Heat stress response for physiological traits in dairy and dual purpose cattle populations on phenotypic and genetic scales

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Adnan Jabbar Jadoa Al-Kanaan

born in Basrah (Iraq)

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First Referee: Prof. Dr. Sven König
Department of Animal Breeding
Faculty of Organic Agriculture
Universität Kassel
37213 Witzenhasuen, Germany

Second Referee: Prof. Dr. Dr. Matthias Gauly
Department of Animal Sciences
Faculty of Science and Technology
Freie Universität Bozen
39100 Bozen, Italy

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ABSTRACT

The main objective of this thesis was to determine the potential impact of heat stress (HS) on physiological traits of lactating cows and semen quality of bulls kept in a temperate climate. The thesis is comprised of three studies. An innovative statistical modeling aspect common to all three studies was the application of random regression methodology (RRM) to study the phenotypic and genetic trajectory of traits in dependency of a continuous temperature humidity index (THI).

In the first study, semen quality and quantity traits of 562 Holstein sires kept on an AI station in northwestern Germany were analyzed in the course of THI calculated from data obtained from the nearest weather station. Heat stress was identified based on a decline in semen quality and quantity parameters. The identified general HS threshold (THI = 60) and the thermoneutal zone (THI in the range from 50 to 60) for semen production were lower than detected in studies conducted in tropical and subtropical climates. Even though adult bulls were characterized by higher semen productivity compared to younger bulls, they responded with a stronger semen production loss during harsh environments. Heritabilities (low to moderate range) and additive genetic variances of semen characteristics varied with different levels of THI. Also, based on genetic correlations genotype, by environment interactions were detected. Taken together, these findings suggest the application of specific selection strategies for specific climate conditions.

In the second study, the effect of the continuous environmental descriptor THI as measured inside the barns on rectal temperatures (RT), skin temperatures (ST), vaginal temperatures (VT), respiration rates (RR), and pulse rate (PR) of lactating Holstein Friesian (HF) and dual-purpose German black pied cattle (DSN) was analyzed. Increasing HS from THI 65 (threshold) to THI 86 (maximal THI) resulted in an increase of RT by 0.6 °C (DSN) and 1 °C (HF), ST by 3.5 °C (HF) and 8 °C (DSN), VT by 0.3 °C (DSN), and RR by 47 breaths / minute (DSN), and decreased PR by 7 beats / minute (DSN). The undesired effects of rising THI on physiological traits were most pronounced for cows with high levels of milk yield and milk constituents, cows in early days in milk and later parities, and during summer seasons in the year 2014.

In the third study of this dissertation, the genetic components of the cow's physiological responses to HS were investigated. Heat stress was deduced from indoor THI measurements, and physiological traits were recorded on native DSN cows and their genetically upgraded crosses with Holstein Friesian sires in two experimental herds from pasture-based production systems reflecting a harsh environment of the northern part of Germany. Although heritabilities were in a low range (from 0.018 to 0.072), alterations of heritabilities, repeatabilities, and genetic components in the course of THI justify the implementation of genetic evaluations including heat stress components. However, low repeatabilities indicate the necessity of using repeated records for measuring physiological traits in German cattle. Moderate EBV correlations between different trait combinations indicate the potential of selection for one trait to simultaneously improve the other physiological attributes.

In conclusion, bulls of AI centers and lactating cows suffer from HS during more extreme weather conditions also in the temperate climate of Northern Germany. Monitoring physiological traits during warm and humid conditions could provide precious information for detection of appropriate times for implementation of cooling systems and changes in feeding and management strategies. Subsequently, the inclusion of these physiological traits with THI specific breeding values into overall breeding goals could contribute to improving cattle adaptability by selecting the optimal animal for extreme hot and humid conditions. Furthermore, the recording of meteorological data in close distance to the cow and visualizing the surface body temperature by infrared thermography techniques might be helpful for recognizing heat tolerance and adaptability in cattle.

Key words: Heat stress, physiological traits, semen traits, genetic parameters, dairy cattle.

ZUSAMMENFASSUNG

Das Hauptanliegen dieser Arbeit war herauszufinden, wie sich Hitzestress (HS) auf die physiologischen Eigenschaften von laktierenden Kühen und auf die Samenqualität von Bullen auswirkt.

Die Arbeit basiert auf drei Studien. Als innovatives statistisches Modell wurde in allen drei Studien das Random Regression Modell (RRM) genutzt, um die phänotypischen und genetischen Veränderungen von physiologischen Merkmalen in Abhängigkeit von einem kontinuierlichem Temperatur- und Feuchtigkeitsindex (THI) festzustellen.

In der ersten Studie wurde Spermaqualität und –menge bei 562 Holstein Bullen auf einer Besamungsstation in Nordwestdeutschland analysiert. Diese wurden mit den passenden Wetterdaten der Region kombiniert, um physiologische Merkmalsreaktionen in Abhängigkeit von THI abbilden zu können. Hitzestress wurde dabei definiert als Verschlechterung der Spermaqualität oder Abnahme der Spermaquantität. Der identifizierte Grenzwert für Hitzestress bei einem THI von 60 und die thermoneutrale Zone (THI von 50 bis 60) für die Samenproduktion von Holsteinbull waren in dieser Studie niedriger verglichen mit Studien zu Hitzestress in tropischen und subtropischen Klimazonen. Trotz der generell größeren Spermaproduktion von ausgewachsenen Bullen im Vergleich zu jüngeren, reagierten die älteren Bullen stärker auf extreme klimatische Bedingungen. Erblichkeiten in einem niedrigen bis moderaten Bereich und additive genetische Varianzen von Spermacharakteristika variierten mit Änderungen des THI. Ebenso wurden aufgrund von genetischen Korrelationen von kleiner 0,80 im gleichen Merkmal, welches in verschiedenen Umwelten gemessen wurde, Genotyp-Umweltinteraktionen nachgewiesen. Die gefundenen Ergebnisse tragen dazu bei, spezifische Selektionsstrategien für besondere Klimabedingungen anzuwenden beziehungsweise spezifische Genetik für spezifische Umwelten zu selektieren.

In der zweiten Studie wurde der THI direkt im Stall gemessen und der THI Einfluss auf die rektale Temperatur (RT), Hauttemperatur (ST), vaginale Temperatur (VT), Atemfrequenz (RR) und Pulsrate (PR) bei laktierenden Holstein Friesian Kühen (HF) und Deutschen Schwarzbunten Niederungsrindern (DSN) analysiert. Ein Anstieg von THI 65 (Grenzwert) auf THI 86 (Maximaler THI) ergab einen Anstieg der RT von 0,6 °C (DSN) und von 1,0 °C (HF), ST von 3,5 °C (HF) und 8 °C (DSN), VT von 0,3 °C bei DSN und RR von 47 Atemzügen pro Minute, sowie eine niedrigere Pulsrate von 7 Schlägen pro Minute bei DSN. Die unerwünschten Effekte von erhöhtem THI auf die physiologischen Eigenschaften waren am deutlichsten bei Kühen mit hoher Milchleistung und hohen Milchinhaltsstoffen, bei Kühen mit hoher Laktationsnummer in der frühen Laktationsphase, insbesondere in der Sommersaison 2014.

In der dritten Studie der Dissertation wurden die genetischen Komponenten der physiologischen Kuhmerkmale in Bezug auf Hitzestress untersucht. Hitzestress wurde abgeleitet von THI-Stallmessungen. Die Merkmalserfassung erfolgte an heimischen DSN Kühen und deren Verdrängungskreuzungen mit HF-Bullen. Hierzu stand zwei Versuchsbetriebe zur Verfügung, die das System einer Weidehaltung praktizieren. Trotz der niedrigen Erblichkeiten für physiologische Merkmale (von 0,018 bis 0,072) ist ausreichen genetische Varianz gegeben, um auf diese Merkmale, auch unter Hitzestressbedingungen, zu selektieren. Die niedrige Wiederholbarkeit der Merkmalserfassung indiziert die Notwendigkeit der Nutzung von wiederholten Aufzeichnungen, um genauere Messungen und somit auch genauer geschätzte Zuchtwerte zu realisieren. Moderate Zuchtwertkorrelationen zwischen verschiedenen physiologischen Merkmalen zeigen, dass die Selektion auf ein Merkmal simultan andere physiologische Merkmale züchterisch gewünscht beeinflusst.

Zusammengefasst bleibt festzuhalten, dass sowohl Bullen auf Besamungsstationen als auch laktierende Kühe anfällig für Hitzestress, auch im gemäßigten Klima Norddeutschlands, sind. Physiologische Merkmale, erfasst bei warmen und feuchtem Wetter, könnten als Indikator genutzt werden, um auch Kühltechnik, Fütterung und Management zu bewerten. Außerdem sollten die physiologischen Merkmale bzw. Zuchtwerte für physiologische Merkmale in Zuchtziele integriert werden, um so die Anpassungsfähigkeiten der Rinder an extrem heiße und feuchte Umweltbedingungen zu verbessern. Die vorliegende Arbeit zeigt, dass meteorologische Aufzeichnungen, gemessen in direkter Nähe der Kuh, in Kombination mit physiologischen Merkmalen, insbesondere der Oberflächentemperatur erfasst mittels Wärmebildkamera, Wertvolle Rückschlüsse zur Adaptionsfähigkeit von Milchrindern liefert.

Schlagworte: Hitzestress, physiologische Merkmale, Merkmale der Bullenfruchtbarkeit, genetische Parameter, Milchvieh, Zweinutzungsrinder

Chapter 1

General introduction

Heat stress

Due to global warming and climatic changes heat stress (**HS**) became a major issue in cattle studies. By the end of the 21st century, experts are expecting an increasing number of hot and dry summer days and an increase of mean earth temperatures of 2 °C in the centre of Europe (IPCC 2014). These climatic changes are predicted to influence dairy production systems in multiple ways, from fodder properties to breeding priorities.

Cattle, as homeothermic animals, require to keep their core body temperature in a relatively steady state, when the external temperature changes. Dairy cattle try to maintain this equilibrium by balancing the heat produced from their metabolism in exchange with the environment. Mammalian try to maintain that balance through losing heat from the body surface by sensible (conduction, convection and radiation) and insensible (evaporation; via panting and sweating) processes. In addition to dissipating high amounts of heat to the environment, cattle attempt to reduce the production of metabolic heat to alleviate the magnitude of HS. Heat production is identified as the total of energy transformations occurring in the body of the animal per unit of time. The nervous and endocrine system controls heat production in cattle directly by modification of appetite and feed digestive processes, and indirectly through alterations in the activity of respiratory enzymes and synthesis of proteins (Yousef, 1985). A decline in feed efficiency mostly occurs due to more energy being used for thermoregulatory processes or to decrease the metabolic heat production (Howard *et al.,* 2013).

