermal load

The reaction of milk-, protein- and fat yield to increasing heat load estimated from THI and cooling THI-day full model, THI only and cooling THI-day only models are presented in Figure 3. For milk yield, a THI threshold of 60 was identified as the breakpoint above which milk yield started to decline due to heat stress as indicated in model 2. Model 1 clearly indicated that the

cumulative heat load above the heat stress threshold caused a further milk yield loss. A loss of -0.14 kg per cow per day was recorded at the maximum cooling THI-day above the THI threshold. Model 2 demonstrated its capability to model both cold and heat stress. Model 3 indicated a milk yield loss of -0.87 kg per cow per day. This was slightly above what was estimated from model 2. Studies that apply model 2, therefore, underestimate the milk yield loss by failing to account for the cumulative heat load. In summary, model 1 is the most efficient for all thermal stress conditions i.e. cold and heat stress and model 3 the most suitable for modeling heat stress if cold stress is not significant and the threshold for heat stress has been established.

The effects of THI and cooling THI-day above the THI threshold for protein yield were about -0.042 and -0.002 kg respectively. From model 2, the protein yield loss above the THI threshold was about -0.039 kg. The loss estimated from model 3 was -0.036 kg per cow per day. The cumulative heat load above the heat stress threshold caused a further protein yield loss.

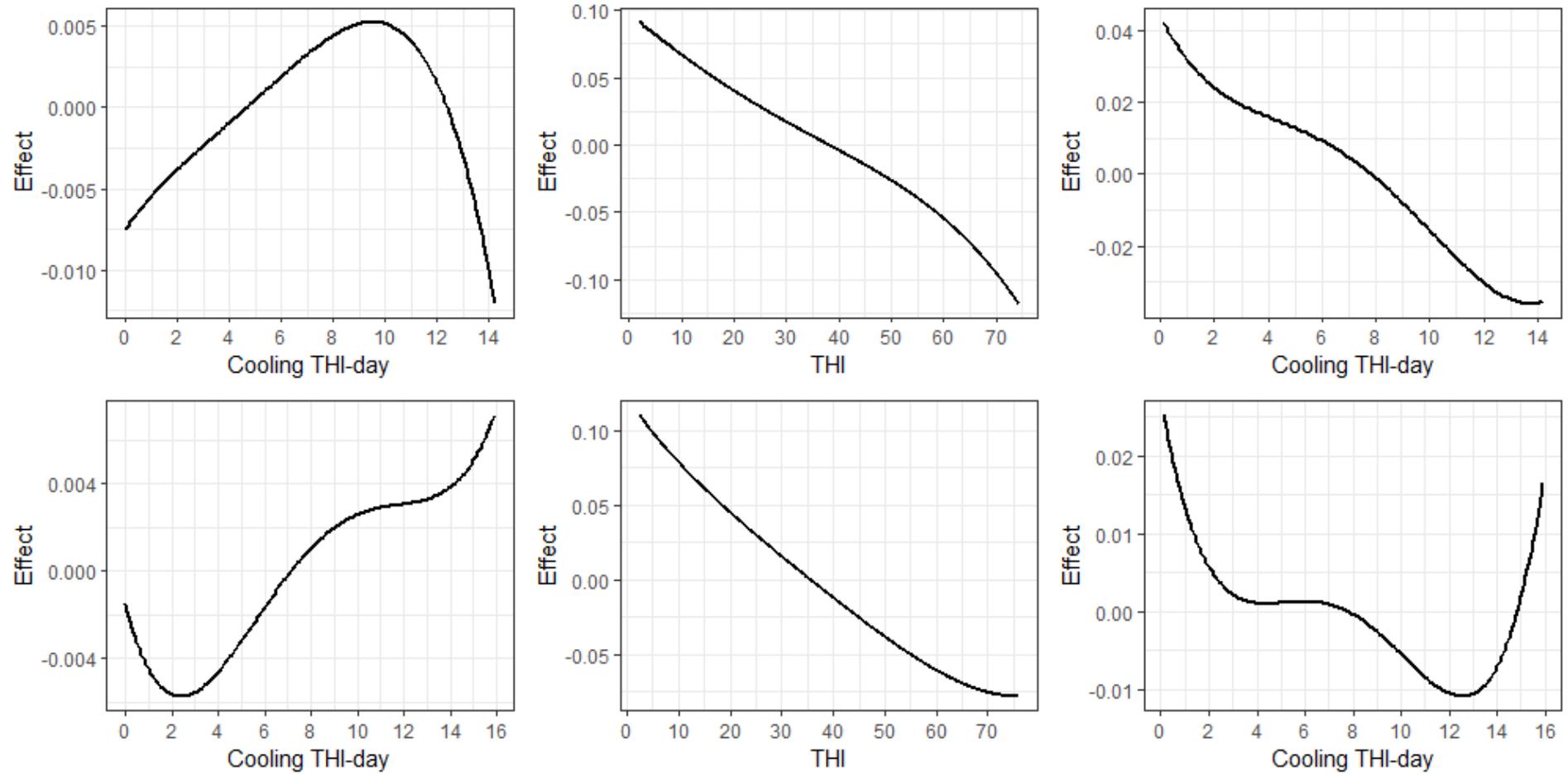
Fat yield did not have a clearly defined threshold as depicted from model 2. The cumulative heat load in model 1 depicted both an increasing and decreasing trend for fat yield with no clear net effect. The general trend from model 3 was a decrease in fat yield with increasing heat load. The cooling THI-day model depicted less decline than the THI model. This is because there was substantial loss before the THI threshold. This fat yield loss before the THI threshold was not accounted for in model 3. The implication is that a cooling THI-day model with the same base THI for different traits would underestimate the effect of heat stress for traits that have a lower or no clearly defined threshold.



**Figure 3.** Reaction of average milk yield (top), protein yield (middle) and fat yield (bottom) to THI and cooling THI-day full model (left; plot is for cooling THI-day), THI (middle) and cooling THI-day (right).

The reaction of protein- and fat content to increasing THI and cooling THI-day are presented in Figure 4. Results from model 2 indicated that protein content decrease with increasing heat stress with no THI breakpoint. From model 1, a slight increase followed by a decrease was observed due to cooling THI-day above a threshold of 60 THI units. However, it was clear from model 3 that protein content continuously declined with increasing heat load.

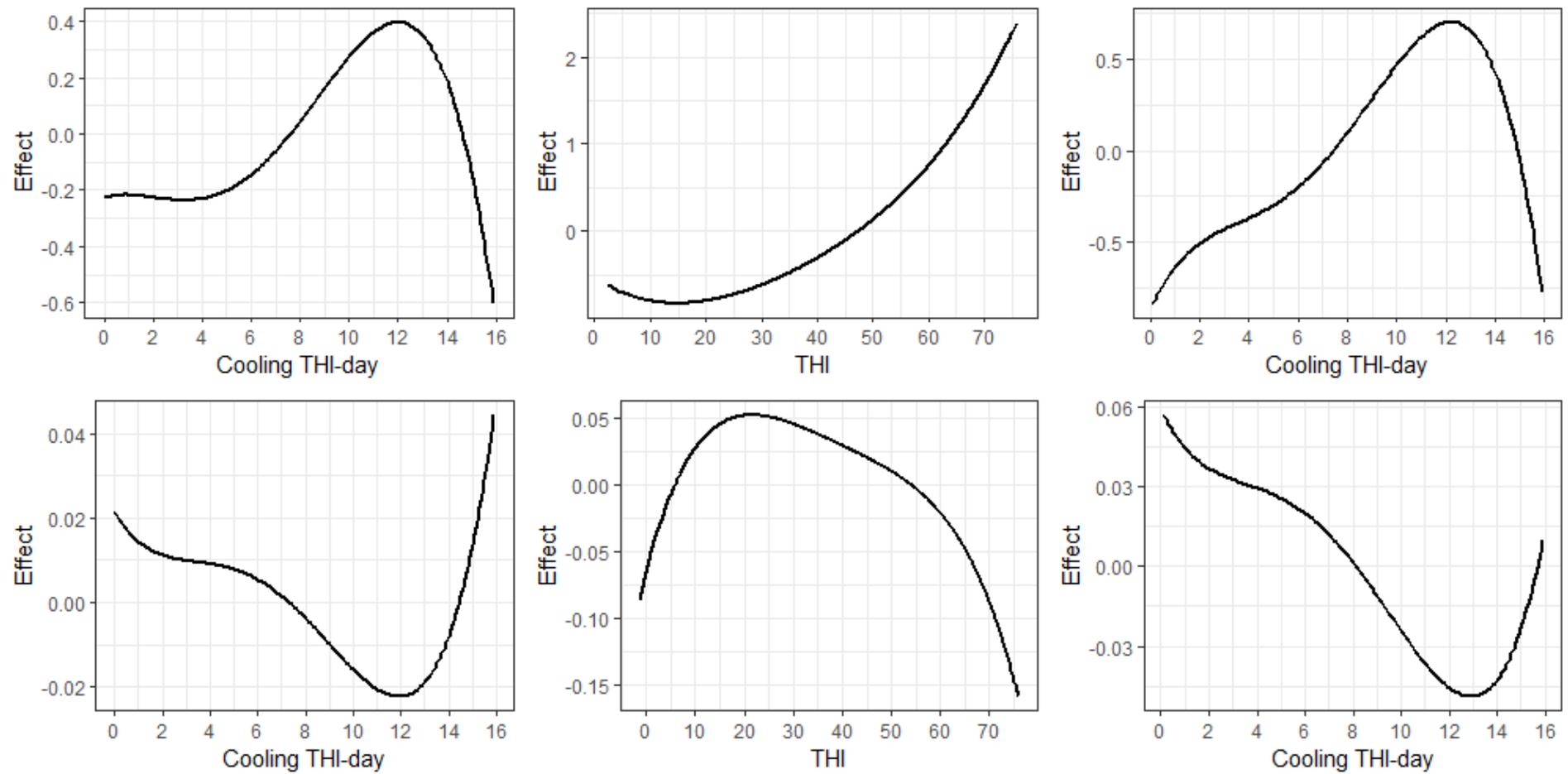
Although fat content declined with no THI threshold when model 2 was applied, cumulative heat stress led to a slight increase. Results from model 3 indicate that cooling THI-day above 12 units would cause the fat content to increase by 0.027 kg per cow per day. Although the THI model indicated decreasing fat content with increasing THI, the cooling THI-day indicated that cumulative heat load causes a counter effect and eventually causes an increase.