The relationship between animals and their thermal environment leads to the concept of a thermoneutral zone (**TNZ**). TNZ is "the range of ambient temperature within which metabolic rate is at a minimum, and within which temperature regulation is achieved by non-evaporative physical processes alone" (Bligh and Johnson, 1973). In the TNZ, bounded by the lower critical temperature (**LCT**) and the upper critical temperature (**UCT**), only a minimum of energy is required for maintaining body temperature of the animal (e.g. vasodilatation of peripheral blood vessels and changing animal posture is sufficient for cooling) (Yousef, 1985). LCT is defined as a threshold for ambient temperature. Below LCT, the rate of heat production of a resting homeothermic animal increases to maintain thermal balance. UCT is the point where evaporative heat loss and/or metabolic rate starts to increase (Silanikove, 2000). Figure 1 describes the physiological responses of animals (body temperature and metabolic heat production) for different environmental temperatures within different thermal zones. The TNZ in cattle differs according to breed, age, sex, reproductive and lactation status, milk yield, and milk composition. However, the difference between climatic types (moderate, subtropical, tropical, sub-arid, or arid climate) is the most important source to explain variations in comfortable zones, with regard to adaptation level of animals.

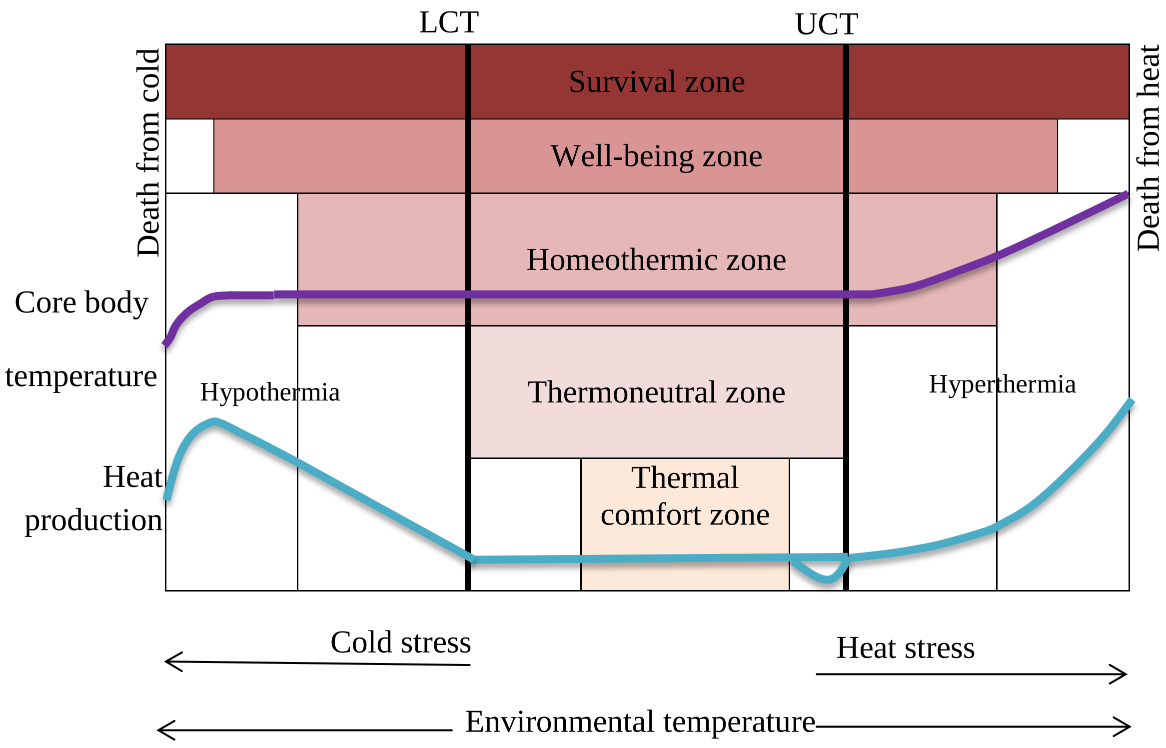

Figure 1. Schematic relationship between core body temperature and heat production of an animal with different environmental zones in relation to thermal stress. Adapted from Silanikove (2000). LCT= lower critical temperature and UCT= upper critical temperature.

Heat stress occurs when the sum of metabolic heat and heat received from the environment exceeds the amount of heat dissipated from the cattle body. HS is caused by an inappropriate combination of environmental factors including ambient temperature, relative humidity, solar radiation, wind velocity, and precipitation. These environmental factors tend to displace physiological variables of the animal from their equilibrium values (West, 2003).

In addition to the expectation of global climatic warming, the problem of HS is growing due to improvements in milk yield of cattle during the last decades. As milk production and body mass increases in dairy cattle, the subsequently rising in the metabolic heat production (as a by-product of digestion of high amount of feed) complicated the regulation of body temperature for cows kept in hot climates, particularly for the high yielding cattle (Hansen, 2007). The intensive selection for milk yield during the last 50 years led to a deterioration in fertility, increasing leg and metabolic problems, declining longevity, and subsequently reduced health and cow welfare (Oltenacu and Broom, 2010). Due to the antagonistic genetic relationship between milk production traits and cattle resistance to heat stress, selection on increased milk yield while ignoring HS expressions caused an impairment of cattle heat tolerance, with a growing environmental sensitivity problem. General responses to thermal stress include a variety of negative (direct and indirect) effects on physiology, behavior, production, reproduction, health, and well-being, which in turn affect the production possibilities and product quality of livestock (Figure 2).

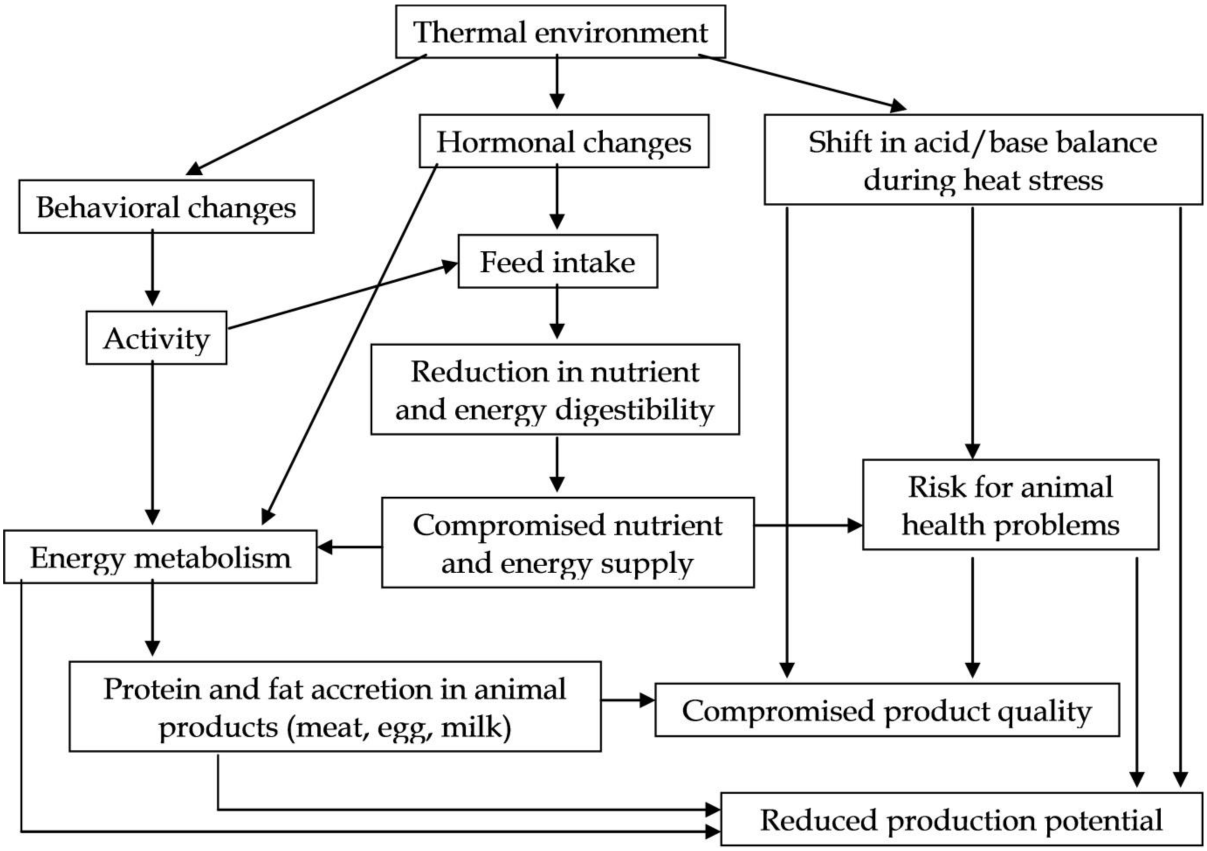

Figure 2. Scheme depicting the relationship of the undesired thermal environment on the quality and quantity of livestock products. Adapted from Babinszky et al. (2011).

Temperature humidity index (THI)

The most widespread indicator of heat stress used in cattle is the temperature humidity index (**THI**). The temperature humidity index was originally developed by Thom (1958) to detect

HS in humans. The THI was improved by Berry *et al.* (1964) to become the world-wide noted HS indicator in cattle. THI is a single value index developed to assess discomfort related to high ambient temperature combined with high relative humidity, and indicates thermal stress intensity. The most important formula to calculate THI is the equation of the National Research Council (1971), defined as follows:

$$\text{THI} = (1.8 * \text{T °C} + 32) - [(0.55 - 0.0055 * \text{RH \%}) * (1.8 * \text{T °C} - 26)],$$

where **T °C** = dry bulb temperature and **RH %** = relative humidity.

This formula was partially modified by using maximum temperature and minimum humidity values on the test day to calculate THI (Ravagnolo *et al.,* 2000), and has mostly been used for detecting the decline in milk yield and composition related to HS in lactating Holstein cows (Brügemann *et al.,* 2012). Bohmanova *et al.* (2007) compared seven THI formulas and concluded that humidity was the limiting HS factor in humid climates, whereas the dry bulb temperature was the limiting factor for heat tolerance in dry climates. These different formulas for THI calculation use at least two of the following parameters: dry bulb temperature, wet bulb temperature, dew point temperature, and relative humidity to assess the combination impact of ambient temperature and humidity on cattle performance. Yamamato *et al.* (1994) developed a thermal index using respiration rate and body temperature as dependent response variables by applying multiple regression in a formula containing ambient temperature and radiation. These authors identified solar radiation as a crucial environmental parameter with regard to heat load of animals. In addition to previous meteorological parameters, scientists developed thermal indices including solar radiation, wind speed and rainfall, and considering duration of exposure (e.g. Gaughan *et al.,* 2008; Mader *et al.,* 2010). These new thermal indices were adjusted to physiological traits and animal specificities (e.g. breed and age). However, Zimbelman *et al.* (2009) evaluated the new indices containing radiation and wind characteristics with regard to physiological heat parameters. They concluded that there is no advantage of replacing THI with the other thermal indices for detecting milk yield losses in high producing cows. Nevertheless, with an abundance of such weather parameters through modern technologies, these thermal indices have been used frequently to investigate the influence of HS on production traits whether in moderate (e.g. Hammami *et al.,* 2013) or in semi-arid (e.g. Milani *et al.,* 2015a, 2015b) climates.