**Figure 4.** Reaction of protein content (top) and fat content (bottom) to THI and cooling THI-day full model (left; plot is for cooling THI-day), THI (middle) and cooling THI-day (right).

Figure 5 presents the reaction of milk urea and SCS to increasing thermal load from different models. Milk urea followed an increasing trend with increasing THI when estimated from model 2. Cumulative heat load in model 1 and 3 did not depict a clear trend of response. An increasing trend was followed by a decreasing trend resulting in an unclear net effect.

Increasing THI caused a decrease in SCS when estimated from model 2. Increasing THI above the threshold caused an unexpected decrease of -0.15 units. However, models 1 and 3 indicated that when cooling THI-day was above 12 units, SCS started to increase. This feature was not captured by the conventional THI model 2.



**Figure 5.** Reaction of milk urea (top) and somatic cell score (SCS, bottom) to THI and cooling THI-day full model (left; plot is for cooling THI-day), THI (middle) and cooling THI-day (right).

Cooling THI-day models were able to detect significant changes in fat content and SCS that THI models did not. A cooling THI-day of about 12 units appeared to be a significant breakpoint for most traits. This is the equivalent of a daily average THI of 72 units. Below this point, the cumulative THI-day did not seem to significantly affect the reaction norm slope.

The heat load is determined by the area under the curve of diurnal THI range and above the base THI. This implies that the choice of base THI is a critical pre-requisite. Furthermore, for traits that don't have a clearly defined threshold, this area under the curve would be underestimated, consequently the effect of heat stress.

The use of THI-day requires high temporal resolution weather data at hourly interval as opposed to daily averages. Observational data are readily available from public weather stations and grid reanalysis datasets may be readily compiled if relevant weather variables are accessible. For instance, Mistry (2019) derived high spatial resolution grid-interpolated degree-days using meteorological variables incorporating satellite and ground-based observations. The spatial extent of such gridded datasets and possibility for regional reanalysis and their free availability to public make them valuable resources for researchers.

This being a novel study applying cooling THI-day on heat stress analysis in cattle, we recommend further studies to validate it.

## **Conclusion**

Accounting for cooling THI-days in conventional THI reaction norms provided some significant improvement because it was able to capture some additional variation. Reaction norm models that do not account for the cumulative heat load above the threshold tend to underestimate the effects on milk production traits. The application of cooling THI-days is not equally important for all milk production traits. In our study, it was not efficient for traits with no definite heat stress thresholds e.g. protein content, fat content and fat yield. This is because a THI threshold is a pre-requisite for cooling THI-day calculation. Calculation of THI-day requires high temporal resolution weather data such as hourly interval as opposed to daily averages.

We recommend further studies with cooling THI-day as a heat stress indicator. This includes genetic studies where it is expected that cooling THI-day would explain more genetic variation and probably improve the heritability estimates.

## **Funding**

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## GENERAL DISCUSSION

Ravagnolo and Misztal (2000) developed a methodology that allowed dairy animals to be evaluated at any degree of temperature humidity index (THI), by merging test-day production data with weather data from public weather stations. In Chapter one, the approaches to improve the resolution of both animal performance and weather information were reviewed. The temporal resolution for animal performance data referred to the amount of time detail in an observation. For weather data, both temporal (time) and spatial resolution i.e. what area and how detailed an observation is, were highlighted as important. Weather information for chapter two was successfully obtained from the NASA Langley Research Center Prediction Of Worldwide Energy Resource (NASA/POWER) Project based upon satellite observations and meteorological data from assimilation models (Stackhouse Jr *et al.*, 2018). For this dataset, the spatial resolution was coarse because parameters were on a global grid of 0.5° latitude by 0.5° longitude. Temporal resolution was daily averages. Gridded weather data is becoming important in studies on heat stress in livestock where weather station data is not readily available or the network is sparse, especially in developing countries (Mutua *et al.*, 2020, Carrara *et al.*, 2021). A regional reanalysis of the Consortium for Small-scale Modeling climate model (COSMO-CLM) over the CORDEX-Africa domain is now available at finer resolution and accessible for application to heat stress studies (Rahimi *et al.*, 2021). Weather data for chapter three were obtained from the German Meteorological Service, (Deutscher Wetterdienst, DWD). Gridded weather was based on COSMO model at a spatial resolution of 6 km and a temporal resolution of one hour. The validation analysis comparing high spatial and temporal resolution weather station data (WSD) and numerical weather prediction (NWP) weather data was outstandingly successful. Further, climatic indices based on gridded data were formulated. Results indicated high correlations between WSD and NWP daily average temperature and THI and moderately high relative humidity. This was in agreement with Razafimaharo *et al.* (2020), that gridded datasets for Germany show good agreement with DWD weather station data. The NWP model COSMO used in this study has been demonstrated to better resolve local extreme events because of the high spatio-temporal resolution (Bollmeyer *et al.*, 2015). However, it was clear that the mountainous Alps posed gridding challenges.

In chapter two, the average (population) heat stress thresholds, expected milk yield loss and individual animal variations for milk yield in dairy cattle under tropical conditions were successfully estimated from test-day records using random regression models. The study of Ravagnolo and Misztal (2000) fitted the days in milk (DIM) classes as fixed effects. However,

to capture the dynamics of heat stress throughout the lactation, random regression models considering both DIM and THI covariates have been implemented (Brügemann *et al.*, 2011, Bohlouli *et al.*, 2013). This approach creates (co)variance components for all available THI × DIM combinations without defining a specific threshold. Extended factor analysis (XFA) was fitted to random regression models to create (co)variance components for all available THI × DIM combinations. The advantages of XFA include the parsimonious description of covariance structures, the scope for direct interpretation of factors and reduced computational requirements (Meyer, 2009).

Part of this study was a genetic analysis to estimate the environmental and genetic variance components for heat stress tolerance. However, it was implausible to disentangle the additive and permanent environmental variance components. The estimated additive variance unrealistically tended towards zero. However, total phenotypic variances were plausible and within the expected range. The results were in agreement that in smallholder systems, standard models do not separate genetic and environmental effects accurately (Selle *et al.*, 2020).

From the dataset used in the analysis for this chapter (chapter two), a valuable percentage of data got lost due to technical, avoidable omissions. A reduction in the total number of test-day records by 41.2%, 39.7% and 38.5% for the first, second and third lactations, respectively, calls for improved data recording.