Impact of heat stress on physiological parameters of AI sires

Artificial insemination (**AI**) is one of the most important reproduction biotechnologies allowing the collection of a large number of fertile and healthy spermatozoa from superior sires. Applicatons of AI contributed to an increased profitability of dairy cattle production. Understanding the relationship between semen quality of AI bulls and environmental climate is necessary to improve reproduction efficiency, also from a genetic improvement perspective. Exposure to a hot climate for a long time before puberty may affect the development of the reproductive system in growing calves (Brito *et al.*, 2012), followed by a decline in semen quality and quantity of these animals. In AI bulls, HS causes several undesired alterations in characteristics of semen quality and quantity, e.g. deterioration of spermatogenesis and epididymal maturation (Meyerhoeffer *et al.*, 1985), subsequently physical and morphological characteristics (Silva *et al.*, 2009), as well as ejaculation and mating behavior (Titto *et al.*, 2011). Additionally, thermal damage is a common cause of testicular degeneration and abnormal scrotal thermograms. Subsequently, high ambient temperatures cause deterioration in semen morphology by a decreasing percentage of the normal sperm and increasing the percentage of abnormal spermatozoa in the ejaculate. The unfavorable thermal stress effects on semen are not limited to the day of semen collection, they start about two weeks before ejaculation and do not return to normality up to eight weeks after the end of stress (Meyerhoeffer *et al.*, 1985). In this context, Fuerst-Waltl *et al.* (2006) illustrate that both periods, epididymal maturation (1-11 days before ejaculation) and spermatogenesis (days 12-65 before ejaculation), are critical intervals were strong effects of HS on semen characteristics could be identified. In AI sires, semen quality traits (=semen volume; **SV**) and semen quantity traits (=semen output; **SO**, semen motility; **SM**, number of semen doses per ejaculate; **NSD**, and semen concentration; **SC**) are described as the most important semen characteristics affected by environmental conditions (Fuerst-Waltl *et al.*, 2006; Teixeira *et al.*, 2011; Al-Kanaan *et al.*, 2013; Snoj *et al.*, 2013). However, the age of the bull, year and the season of semen collection, the intervals between semen collection dates, and the breed are some of the major factors affecting these semen traits (Fuerst-Waltl *et al.*, 2006; Al-Kanaan *et al.*, 2013).

Impact of heat stress on physiological parameters of dairy cows

When the environment evolves into harsh conditions, animals react with behavioral and physiological responses in order to maintain the thermal body balance. Behavioral responses to heat stress include an increase in shade seeking and water consumption, with a reduction in feed intake and lying time (Schütz *et al.*, 2014). Mammalian attempt to increase body heat loss by activating evaporation (by accelerating respiration and sweating rates), and enhancing body temperature as physiological responses to heat stress. Thus, and in addition to recording the animal health status, monitoring physiological attributes could provide reliable information about the degree of adaptability of livestock to their climatic environment.

Physiological traits such as rectal temperature (**RT**), skin temperature (**ST**), vaginal temperature (**VT**), respiration rate (**RR**), and pulse rate (**PR**) are widely used as indicators for HS in dairy cattle (e.g. Silanikove, 2000). These parameters are normally used as indices for HS in studies of feeding strategy and feeding efficiency (Umphrey *et al.*, 2001; Kargar *et al.*, 2015; Martello *et al.*, 2015). Brown-Brandl *et al.* (2005) confirmed that physiological parameters respond earlier to HS than production-related parameters (feed intake and feeding behavior) or behavior changes (shade usage). Respiration rate is considered as the most appropriate indicator to monitor thermal stress, i.e. RR is the most valuabe indicator among all physiological, production, and behavioral indicators. In another study, Ominski *et al.* (2002) used the increasing in VT and RR as indicators during the moderate HS to suggest that shifting feeding from morning to evening did not relieve the decline in milk yield and milk composition. Physiological traits (RT and RR) were also used as predictors of the decline in milk production traits and feed intake during moderate stress in multiparous US Holstein (Spiers *et al.*, 2004).

In studies concerning the requirement of shade, physiological traits are used as an indicator of the decline in production traits and metabolism (Van Laer *et al.*, 2015a). They show the cattle preference for shade in comparison to sprinklers (Schütz *et al.*, 2011) during summer HS in the pasture. In the same context, respiration rate and panting scores with behavioral traits were indicators for detecting the amount of shade required for lactating cows during hot summers in the temperate climate of New Zealand (Schütz *et al.*, 2014) and Belgium (Van Laer *et al.*, 2015b).

Regarding developped cooling systems, the undesired increasing in RT, ST and RR was used as HS indicator for the modification of evaporative tunnel cooling (Smith *et al.*, 2006), evaluation of conductive cooling (Ortiz *et al.*, 2015), and voluntary use of shower heads (Legrand *et al.*, 2011) in lactating US Holstein.

Recently, there has been a distinct interest in assessing the cows' physiological aspects with genome-wide association studies (Dikmen *et al.*, 2013; Dikmen *et al.*, 2014; Deb *et al.*, 2015;

Sajjanar *et al.,* 2015; Verma *et al.,* 2015). Genome-wide associations might contribute to a deeper understanding of thermotolerance in dairy cattle. Subsequently, application of genomic selection using the genetic variation of physiological traits during HS could help to improve cattle heat tolerance by detecting genes involved in physiological responses to HS (Dikmen *et al.,* 2013). I dentifications of specific HS single nucleotide polymorphism (**SNP**) (Dikmen *et al.,* 2013; Dikmen *et al.,* 2014) and specific HS biomarkers (i.e. heat shock protein; **HSP** family) (Deb *et al.,* 2015; Sajjanar *et al.,* 2015; Verma *et al.,* 2015) associated with physiological traits will lead to a better understanding of cellular thermotolerance, and the mechanism of HS effects on productivity of animals.

For improving heat tolerance of cattle during hot summers in the temperate climate of Germany, it might be interesting to study responses of local breeds adopted to harsh environments. The dual-purpose German black pied cattle (*Deutsches Schwarzbuntes Niederungsrind*; **DSN**) is one endangered breed in this regard. DSN cattle are the original founder breed of the modern German Holstein Friesian (**HF**; Brade and Brade, 2013). Physiological responses of this endangered-maintained breed might be new selection criteria, in order to achieve adaptation to hot conditions. As a small cattle breed (compared to HF), DSN cows produced low metabolic heat due to their low requirements for maintenance and low milk production (Jürgens *et al.,* 2015). Also, DSN cattle have the ability to digest low quality feed and convert it to appropriate milk (ranges between 5000 and 7000 kg/ year) with high quality milk composition (4.2 % fat and 3.5 % protein) (Brade and Brade, 2013). Due to this pasturing ability, breeders mostly prefer to keep DSN cows in pasture-based dairy systems, especially in organic farms (Jürgens *et al.,* 2015). On the other hand, little information is known about physiological traits of DSN and its adaptation level to the moderate climate, compared to the adaptability of HF, the more popular breed in German farms with its larger body size and higher milk production.

Infrared thermography (IRT)

Since cattle gain heat from the environment and dissipate most of the heat produced in body through the skin, skin is considered to be an important indicator for the heat load. Skin temperature is the result of the exchange of heat between body core and skin, which is normally regulated by blood flow (Collier *et al.,* 2006). Recently, measuring the surface body temperature by infrared thermography (**IRT**) has become more feasible through the technological advances. IRT is a method to visualize and analyze local and temporal changes in surface body temperatures. As a modern technology, IRT is used to capture thermophotography pictures of different parts of the cattle's body for detecting reproduction-related attributes (Talukder *et al.*, 2014; Talukder *et al.*, 2015) as well as a diagnostic tool for hoof (Oikonomou *et al.*, 2014; Alsaaod *et al.*, 2015) and udder (Polat *et al.*, 2010) diseases.

Infrared thermography is applied to study ST patterns and to predict heat production in dairy cattle, as summarized by Montanholi *et al.* (2008). These researchers also mentioned the potential use of IRT for assessing methane production by analysis of the temperature difference between left and right flanks. In a study by Ortiz *et al.* (2015), IRT was used to measure skin temperature in the rump, tail, head, and shoulder area in 6 multiparous Holstein dairy cows to identify heat load in lactating cattle. Martello *et al.* (2015) measured RT and RR with visualized ST by IRT from eight body areas to conclude that the frontal head is the best body part to identify associations with feed intake in Nellore cattle. Metzner *et al.* (2014) found a high relationship between RT and udder ST, and they put a framework for automated monitoring of mastitis and predicting udder health using IRT in dairy cows. In brief, infrared thermography may represent a promising non-invasive diagnostic technique as a direct indicator of heat stress by measuring the cattle's skin temperature.

Identification of THI thresholds for physiological traits of dairy cows and AI sires

The knowledge about a specific THI threshold (=the upper critical THI) is important for understanding the onset of HS and the level of adaptation in cattle. This information might help farmers to make a decision about the implementation of cooling systems and necessary changes of their management procedures. According to critical THI values, which are mostly adjusted to physiological body parameters, HS in cattle is categorized into different levels: no heat stress, moderate heat stress, severe heat stress, fatal heat stress, and death due to heat stress. Ranges of these thermal stress categories differ between breed, age and sex of cattle and according to production and reproduction status, as well as the adaptation level of the animal.

The THI threshold for milk production and reproduction traits was THI 72 for tropical and subtropical climates (Silanikove, 2000; Kadzere *et al.*, 2002; West, 2003). For countries located in hotter regions like deserts or arid climates, such THI thresholds were higher (Boonkum *et al.*, 2011). Nevertheless, new studies conducted in temperate climate emphasized that using THI less than 68 is a suitable threshold for cattle performance and welfare in the center of Europe (Gauly *et al.*, 2013). Recently, researchers have indicated that the low climatic adaptation and high environmental sensitivity of Holstein cows contributed to a lower THI threshold in German (Brügemann *et al.,* 2011; Brügemann *et al.,* 2012; Brügemann *et al.,* 2013), Belgian (Hammami *et al.,* 2013; Van Laer *et al.,* 2014; Hammami *et al.,* 2015), or even in Arizona-US (Zimbelman *et al.,* 2009).

For physiological traits of semen characteristics or physiological traits of lactating cows kept in German production systems, so far there was no identification of THI thresholds. Using THI thresholds identified in the tropical and subtropical countries might not be suitable for a moderate climate region (i.e. Germany), due to the low adaptation of European cattle (Gauly *et al.,* 2013). From this perspective, the importance of defining new proper heat stress thresholds for these physiological parameters, particularly for cattle bred in and adapted to the moderate climate of Germany, becomes evident. The new definition of physiological HS thresholds in German lactating cattle will support breeders and researchers to understand the effect of HS on cattle performance, because of the strong relationship between HS and such physiological factors.

Genetic components of heat stress

The genetic background is an important factor to infer antagonistic effects of heat stress on cattle vitality. In lactating cattle, the genetic components of heat stress have been extensively studied including production, reproduction, health and welfare traits (Aguilar *et al.,* 2009, 2010; Boonkum *et al.,* 2011; Brügemann *et al.,* 2011; Bohlouli *et al.,* 2013; Brügemann *et al.,* 2013; Hammami *et al.,* 2015). Moreover, genetic parameters of heat stress were also discussed for semen traits of bulls from US (Taylor *et al.,* 1985; Carabaño *et al.,* 2014), Austria (Gredler *et al.,* 2007) or Spain (Karoui *et al.,* 2011). However, there is a still need for more studies related to effects of HS on physiological cow traits or semen traits in German cattle.