In chapter three, herd average reaction norms were estimated for daily average temperature and THI heat stress indicators comparing weather information obtained from weather stations and grid interpolation. Evaluation of WSD and NWP temperature and THI as thermal stress indicators for milk production traits were successful. Thermal stress thresholds for herd average milk production traits were identified for temperature and THI. For milk yield, the models were able to predict both cold and heat stress regions. The general herd-level heat stress threshold identified for milk yield of 60 THI units was in agreement with estimates from a similar THI evaluation method of hourly THI averaged over a day in the lower Saxony region of Germany (Brügemann *et al.*, 2012). For fat yield, fat content and protein content, no definite heat stress thresholds were observed. This was in agreement with other studies in the region but in Holsteins (Brügemann *et al.*, 2012, Gorniak *et al.*, 2014, Lambertz *et al.*, 2014). The response of milk urea was an increase with increasing heat stress. This was in agreement with other studies in the region (Gernand *et al.*, 2019). Somatic cell score did not seem to be adversely affected by heat stress. Studies on the traits response to heat stress have generally been contradictory.

Several THI calculation formulae and heat stress thresholds have been presented in the literature (Bohmanova *et al.*, 2007, Dikmen and Hansen, 2009). As a result, there is no single standard formula for calculating THI from temperature and relative humidity data. The temperature and THI thresholds for milk yield obtained for a tropical environment (chapter two) were different from those of a temperate environment (chapter three). Although the results are not directly comparable, the heat stress thresholds for the temperate environment were much lower. It is clear, therefore, there is no universal threshold for the beginning and extent of production loss due to heat stress in dairy cattle. Different heat stress indicators may be required for different geographic locations.

In chapter four, weather station data was used to calculate the THI and the concept of degree-days used to compute THI-days. Cooling THI-day was then successfully used as a new heat stress indicator for milk production traits in cattle. Herd average reaction norms for cooling THI-days were estimated with and without the conventional THI. In calculating the cooling degree days (CDD), the base or threshold temperature is defined as the temperature below which growth or development is effectively zero (Chezik *et al.*, 2014). In our study, the threshold THI was the THI below which heat stress was effectively zero i.e. the thermal comfort zone. The set THI threshold determines the summable area under the curve and, therefore, the thermal load. The implication is that the THI threshold for the trait under consideration need to be precisely estimated. Three reaction norm models were evaluated: a full model with THI and cooling THI-day, a reduced model for THI only and a reduced model for cooling THI-day only. Results indicated that the full model was the most efficient for all thermal stress conditions i.e. cold and heat stress. Cooling THI-day model was the most suitable for modeling heat stress if cold stress is not significant and the threshold for heat stress has been established. Since THI-days have not been used before in evaluation of heat stress in cattle, there were no comparable parameters in the literature.

From the results, cooling THI-day explained more of the variation between herds than average THI alone. Cooling THI-days can be computed with higher temporal resolution if data are available e.g. hourly temperature and humidity. Better milk recording data in some cases may be achieved by better data curation and quality checks and/or also by more frequent recording. While the latter may be less important if the goal is to estimate the lactation milk yield of a cow, it may be helpful if the aim is estimating the accumulated milk loss due to a series of hotter days. So, better estimates of economic losses could be generated by combining better indicators of heat stress, such as cooling degree days or THI-days, with more frequent recordings of milk

yield. Such an approach is presented by Fodor *et al.* (2018), whereby sub-daily and daily THI are used to predict both the milk yield loss and the economic consequence due to heat stress.

The number of days that cattle experience heat stress is limited in the temperate regions. This means that cows experience little pressure to adapt to heat stress. However, climate change is causing an increase in temperature and frequency of weather events. In the short-term, cooling mechanisms could reduce the milk yield loss and changes in milk composition while improving the herd's welfare. Long-term strategies should focus on breeding for heat tolerance/ resilience. For genetic selection, individual reaction norms are the prerequisites rather than reaction norms on population or herd level. Biases in heat stress indicators, caused e.g. by extraordinary elevation or distance of weather stations, may hamper the effectiveness of genetic evaluations for heat stress resilience. Better indicators, on the other side, may reveal a higher amount of genetic and non-genetic variability on the individual scale.

Because of the long generation intervals in dairy cattle, traditional selective breeding has slow rates of genetic gain (Garner *et al.* 2016). Due to the increased frequency of heat stress events, faster genetic gains are desired. Genomic selection will allow young bulls and heifers to be selected based on their genomic estimated breeding values (GEBV), thereby accelerating genetic gain (Garner *et al.* 2016). Other advanced assessment methodologies and biotechnological tools necessary to breeding for heat tolerance in dairy have been reviewed (Carabano *et al.*, 2017, Rashamol *et al.*, 2019). More recently, GEBV for heat tolerance have been developed for the Australian dairy cattle (Nguyen *et al.* 2017). However, the knowledge on individual genes and functional pathways associated with the cow's ability to produce milk under heat stress condition is still limited (Sigdel *et al.*, 2019). In cattle, a major gene known as *slick hair* gene has been found to confer thermotolerance (Olson *et al.*, 2003). Lactating Holstein cows with the *slick hair* gene were reported to have superior thermoregulatory ability and experience less drastic depression in milk yield than non-slick animals (Dikmen *et al.*, 2008, Dikmen *et al.*, 2014). The gene is now being introgressed into dairy breeds to produce heat tolerant *Bos Taurus* cattle (Davis *et al.*, 2017, Hansen, 2020).

Heat tolerant cows might not be cold resistant and vice versa which require consideration in the temperate regions. In terms of selection, the option lies in selection for resilience where animals that are more able to cope with future changes or short term fluctuations e.g. spikes of heat or cold are selected (Misztal, 2017). One of the approaches for improving heat tolerance in the tropics would be organized crossbreeding of high performance breeds with locally adapted breeds. The thermotolerance ability of indigenous genotypes can be utilized through

crossbreeding with highly selected dairy cattle breeds in commercial herds to take advantage of the specific abilities. Heterosis achieved by crossbreeding could be exploited to confer thermotolerance in dairy cattle. Another alternative would be selection among crossbreds and creating new synthetic breeds.

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## SUMMARY

Thermal stress effects can be harmful for milk production and quality in cattle. High temporal and spatial resolution weather data are necessary for evaluation of the effects of thermal stress on milk production traits for management purposes to mitigate production losses. Data from a number of meteorological stations and interpolated onto a grid to provide high spatio-temporal resolution weather variables at locations away from the observing stations were used. Weather stations and grid-interpolation methodologies provide independent weather parameters. Gridded weather index, in this case gridded temperature humidity index (THI) was developed. The grid-interpolated average temperature and grid-interpolated temperature humidity index (THI) were suitable indicators of heat stress for dairy cattle in Kenya. For these indicators, heat stress thresholds for milk yield were estimated.

To compare the correlation between observational weather station data (WSD) and gridded numerical weather prediction (NWP) model data, a validation analysis was performed. Results indicated high correlation coefficients ( $r$ ) of 0.97 for both daily average temperature and THI and 0.84 for relative humidity in southern region of Bavaria, Germany. This implied that missing weather station data can be adequately filled-up by grid-interpolated weather data. However, care should be taken in the mountainous regions to account for both vertical and horizontal topographical profiles. Both WSD and NWP daily average temperature and THI were suitable indicators of heat stress in cattle. Herd average reaction norms for milk production traits and the thresholds for heat stress were estimated for Bavarian conditions.

After determining the heat stress thresholds for some milk production traits, the effect of cumulative heat load above the threshold was investigated. Cooling THI-day (average of hourly THI values above the threshold) was formulated and applied as the heat stress indicator for this analysis. Heat stress models combining THI and cooling THI-days, proved to be superior to conventional THI models. Results show that the new heat stress indicator, cooling THI-day would best suited for modeling heat stress when cold stress is not significant and the threshold for heat stress for the given trait can be estimated.

## ZUSAMMENFASSUNG

Hitzestress kann die Leistung von Milchkühen und die Qualität ihrer Milch beeinträchtigen. Zeitlich und räumlich hochaufgelöste Wetterdaten sind für die Abschätzung der Auswirkungen von Wärmestress auf Milchproduktionsmerkmale erforderlich, um Produktionsverluste zu mindern. Es wurden sowohl Daten von einzelnen Wetterstationen als auch auf ein Gitter interpolierte Wettervariablen eingesetzt, um alternative Hitzestressindikatoren mit hoher räumlicher und zeitlicher Auflösung für Milchviehbetriebe zu erhalten. Der gitterinterpolierte Temperatur-Feuchte-Index (THI) und die gitterinterpolierte Durchschnittstemperatur eigneten sich als Indikatoren für Hitzestress bei Milchvieh in Kenia. Für diese Indikatoren wurden Hitzestress-Schwellenwerte für die Milchleistung geschätzt.