Misztal (1999) proposed the use of a model fitting a comfort zone to study the genetic component of HS with no effect of temperature on production until a certain level of THI, and then the production linearly declines above that level. This model assumes that selection of heat tolerant animals is possible, if the variation in the slopes contains large genetic additive components. Therefore, if the additive genetic variation for HS is substantial enough, then the genetic improvement of cattle could become the best option to overcome HS as an alternative to change cooling, shading, nutrition, and management strategies. Ravagnolo and Misztal (2000) applied this model to confirm the possibility of selection for HS when they found that the additive genetic variance above the THI threshold was similar to the additive variance below the threshold for milk production traits. Bohmanova *et al.* (2005) found that Holstein bulls with higher heat tolerances had daughters with lower milk yield, but those cows had higher milk contents and udder health, longer longevity and higher daughter conception rates.

Ravagnolo and Misztal (2000) mentioned that mating high yielding Holstein with breeds of high heat tolerance will have little success to alleviate HS because crossbreeds usually produce less milk than Holsteins. Hence, the best choice is an estimation of genetic components of heat resistance and selection of animals within a breed for higher heat tolerance. Moreover, due to the small genetic correlation between physiological and productive parameters (Ravagnolo and Misztal 2000), a combined selection for both is possible.

Random regression methodology (RRM)

The application of random regression models (**RRM**) allows to study animal traits on phenotypic and genetic scales in dependency of continuous environmental descriptors. RRM is a useful method to evaluate the impact of environmental parameters that have continuous distributions (e.g. ambient temperature and relative humidity). Therefore, applying RRM to study the phenotypic and genetic trajectory of physiological traits in dependency of the continuous environmental THI is an alternative to distinct environmental classes combined with multiple trait models. The advantage of RRM compared to the fixed regression model is the following: the random regression curve can be modelled within each random effect and therefore allows the estimation of heritabilities, repeatabilities, and genetic variances for specific THI. Studies addressing the genetic component of heat tolerance identified RRM superior over repeatability models, especially when aiming to analyze the dynamics of HS (Ravagnolo and Misztal, 2000; Ravagnolo *et al.,* 2000). Recently, more investigations for analysing phenotypic (Brügemann *et al.,* 2012; Hammami *et al.,* 2013) and genotypic (Brügemann *et al.*, 2011, 2013; Bernabucci *et al.*, 2014; Hammami *et al.*, 2015) parameters of production and reproduction (Brügemann *et al.*, 2013) traits used RRM to identify THI thresholds and genotype by environmental interaction (**GxE**) in dairy cattle.

Objectives of the thesis

Studies on THI as an indicator of heat stress and its effects on physiological traits of dairy cows and AI bulls under the moderate climatic conditions of Europe are limited: a limited number of studies and designed specific experiments; with a limited number of animals, without specifically focusing on the direct impact of climate on farm animals.

The objective of this thesis is to determine the effect of heat stress on physiological traits of cows and AI bulls in Germany. Towards this aim, physiological characteristics of semen and lactating cows were analyzed on a phenotypic and genotypic continuous THI scale. Further, comfort zone and THI threshold has to be identified and genetic parameters estimated for these physiological traits, with investigation of GxE interaction.

The thesis contains *five chapters* in the style of a cumulative thesis with three papers:

The first chapter addresses the general introduction of the thesis. This section includes literature reviews of the general impact of heat stress on cattle performance, focusing on the impact of heat stress on physiological traits of cows and semen traits of AI bulls.

In *Chapter 2,* physiological traits of bulls represented by semen characteristics were analyzed with longitudinal data of Holstein sires kept on an AI center located in the north-western part of Germany. Semen characteristics contain traits of semen quality (=SV) and semen quantity (=SO, SM, NSD, and SC). In the statistical model, the vector of fixed effects includes: age of the bull, year-season of semen collection, and intervals between semen collection dates. The impact of heat stress was analyzed for different intervals previous to semen collection date (up to 65 days before ejaculation) focusing on the period of epididymal maturation and the period of spermatogenesis. Phenotypically, the aim of this study was to assess the impact of HS on semen traits in dependency of the continuous THI scale using regression models with third-order Legendre polynomials. A further task was the identification of the upper THI threshold and the optimal THI range for physiological semen traits in the moderate climate of Germany. On a quantitative genetic scale, the linear RRM was applied to determine the effects of HS on genetic parameters in the course of THI, and to identify the existence of GxE interaction.

In *Chapter 3,* physiological traits of lactating cows were analyzed on the phenotypic scale in dependency of the continuous environmental heat stress descriptor THI. During the years 2012, 2013, and 2014, physiological cows traits (=RT, ST, VT, RR, and PR) were measured using 83 Holstein and 155 dual purpose DSN milking cows in two farms located in northern Germany. Meteorological data were calculated inside barns, exactly at the time when measuring physiological traits. In the statistical model, the fixed effects were: classes for milk yield and milk composition (fat %, protein %, fat to protein ratio, milk urea nitrogen, and somatic cell counts), days in milk, parity, season and year of measurements, and the individual cow's heat stress sensitivity. Random regression models were applied, with a regression for the coverable THI using third-order Legendre polynomials nested within fixed effect groups. This study contains three main objectives: 1- to evaluate the potential impact of HS on physiological traits of dairy and dual purpose lactating cows; 2- to identify the THI threshold and the optimal THI range of physiological traits in German cattle; 3- to determine the effect of different levels of fixed effects on physiological cows traits.

In *Chapter 4*, physiological traits of milking cows were analyzed on a genetic scale, and again using the continuous environmental descriptor THI. Physiological parameters were recorded in two herds of DSN and their genetically upgraded crossed with Holstein-Friesian sires. The objective of this study was to explore genetic (co)variance components of physiological traits considering THI recorded inside the barn. Moreover, the concept of GxE interactions, and suggestions for physiological data recording (i.e., defining the optimal longitudinal data structure) were major subject of this chapter.

Chapter 5 represents the general discussion of the thesis. This chapter address the relationship between HS and physiological cow and bull traits, beyond the topics covered in chapters 2, 3, and 4. Furthermore, additional results from these studies are shown and discussed in the context of literature published recently on this topic. At the end of this section, some suggestions for future HS studies are made. Finally, specific suggestions to reduce HS in dairy cattle, also from a management perspectice, are considered.

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Chapter 2

Effects of heat stress on semen characteristics of Holstein bulls estimated on a continuous phenotypic and genetic scale

A. Al-Kanaan[*,§], S. König[*] and K. Brügemann[*]

[*] Department of Animal Breeding, University of Kassel, 37213 Witzenhausen, Germany

[§] Department of Animal Production, College of Agriculture, University of Basrah, Basrah, Iraq

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ABSTRACT

Semen volume (SV), semen output (SO), semen motility (SM), number of semen doses per ejaculate (NSD) and semen concentration (SC) were analyzed on a phenotypic and quantitative genetic scale in dependency of the continuous environmental descriptor THI (= an index describing combinations of temperature with humidity). Longitudinal semen data included 10,341 observations from 562 Holstein sires kept on an AI station in the northwestern part of Germany. Statistical modeling was based on fixed and random regression methodology (RRM). In this context, the phenotypic and genetic trajectory of traits in dependency of THI was modeled by orthogonal polynomials, i.e. Legendre polynomials of order 3. A general heat stress threshold of THI = 60 was identified, and indicating detrimental effects of heat stress beyond this threshold, especially on SV, SO, NSD, and SC. Least square means for semen productivity were generally higher for adult bulls, but compared to young bulls, adult bulls responded with stronger semen production losses for extremely high THI. The optimal environment for semen production was a THI in the range from 50 to 60. Detrimental impact of heat stress was reduced for bulls with longer intervals between consecutive semen collection dates. Heritabilities for semen traits were in a low to moderate range with the following maximal values: 0.18 for SV at THI 30, 0.29 for SM at THI 70, 0.26 for SO at THI 30, 0.26 for NSD at THI 58, and 0.27 for SC at THI 53. Genetic values of sires and additive genetic variances changed with alterations of THI, with highest genetic variances at the extreme ends of the continuous environmental descriptor. Genetic correlations in same traits from THI levels in great distance were lower than 0.80, and indicated genotype by environment interactions. Application of RRM allow to select sires according to THI specific breeding values, which implies an optimization of cow sire and bull sire selection for harsh environments.

Key Words: Heat stress, longitudinal semen traits, genetic parameters

1. INTRODUCTION

Artificial insemination (AI) is one of the most important reproduction biotechnologies in dairy cattle. Artificial insemination enabled a rapid dissemination of superior genetic material across country borders, and boosted selection response in production and, more recently, also in functional traits. Maximizing genetic gain for the overall breeding goal implies widespread use of outstanding sires from national and international genetic evaluations (König *et al.*, 2007). Reasons for sub-optimal utilization of outstanding sires include deficiencies of semen production, or even male infertility due to environmental or genetic impact. The physiological and genetics background for male infertility was described in detail in humans (Carell, 2007), and infertility was also reported for influential bulls kept on AI stations.

Artificial insemination with fresh and frozen semen plays a role of increasing importance in countries with tropical or subtropical climate located in Asia, Africa, or South America (Thibier and Wagner, 2002). The detrimental impact of heat stress in terms of high temperatures and humidities, or a combination of both (temperature x humidity index (THI)), on cattle physiology was summarized by Hoffmann (2010). Physiological alterations included male and female fertility traits, and also the risk of mortality. For female fertility traits, Brügemann *et al.* (2012) focused on temperate zones in middle Europe and identified environmental sensitivity especially for dairy cows with highest production levels and highest genetic values for test-day milk yield. Consequences of adaptation to harsh environments (= high THI), and addressing male and female pathways of selection, were evaluated by stochastic simulations on the basis of a longitudinal data structure (Yin *et al.*, 2014).

From a phenotypic perspective, most studies designed specific experiments to assess the impact of heat stress on male fertility traits. Detrimental effects included reduced sperm counts and the phenomenon of "summer sterility", i.e. the depression of sexual activity due to heat stress (Setchell, 1998); semen deterioration based on in-vitro experiments for bulls (Rahman *et al.*, 2013a) and rams (Malama *et al.*, 2013); decreased fertilization and embryo development (Rahman *et al.*, 2013a; Rahman *et al.*, 2013b); high percentages of abnormal sperms (Al-Makhzoomi *et al.*, 2008; Silva *et al.*, 2009); as well as high saturated fatty acids, low polyunsaturated fatty acids and low cholesterol concentrations in semen (Argov-Argaman *et al.*, 2013). Direct climatic heat stress revealed undesired effects on semen ejaculate volume and on semen concentration (Teixeira *et al.*, 2011; Snoj *et al.*, 2013).

From a genetics perspective, heritabilities for semen quantity and semen quality traits were estimated in different countries, but without specifically focusing on climatic descriptors. Across country and across breed comparisons reviewed in Table 1 illustrate a broad range of heritability estimates for the following semen traits: volume, motility, output, number of doses per ejaculate and concentration.

Table 1. Literature overview of heritabilities for the following semen traits of AI sires: Semen volume (SV), semen motility (SM), semen output (SO), number of semen doses per ejaculate (NSD), and semen concentration (SC).