Um die Korrelation zwischen Beobachtungsdaten von Wetterstationen (WSD) und gittergestützten numerischen Wettervorhersagemodellen (NWP) zu vergleichen, wurde eine Validierungsanalyse durchgeführt. Die Ergebnisse zeigten hohe Korrelationskoeffizienten ( $r$ ) von 0,97 für die tägliche Durchschnittstemperatur und den THI und 0,84 für die relative Luftfeuchtigkeit in der südlichen Region Bayerns. Dies bedeutet, dass fehlende Wetterstationsdaten durch rasterinterpolierte Wetterdaten adäquat aufgefüllt werden können. Allerdings sollte in den Gebirgsregionen darauf geachtet werden, dass sowohl vertikale als auch horizontale topographische Profile berücksichtigt werden. Sowohl die WSD- als auch die NWP-Tagesdurchschnittstemperatur und der THI waren geeignete Indikatoren für Hitzestress bei Rindern. Durchschnittliche Reaktionsnormen für Milchleistungsmerkmale und Schwellenwerte für Hitzestress wurden für Bayerische Verhältnisse geschätzt.

Nach der Bestimmung der Hitzestressschwellen für mehrere Merkmale wurde die Auswirkung der kumulativen Hitzebelastung oberhalb der Schwelle untersucht. Als Hitzestressindikator für diese Analyse wurde der THI-Tag (Durchschnitt der stündlichen THI-Werte über der Schwelle) berechnet. Hitzestressmodelle, die den THI und die THI-Tage kombinieren, erwiesen sich gegenüber den herkömmlichen THI-Modellen als überlegen. Die Ergebnisse zeigen, dass der neue Indikator THI-Tag für die Modellierung von Hitzestress am besten geeignet ist, wenn Kältestress nicht bedeutend ist und für das Merkmal eine gut definierte Hitzestressschwelle geschätzt werden kann.

## APPENDIX

**ASReml job control file for factor analytic model to analyse heat stress in Kenya dairy cattle (Mbuthia *et al.*, 2021). The model presented was for average temperature.**

```
!Workspace 5024
Milk yield data for Kenyan dairy cattle. First three parities arranged as
univariate
Animal      !A    9563      #Cows_ID
Breed       !A                #Breed of cow
HTD         !A    9531      #Herd-Test-Day
CS          !A     4        #Calving Season
Temp1       #Average temperature 1 day prior to Test-Day
Temp2       #Average temperature 2 days prior to Test-Day
Temp3       #Average temperature 3 days prior to Test-Day
TEMPMAX1    #Maximum temperature 1 day prior to Test-Day
TEMPMAX2    #Maximum temperature 2 days prior to Test-Day
TEMPMAX3    #Maximum temperature 3 days prior to Test-Day
THI1        #THI 1 day prior to Test-Day
THI2        #THI 2 days prior to Test-Day
THI3        #THI 3 days prior to Test-Day
HLI1        #Heat Load Index 1 day prior to Test-Day
HLI2        #Heat Load Index 2 days prior to Test-Day
HLI3        #Heat Load Index 3 days prior to Test-Day
DIM          #Days in milk
LACT        !A     3        #Lactation number
MY          #milk yield

zero 3 !=0

Heat_final_long.txt      !MVREMOVE  !AISING !MAXIT 200  !DDF !FCON
!CONTINUE

MY~ mu LACT Breed LACT.Breed CS LACT.leg(Temp3,-3) LACT.leg(DIM,3).Breed.CS
+ !r HTD !{LACT.leg(DIM,3).Animal LACT.leg(Temp3,-3).Animal zero.Animal
xfa3(21).Animal !}
residual sat(LACT).units
```