Author	Number of records / bulls	Region	Breed	Heritability				
				SV	SM	SO	NSD	SC
Knights *et al.* (1984)	717 bulls	Anita, Iowa, USA	Angus		0.13	0.24		0.13
Taylor *et al.* (1985)	149,339 records	Ohio ,USA	Holstein	0.16-0.18		0.03-0.05		0.10-0.16
Stålhammar *et al.* (1989)	215 bulls	Sweden	Swedish R&W + Swedish Friesian	0.2	0.18		0.17	
Ducrocq and Humblot (1995)	2,387 records	L'Aigle, France	Normande	0.65			0.23	0.37
Diarra *et al.* (1997)	294 bulls	Québec, Canada	Holstein	0.53	0.51			0.37
Mathevon *et al.* (1998a)		Guelph, Canada	Holstein	0.24-0.44	0.01-0.31	0.38-0.54		0.36-0.52
Mathevon*et al.* (1998b)	16,242 records	Ceyzeriat, France	Montbéliard	0.08-0.49		0.12-0.15		0.08-0.32
Gredler *et al.* (2007)	12,746 records	Austria	Simmental	0.18	0.04	0.22		0.14
Kealey *et al.* (2006)	841 bulls	Montana, USA	Hereford	0.09	0.22			0.21
Carabaño *et al.* (2007)	8,773 records	Madrid, Spain	Holstein	0.23-0.36				
Druet *et al.* (2009)	2,131 records	Jouy-en-Josas, France	Holstein	0.22	0.43	0.09		0.19
Karoui *et al.* (2011)	42,348 records	Aberekin, Spain	Holstein	0.22	0.16	0.18		0.19
Silveira *et al.* (2012)	5,903 bulls	São Paulo, Brazil	Nellore		0.11			
Siqueira *et al.* (2012)	21,186 bulls	São Paulo & Mato Grosso, Brazil	Nellore		0.08-0.18			

The aim of the present study was to extend heat stress analyses by considering longitudinal data for semen quantity and quality traits via random regression methodology (RRM). Applications of RRM allow to infer genetic parameters and to study the phenotypic trajectory of male fertility traits as a function of continuous environmental descriptors. Brügemann *et al.* (2013) used THI as continuous environmental descriptor and applied RRM to longitudinal female fertility data. Following their results in cows, we hypothesize environmental sensitivity for semen traits of AI sires, i.e. the identification of heat stress thresholds, alterations of additive-genetic variances and heritabilities with increasing THI, and indications for genotype by environment interactions.

2. MATERIALS AND METHODS

2.1 Data

Semen quality and quantity traits in the present study were: semen volume (SV, in ml), semen output (SO, in no. of sperm), semen motility (SM, in %), number of semen doses per ejaculate (NSD) and semen concentration (SC, in no. of sperm per ml ejaculate). Data included 10,341 observations for all traits from 562 Holstein Friesian sires kept on one AI station in the federal state of Lower Saxony in the north-western part of Germany. Traits were recorded in the years 2009, 2010, and 2011 from 10 to 136 months old bulls. Semen volume is the quantity of a bull's ejaculation as measured in a scaled tube. Semen concentration was determined in the laboratory of the AI center using a NucleoCounter®. Semen output is the product of SV with SC and reflects the no. of sperm per ejaculation. Semen motility (percentage of motile sperm in relation to all sperm) was visually analyzed with a microscope by trained technicians. Number of semen doses strongly depends on SO, i.e. a single semen doses requires 15 millions of motile sperm. Descriptive statistics of semen traits is summarized in Table 2.

Table 2. Descriptive statistics for semen traits of AI sires as used in the present study.

Semen trait	Unit	Mean	SD	Min	Max
Semen volume (SV)	[ml]	5.97	2.18	0.5	17
Semen motility (SM)	[%]	69.45	2.71	65	75
Semen output (SO)[1]	[no. of sperm]	7.89	4.02	0.33	30.80
No. of semen doses (NSD)	[count variable]	449.53	276.24	3	2008
Semen concentration (SC)[1]	[no. of sperm/ml ejaculate]	1.33	0.46	0.09	3.00

[1] x 10^9

Meteorological data were daily average measurements for ambient temperature (in °C) and relative humidity (in %) from two weather stations at a distance of 24 and 39 km from the AI station. Daily average THI was calculated by applying the formula of the National Research Council (1971):

$$THI = (1.8 * T\,°C + 32) – [(0.55 - 0.0055 * RH\,\%) * (1.8 * T\,°C - 26)]$$

where T °C = temperature of air measured by a thermometer and RH% = relative humidity.

Maximal daily THI (69.64) and minimal daily THI (30.29) were realized in June 2010 and in December 2010, respectively. Highest monthly THI indicating periods of heat stress in dairy cattle (THI > 60) were observed in June 2010 and 2011, and in July and August for all three years (Figure 1).

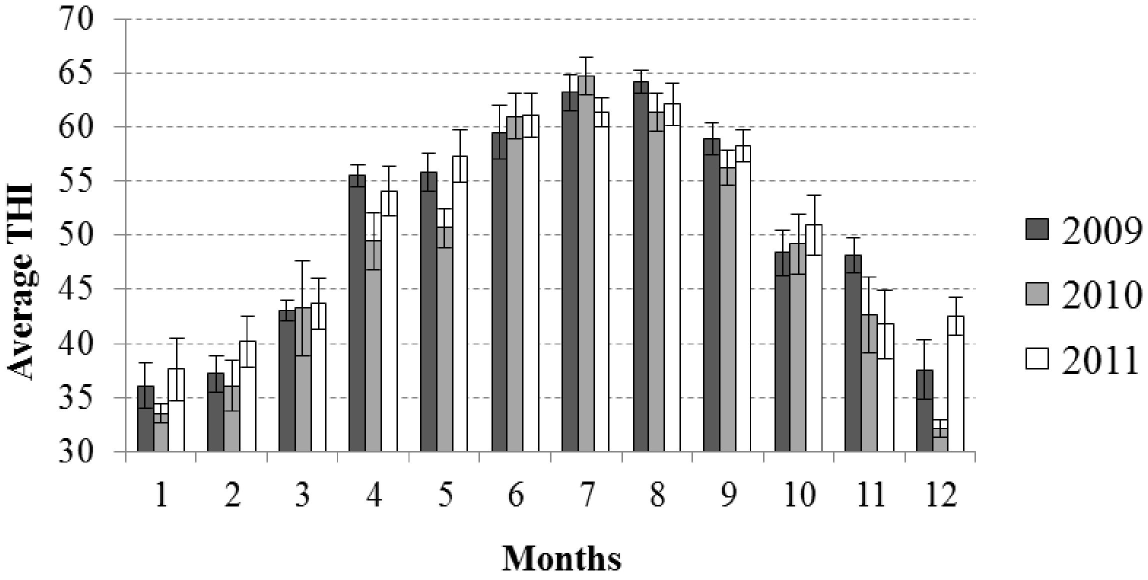

Figure 1. Average monthly temperature-humidity indices (THI) for the years 2009, 2010, and 2011. (Stripes in the bars indicate daily minima and maxima for THI).

2.2 Statistical analysis

2.2.1 Analyses on the phenotypic scale

A fixed regression model was used to study alterations of semen traits by THI on the phenotypic scale. For this purpose, a linear mixed model as implemented in the SAS 9.2 procedure PROC MIXED (SAS Institute, Cary, NC) was applied. The statistical model 1 in matrix notation used for analysis of variance (test of significance of fixed effects based on sum of squares (SS), type 3) was defined as follows:

$$\mathbf{y} = \mathbf{X\text{ß}} + \mathbf{Zu} + \mathbf{e} \qquad \textbf{[1]}$$

where **y** = vector of observations for semen traits, **ß** = vector of fixed effects including age of sire (age groups: <12, 12-18, 18-48 and > 48 months), year-season of semen collection (years: 2009, 2010 and 2011; seasons: spring = March-May, summer = June-August, autumn = September-November, and winter = December-February), the intervals between consecutive semen collection dates (intervals: < 3 days and ≥ 3 days), and regressions for the covariate THI from the ejaculation date (regressions were modelled using Legendre polynomials) nested within age classes of bulls, **u** = vector of random bull effects, and **X** and **Z** are the associated incidence matrices, respectively. Model evaluation for regression specification was based on AIC-values and BIC-values for models with either Legendre polynomials of order 3 and of order 4 in consecutive runs. Based on previous experiences with longitudinal THI simulation studies (Yin *et al.*, 2014), and based on smaller AIC- and BIC values, we chose Legendre polynomials of order 3 for the final modeling of THI regressions.

For the analyses of the physiological background of semen traits in detail, not only THI measured at the ejaculation dates (= semen collection dates) were considered. In different runs, we analyzed the impact of THI from dates previous to semen collection date on semen traits. Fuerst-Waltl *et al.* (2006) identified that the period of epididymal maturation (1-11 days before semen collection) and the period of spermatogenesis (days 12-65 before semen collection) are important intervals when analyzing semen traits. Hence, average THI from the following intervals was used as continuous environmental descriptor: Interval I = 1-11 days, interval II = 12-65 days, interval III = 22-28 days, and interval IV = 29-35 days before the ejaculation date.

2.2.2 Analyses on the genetic scale

For genetic analyses, a linear RRM as used by Brügemann *et al.* (2012) for heat stress studies of female fertility traits, and considering the full relationship matrix among animals, was specified. Inclusion of random additive-genetic effects of bulls via the genetic relationship matrix allow to separate random bull effects into genetic and permanent environmental effects. In matrix notation, the RRM (model 2) was:

$$\mathbf{y} = \mathbf{X\ß} + \mathbf{Za} + \mathbf{Wpe} + \mathbf{e}\ \mathbf{[2]}$$

where **y** = vector of observations for semen traits, **ß** = vector of fixed effects and regressions as specified for model 1, **a** = vector of additive genetic effects for random regression coefficients on THI using third-order Legendre polynomials, **pe** = vector of random permanent environmental effects using third-order Legendre polynomials, **e** = vector of random residual effects, and **X**, **Z** and **W** are the associated incidence matrices, respectively.

The (co)variance structure of random effects was defined as:

$$\mathrm{Var}\begin{bmatrix}\mathbf{a}\\ \mathbf{pe}\\ \mathbf{e}\end{bmatrix} = \begin{bmatrix}\mathbf{G}\otimes\mathbf{A} & \mathbf{0} & \mathbf{0}\\ \mathbf{0} & \mathbf{P}\otimes\mathbf{I_{pe}} & \mathbf{0}\\ \mathbf{0} & \mathbf{0} & \mathbf{R}\otimes\mathbf{I_{n}}\end{bmatrix}$$

where **G** = additive genetic (co)variance matrix of random regression coefficients, **A** = additive genetic relationship matrix considering genetic relationships traced back to base animals born in 1924, **P** = permanent environmental variance matrix of random regression coefficients, $\mathbf{I_{pe}}$= identity matrix for 562 bulls, **R** = residual variance matrix, $\mathbf{I_n}$ = identity matrix for *n* observations, and ⊗ = Kronecker product.