**ASReml job control file for the effect of weather station data on milk yield for Bavaria data. Comparison models were similar but substituted the traits and heat stress indicator as appropriate.**

```

!WORKSPACE 20 !RENAME !ARGS 1 !OUT !SLN
LKV dairy transformed proportions of fixed effects - weighted by number of
cows
#records for year 2010 removed. herds with 1 cow in a testday, and less
than 2 cows on average removed
herd !A 9726
Testday !A 3165
parity !G 3
parityLS_1 !G 10
parityLS_2 !G 10
parityLS_3 !G 10
calvingtype !G 2 #single twins
calvingease !G 6 #calvease class 0,1,2,3,4,9
Latitude
Longitude
ncows
avgmilk
FatContent
Protein
CellCount #Somatic Cell Count
Urea
SCS #Somatic Cell Score
proteinyield
fatyield
tempstation_td
tempstationlag1
tempstationlag2
tempstationlag3
tempstationMA
tempgrid_td
tempgridlag1
tempgridlag2
tempgridlag3
tempgridMA
thistation_td
thistationlag1
thistationlag2
thistationlag3
thistationMA
thigrid_td
thigridlag1
thigridlag2
thigridlag3
thigridMA
gemeinde
altitude
distance
altitudediff
year !I
month !A
tdnumber !I 103
season !A

lkv_performance_weather.csv !SKIP 1 !DDF !FCON !MVREMOVE
!MAXIT 100 !NODISPLAY !CONTINUE

```

```
avgmilk !WT 46~ mu parity parityLS_1 parityLS_2 parityLS_3 calvingtype
calvingease month year year.season leg(tempstationlag3,4) !r
idv(herd).ar1(tdnumber) !f herd
PREDICT tempstationlag3          !PRINTALL
```

**ASReml job control file for the effect of THI and cooling THI-day on milk yield for Bavarian data. Comparison of models was performed by alternatively excluding one heat stress indicator (THI or cooling THI-day) from the model.**

```
!WORKSPACE 20 !RENAME !ARGS 1 !OUT !SLN
THI-days. Cumulative THI above the threshold of 60 in a day
#thihours are the number of hours THI was above the threshold. thiday is
the cumulative THI above the threshold in a day
herd !A 9726
Testday !A 3165
parity !G 3
parityLS_1 !G 10
parityLS_2 !G 10
parityLS_3 !G 10
calvingtype !G 2 #single twins
calvingease !G 6 #calvease class 0,1,2,3,4,9
Latitude
Longitude
ncows
avgmilk
FatContent
Protein
CellCount #Somatic Cell Count
Urea
SCS #Somatic Cell Score
proteinyield
fatyield
thistationlag3
year !I
month !A
tdnumber !I 103
season !A
thiduration
thiday

thidd_milk.csv !SKIP 1 !DDF !FCON !MVREMOVE !MAXIT 20 !NODISPLAY
#!CONTINUE

avgmilk !WT 44 ~ mu parity parityLS_1 parityLS_2 parityLS_3 calvingtype
calvingease month year year.season leg(thistationlag3,4) leg(thiday,4) !r
idv(herd).ar1(tdnumber) !f herd
```

## Reference

Mbuthia, J., M. Mayer, and N. Reinsch. 2021. Modeling heat stress effects on dairy cattle milk production in a tropical environment using test-day records and random regression models. *Animal* 15(8):100222.

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## Selbständigkeitserklärung

M.Sc. Jackson Mwenda Mbuthia

Hiermit erkläre ich an Eides statt, dass ich die vorgelegte Dissertation mit dem Titel „ **Modeling heat stress in dairy cattle using high spatio-temporal resolution observational and grid-interpolated weather data**“ selbständig und ohne unerlaubte Hilfe angefertigt habe und dass ich die Arbeit noch keinem anderen Fachbereich bzw. noch keiner anderen Fakultät vorgelegt habe.

Dummerstorf, 17.11.2021

M.Sc. Jackson Mwenda Mbuthia

Hiermit erkläre ich, dass die Dissertation nach den Regeln guter wissenschaftlicher Praxis (Standard wissenschaftlichen Arbeitens nach den Empfehlungen der DFG) abgefasst wurde.

Dummerstorf, 17.11.2021

M.Sc. Jackson Mwenda Mbuthia

Hiermit erkläre ich, dass gegen mich kein strafrechtliches Ermittlungsverfahren schwebt.

Dummerstorf, 17.11.2021