Genetic statistical analyses were done in a Bayesian framework using the THRGIBBS1F90 software-package (Tsuruta and Misztal, 2006). In total, 100,000 Gibbs samples were generated, whereof 10,000 samples were used for the “burn-in period”, and 90,000 samples were used to calculate posterior statistics. Assessment of the length of the burn-in sample and of the sampling period was based on visual inspections of (co)variances for genetic effects, and is illustrated for the additive genetic variances of SC at THI 50 (Figure 2).

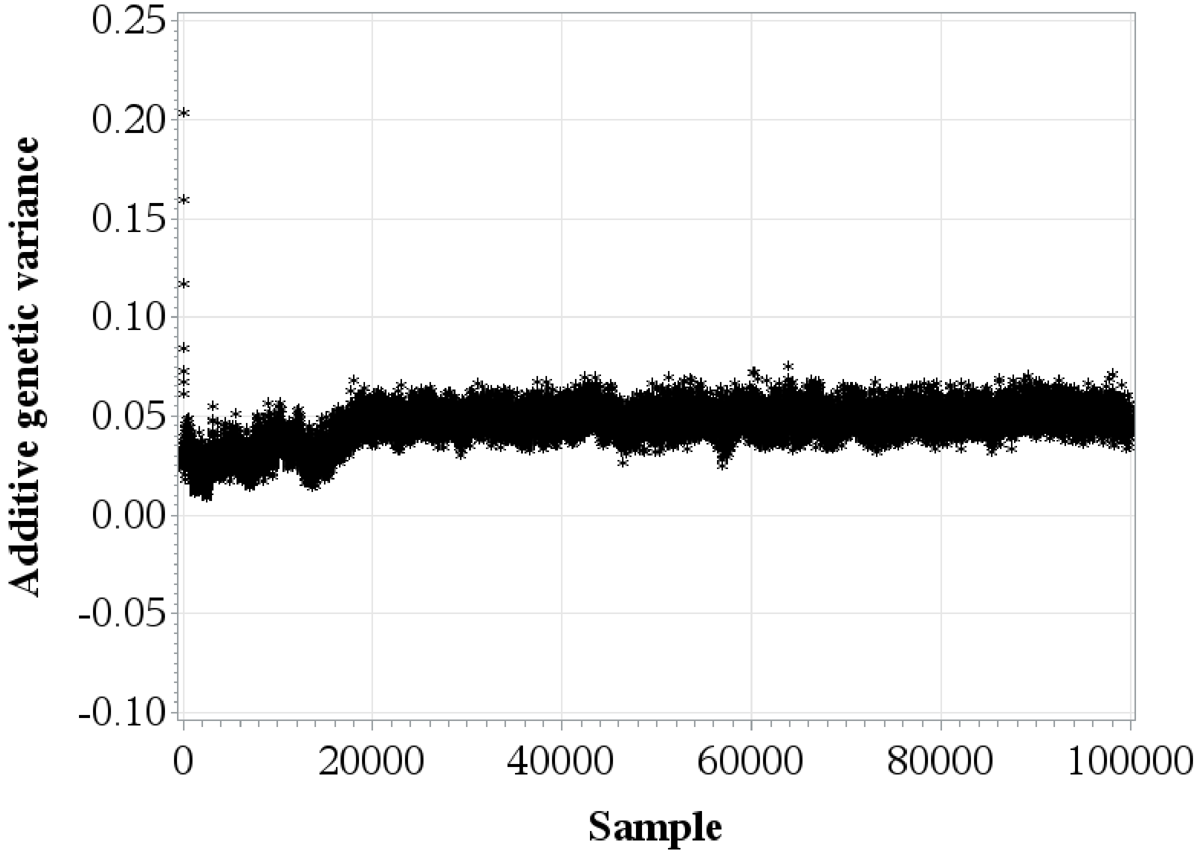

Figure 2. Genetic variances for semen concentration at THI 50 for the different rounds of iterations (samples).

3. RESULTS AND DISCUSSION

Results from the fixed effects model (1) were used to stretch the topic of environmental sensitivity for bulls kept on AI stations (section 3.1: Environmental impact on semen traits). This topic of environmental sensitivity includes comparisons of least square means for semen traits within classes of effects for ejaculation seasons and ejaculation years. Simultaneously, the effects of THI (continuous environmental descriptor) from the ejaculation date on semen traits was studied, and allowing the identification of so called heat stress thresholds. Heat stress thresholds indicate a THI value associated with an obvious increase or decrease of semen traits. The THI threshold in the present study was identified at THI 60 for all semen traits, and indicating detrimental effects on SV, SO, NSD, and SC. Only SM slightly increased with increasing THI. Significant effects *($P < 0.05$)* of years and of seasons reflect the impact of feeding and of husbandry, and of daylight changes on semen traits. Furthermore, environmental impact includes the management of semen collection as practiced on AI stations. A close interval between consecutive ejaculation dates (< 3 days) was associated with impaired male fertility, and might be seen as an additional stress component for AI bulls.

Model (1) was used in ongoing runs to study the THI effect from dates previous to the ejaculation date on semen traits. Significant THI effects were identified for THI from 1 to 11 days previous to the ejaculation date, and from 29 to 35 days before the ejaculation date. These results underline that heat stress during the periods of epididymal maturation (1-11 days before semen collection) and of spermatogenesis (days 12-65 before semen collection) negatively effects semen quantity and quality.

Model (2) additionally includes the additive-genetic component via the full relationship matrix among animals, and allowing the estimation of heritabilities for semen traits for specific THI (section 3.2: Genetic impact on semen traits). We identified alterations of heritabilities for semen traits with alterations of THI. Model (2) also allows to prove the existence of genotype by environment interactions. A genetic correlation lower than 0.80 in same traits measured at different THI indicate genotype by environment interactions, and was identified for THI in great distance (e.g. SV measured at THI 30 = trait 1 with SV measured at day 60 = trait 2).

3.1 Environmental impact on semen traits

3.1.1 Temperature-Humidity index (THI)

Analyses of variance (Type 3 test of fixed effects) revealed highly significant impact ($P < 0.001$) of THI from the ejaculation date modeled with Legendre polynomials of order 3 on SV, SO, and NSD. Significant impact of THI at the ejaculation date ($P < 0.05$) was identified for SM and SC. Least square means from model (1) as a function of THI, and stratified by age classes of bulls, are depicted on the phenotypic scale for SV (Figure 3), SM (Figure 4), SO (Figure 5), NSD (Figure 6), and SC (Figure 7) . Semen traits were quite constant or only showed minor fluctuations for THI < 60. Such environmental comfort zones for dairy cattle were described by e.g. Aceves *et al.* (1987). Detrimental impact of heat stress on semen productivity and semen quality was identified for THI > 60, and indicating decreasing SV, SO, NSD and SC beyond this upper critical "THI threshold". The identified upper THI threshold for semen quality and semen quantity traits from Holstein bulls is in agreement with results from Holstein cows located in a temperate climatic zone in the northern part of

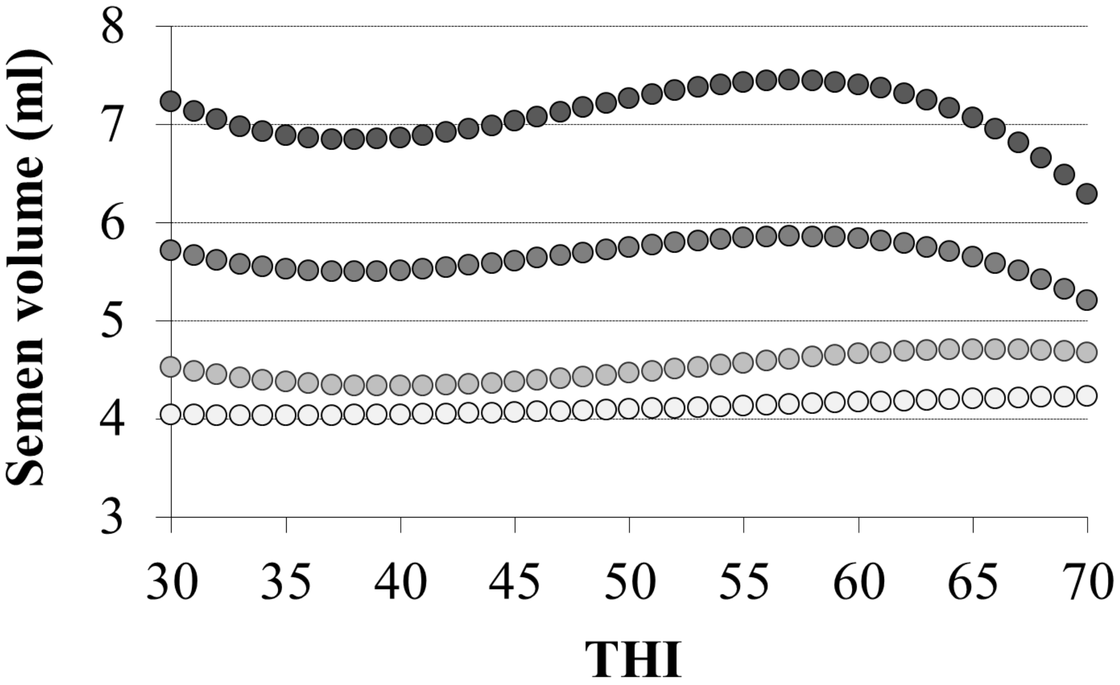

Figure 3. Least squares means for semen volume (SV) of AI sires by THI measured at the ejaculation date for different age classes of bulls. (Age classes of bulls: From light to dark grey: <12 months, 12-18 months, 18-48 months and > 48 months).

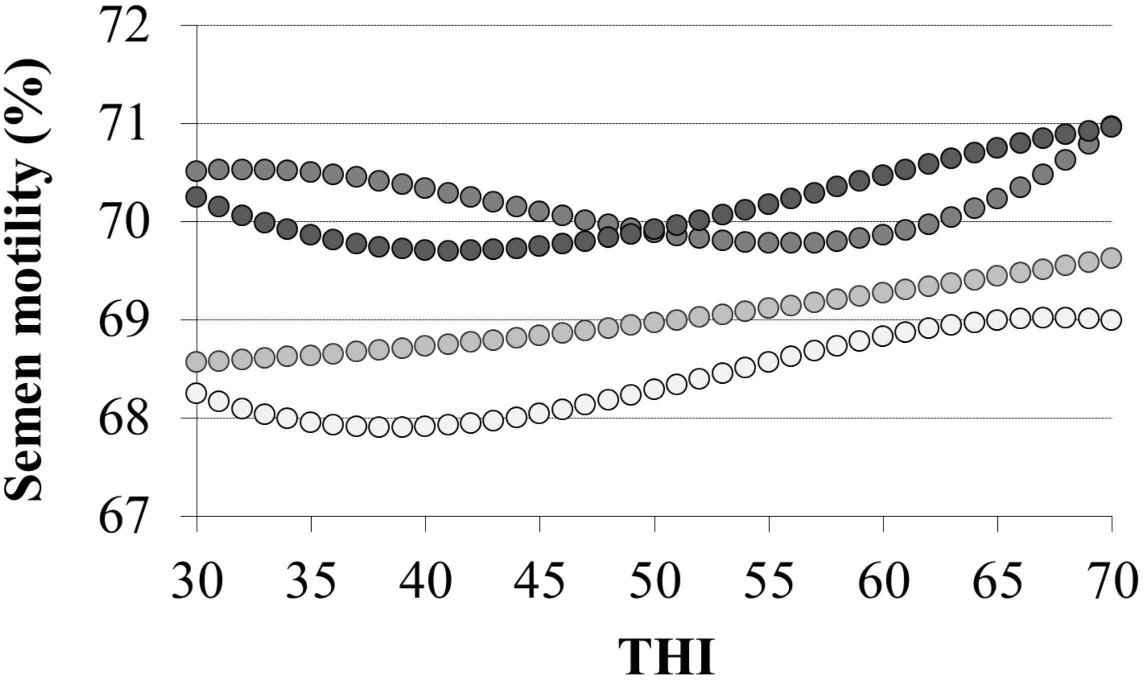

Figure 4. Least squares means for semen motility (SM) of AI sires by THI measured at the ejaculation date for different age classes of bulls. (Age classes of bulls: From light to dark grey: <12 months, 12-18 months, 18-48 months and > 48 months).

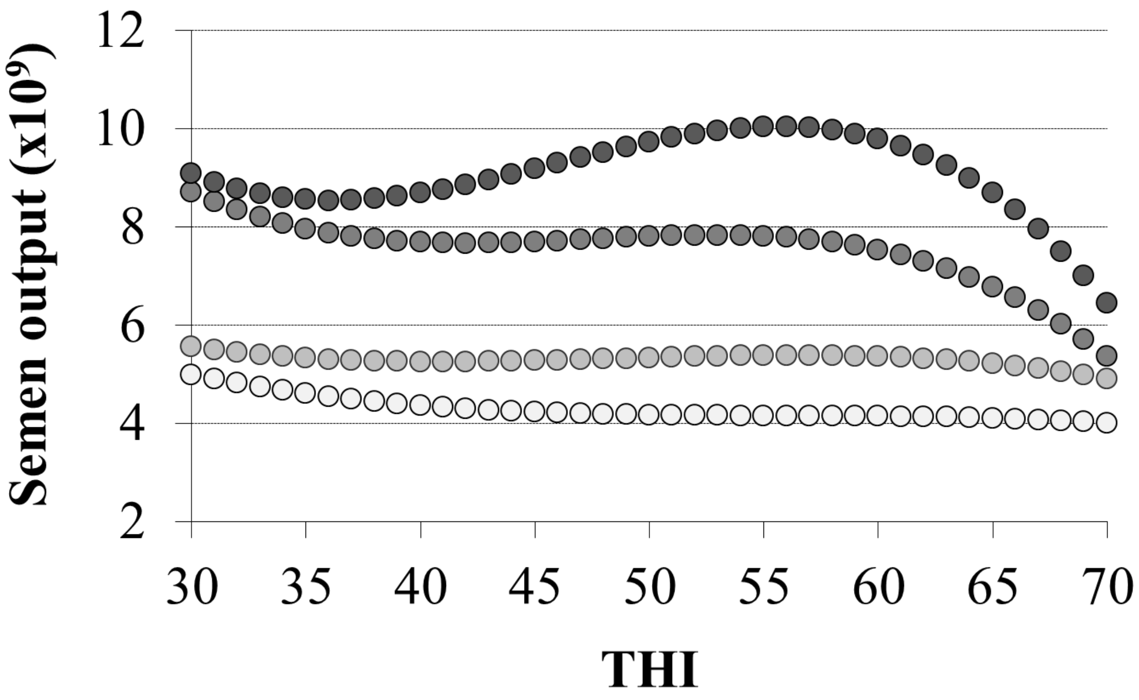

Figure 5. Least squares means for semen output (SO) of AI sires by THI measured at the ejaculation date for different age classes of bulls. (Age classes of bulls: From light to dark grey: <12 months, 12-18 months, 18-48 months and > 48 months).

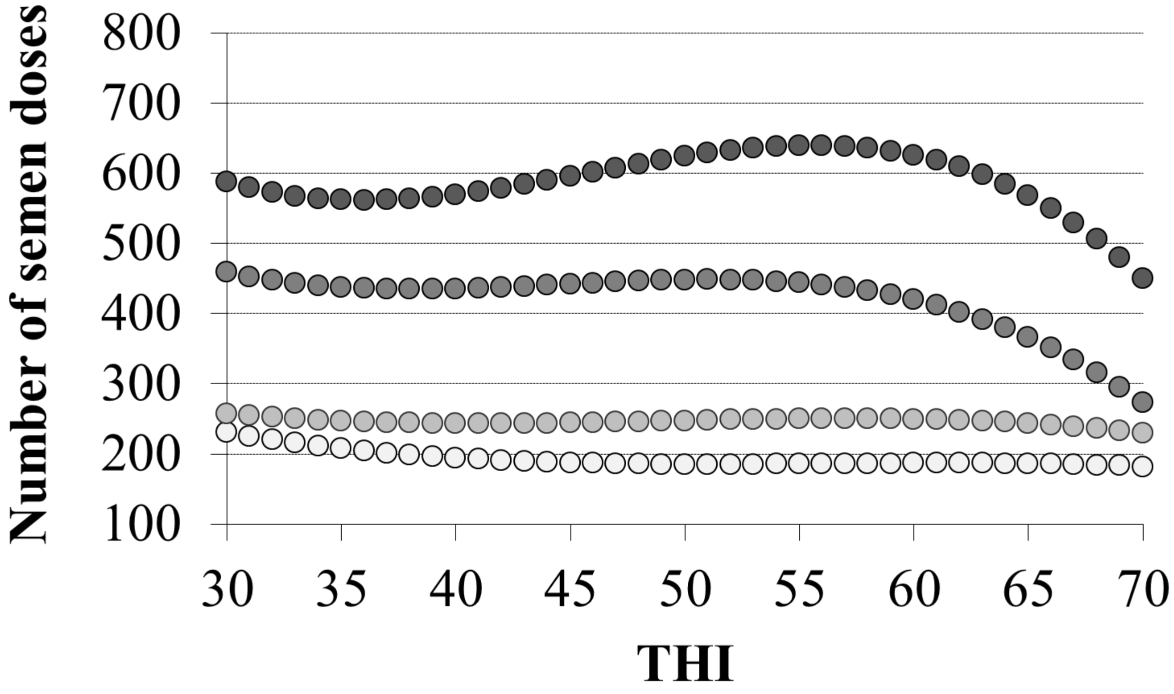

Figure 6. Least squares means for number of semen doses per ejaculate (NSD) of AI sires by THI measured at the ejaculation date for different age classes of bulls. (Age classes of bulls: From light to dark gray: <12 months, 12-18 months, 18-48 months and > 48 monthes.

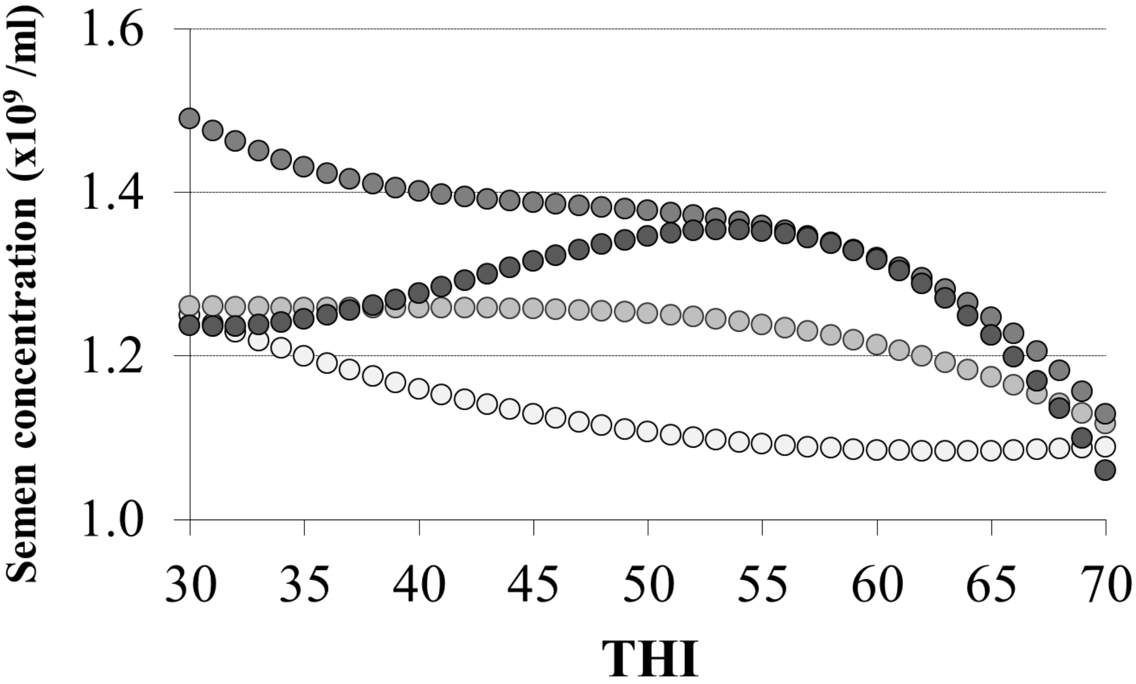

Figure 7. Least squares means for semen concentration (SC) of AI sires by THI measured at the ejaculation date for different age classes of bulls. (Age classes of bulls: From light to dark grey: <12 months, 12-18 months, 18-48 months and > 48 months).

Germany for test-day protein yield (Brügemann *et al.*, 2011), and for female fertility traits (Brügemann *et al.*, 2012). Only SM steadily increased for THI > 60. However, apart from 18 to 48 months old bulls, increase was small (on average lower than 1% between THI 60 and THI 70), and within the range of standard errors (SE) for SM at the extreme end of the THI scale. Due to the reduced dataset for extremely low and extremely high THI, SE of least square means were larger at the extreme ends of the environmental scale. In the case of SM, SE was 0.11% at THI 30, decreased to 0.03% at THI 50, and again increased to 0.08% at THI 70. Gredler *et al.* (2007) found a slightly negative phenotypic correlation between SM and SV indicating an antagonistic association between both traits for the whole dataset of dual-purpose Simmental bulls on the phenotypic scale. Following the results from the present study (comparison of Figure 3 with Figure 4), a decrease of SV and an associated increase of SM was most obvious under heat stress conditions for adult bulls. This finding was partly verified when using THI measurements in greater distance from the ejaculation date (Suppl. Figure S1).

For SV, SO, and NSD, we identified an optimal THI range for semen production, i.e. THI 50 to THI 60. This range of optimal THI is in agreement with results for Simmental bulls kept in moderate climates of Austria (Fuerst-Waltl *et al.*, 2006). These authors suggested an optimal ambient temperature for semen production ranging between 5 and 15 ºC. The optimal ambient temperature for semen production of AI bulls kept in hot environments of the US was higher (15-21ºC, Parkinson 1987; Taylor *et al.*, 1985), and indicating adaptation to the production environment. An impressive example for adaptation is given by Farooq *et al.* (2013). They found that physical and biochemical semen parameters in Pakistani Zebu bulls adapted to hot environments were in a desired range only during hot seasons. In contrast, detrimental effects of heat stress on semen traits in dairy and beef cattle bulls from commercial populations were most obvious under heat stress scenarios. Such findings were described by Boujenane and Boussaq (2013) for semen production of Holstein bulls housed in Morocco during the hot season, or by Meyerhoeffer *et al.* (1985) for decreasing percentages of motile and normal spermatozoa of Angus bulls. Biological explanations were given by Setchell (1998), e.g. physiological limits for the testicular temperature because of increasing oxygen demand.

Critical heat stress periods also included intervals I (interval I = THI calculated from 1- 11 days before the semen collection date) and IV (interval IV = THI calculated from 29 - 35 days before the semen collection date). Especially for adult bulls, pattern of solutions for semen traits in dependency of THI in the period previous to the ejaculation date reflect result from THI measured at the ejaculation date (Suppl. Figure S1). Undesired impact of heat stress measured before the ejaculation date on semen production is in agreement with result by Fuerst-Waltl *et al.* (2006) for dual-purpose cattle. These authors found undesired effects of high ambient temperature during epididymal maturation, and during spermatogenesis. These authors referred to Dorst (1991), who reported that spermatogenesis and epididymal maturation include a time period of 65 days. Time lagged detrimental heat stress impact of ~2 weeks on semen quality was identified by Meyerhoeffer *et al.* (1985) for AI sires in the US. In the study by Vogler *et al.* (1993), spermatogenic processes were more sensitive to heat stress compared to sperm maturation in the epididymis.

3.1.2 Year-season of semen production

Statistical modeling (model 1) simultaneously considered effects of year-season and of THI. Seasonal effects are strongly confounded with temperature and humidity, but additional seasonal characteristics include animal associated components (e.g. changing sexual activity of mammals during the year (Swanson and Herman, 1944)), as well as further environmental descriptors (e.g. length of daylight, fodder components). Despite consideration of THI, the effect of year-season was highly significant ($P < 0.0001$) for all semen traits. When comparing solutions from different seasons for the years 2009, 2010, and 2011, SV and SM were throughout highest in 2010 (Table 3). Such a strong effect of the year might be due the quality of the feeding ratio, which especially depends on the quality of the first cut of grass silage. Grass silage is the major component of the feeding ration of bulls which are kept indoors on AI station throughout the whole year.

Solutions for seasonal effects in our study revealed inconsistencies in different years. This might be due to our statistical modeling by including both environmental effects season and THI, whereas most of the previous "heat stress studies" solely analyzed seasonal impact on semen traits. Provided that bull management and feeding rations are quite constant throughout the year, seasonal impact is considerably explained by variations of temperature and humidity. Further seasonal impact on semen traits uncoupled from THI include changes of daylight. In our study, "Autumn-2011" was identified as a year-season combination with highest values for SO and NSD. Lowest values for SO and NSD in "Autumn-2009" indicate a pronounced effect of the year. During summer and spring seasons 2009 and 2010, SO and NSD were higher compared to semen production levels as achieved in autumn and winter. Despite the detrimental effects of temperature and humidity on semen characteristics, a multiplicity of studies (van Os *et al.*, 1997; Nichi *et al.*, 2006; Teixeira *et al.*, 2011; Chacur *et al.*, 2013) also reported highest ejaculate volumes of bulls during the summer months.

Based on their 31-year retrospective study conducted under moderate climatic conditions in Slovenia, and including four Bos Taurus breeds, Snoj *et al.* (2013) reported highest ejaculate volumes and highest total sperm output in summer, and lowest values in winter. In our study, SC was not significantly different in different year-season combinations (significantly higher values ($P > 0.05$) only in "autumn-2011"). In analogy, Snoj *et al.* (2013) also denied relationships between seasons and levels of SC. In 2010 and 2011, SM was lowest in the summer season (Table 3). Argov-Argaman *et al.* (2013) found almost identical semen production levels (SV and no. of sperms) across seasons, but in their study, SM was higher in

Table 3. Least square means with corresponding standard errors within classes of fixed effects for the following semen traits of AI sires: Semen volume (SV), semen motility (SM), semen output (SO), number of semen doses per ejaculate (NSD), and semen concentration (SC).

Effects	Groups	SV ±SE	SM ±SE	SO ±SE	NSD ±SE	SC ±SE
Year-season	Spring-2009	5.37^{a} ±0.09	69.23^{a} ±0.13	6.70^{a} ±0.18	372.90^{a} ±11.03	1.26^{a} ±0.02
	Spring-2010	5.59^{b} ±0.07	70.09^{b} ±0.11	6.80^{a} ±0.15	382.22^{a} ±9.28	1.25^{a} ±0.02
	Spring-2011	5.35^{a} ±0.08	68.99^{a} ±0.12	6.85^{a} ±0.16	366.16^{a} ±9.94	1.27^{a} ±0.02
	Summer-2009	5.33^{a} ±0.10	69.72^{a} ±0.15	6.59^{a} ±0.20	363.23^{a} ±12.14	1.26^{a} ±0.03
	Summer-2010	5.62^{b} ±0.09	68.98^{b} ±0.13	6.78^{a} ±0.17	375.72^{a} ±10.74	1.24^{a} ±0.02
	Summer-2011	5.25^{a} ±0.09	68.83^{b} ±0.14	6.66^{a} ±0.19	364.43^{a} ±11.55	1.25^{a} ±0.03
	Autumn-2009	5.16^{a} ±0.09	69.85^{a} ±0.13	6.09^{a} ±0.18	335.90^{a} ±11.26	1.22^{a} ±0.02
	Autumn-2010	5.54^{b} ±0.07	69.31^{b} ±0.11	6.56^{b} ±0.15	367.43^{b} ±9.24	1.22^{a} ±0.02
	Autumn-2011	5.28^{a} ±0.08	69.30^{b} ±0.12	7.34^{c} ±0.16	414.79^{c} ±10.21	1.37^{b} ±0.02
	Winter-2009	5.29^{a} ±0.11	69.62^{a} ±0.16	6.24^{a} ±0.22	346.49^{a} ±13.37	1.20^{a} ±0.03
	Winter-2010	5.56^{b} ±0.12	69.60^{a} ±0.18	6.54^{a} ±0.24	357.83^{a} ±15.02	1.22^{a} ±0.03
	Winter-2011	5.12^{a} ±0.08	69.61^{a} ±0.13	6.17^{a} ±0.17	337.62^{a} ±10.32	1.24^{a} ±0.02
Age of bull (in months)	≤ 12	4.11^{a} ±0.10	68.44^{a} ±0.15	4.25^{a} ±0.19	190.34^{a} ±12.07	1.13^{a} ±0.03
	12-17	4.52^{b} ±0.06	69.03^{b} ±0.09	5.31^{b} ±0.12	246.66^{b} ±7.20	1.23^{b} ±0.01
	18-47	5.69^{c} ±0.08	70.08^{c} ±0.11	7.59^{c} ±0.15	424.77^{c} ±9.26	1.35^{c} ±0.02
	≥ 48	7.16^{d} ±0.05	70.15^{c} ±0.08	9.28^{d} ±0.11	599.81^{d} ±6.60	1.30^{c} ±0.01
Interval (in days)	< 3	4.97^{a} ±0.10	69.53^{a} ±0.15	5.61^{a} ±0.19	302.93^{a} ±12.03	1.11^{a} ±0.02
	≥ 3	5.77^{b} ±0.03	69.32^{a} ±0.04	7.60^{b} ±0.06	427.86^{b} ±3.74	1.32^{b} ±0.01

Different superscripts ^{a,b} denote significant differences ($P < 0.05$) within blocks of effects (blocks are separated by horizontal lines).

winter than in summer. Detrimental effects of summer seasons on semen quality were shown by Teixeira *et al.* (2011), who investigated sperm morphology and acrosome integrity.

3.1.3 Age of bulls

For all levels of THI, semen production characteristics SV (Figure 3), SO (Figure 5), and NSD (Figure 6) were throughout highest for oldest bulls (> 48 month). For all traits in our study (also including SM; Figure 4 and SC; Figure 7), youngest bulls (< 12 month) achieved lowest values. Solutions (least square means) for fixed effects of defined "age of sire classes" (Table 3) generally support superiority of older bulls, and reflect results from previous studies (e.g. Boujenane and Boussaq, 2013; Snoj *et al.*, 2013). In the study by Brito *et al.* (2002), ejaculate volume was strongly related to age classes of bulls, because increasing body mass was correlated with testicular growth. In causality, increase in semen quantity was positively correlated with the number of mature spermatozoa, and with pronounced progressive semen motility (Majić Balić *et al.*, 2012).

Adult bulls are characterized by higher semen productivity than younger bulls, but they reacted with a stronger decline in production levels on increasing THI beyond the identified THI threshold (THI > 60). Higher environmental sensitivity for older bulls might be due to the increasing distance between the testicular artery and venous blood with increasing age. Hence, a strong decline in semen quality with increasing THI is explained by reduced heat transfer efficiency between arterial and venous blood, and impaired testicular thermoregulatory ability (Brito *et al.*, 2012). Based on negative correlations between THI and SM, Majić Balić *et al.* (2012) reported environmental sensitivity to increasing ambient temperatures for young Simmental bulls. These authors identified an intensification of pro-oxidative processes in semen plasma and in spermatozoa from young bulls as a major reason for decreasing sperm progressive motility, along with semen quality deterioration. König *et al.* (2005a) studied environmental sensitivity of health disorders by parity classes of cows. They identified trait specific reactions: For some traits, incidences decreased with increasing parity (effects of resistance, adaptation, and also of selection), but for other traits, opposite results were found.

3.1.4 Intervals between semen collection dates

Apart from SM, the interval between consecutive semen collection dates was highly significant ($P < 0.0001$) for all semen traits. Traits SV, SO, NSD and SC achieved higher values with longer intervals (more than 3 days) compared to a shortened time period (less than 3 days; Table 3). Results are in accordance with studies by Everett *et al.* (1978), Everett and Bean (1982), Mathevon *et al.* (1998a), Fuerst-Waltl *et al.* (2006), Karoui *et al.* (2011), and Boujenane and Boussaq (2013). These authors found increased ejaculate volume, sperm concentrations, and number of spermatozoa per ejaculate with an increasing time span between consecutive semen production dates.

3.2 Genetic impact on semen traits

3.2.1 Heritabilities of semen traits in dependency of THI

Genetic parameters for semen traits as listed in Table 1 are mostly the outcome from univariate or multiple trait models. An RRM with Legendre polynomials was applied by Carabaño *et al.* (2007), who studied the genetic trajectory of ejaculate volume by alterations of a time dependent covariate (age at semen collection). Their statistical modelling focussed on different polynomial structures, i.e. Legendre polynomials of order 3 to order 6 for both animal genetic and permanent environmental effects, but heritabilities from different models were almost identical at identical collection dates. In their study, heritabilities for ejaculate volume were highest at the extreme ends of the time scale. This finding is in line with results from our study showing highest heritabilities for semen traits SV, SM, SO, NSD, and SC at the extreme ends of the continuous THI scale (Figure 8). However, extremely high additive-genetic variances and heritabilities at the extreme ends of the environmental scale might be attributed to the artefacts of the chosen polynomial structure for RRM, and to the reduced dataset for minimal and maximal THI. Carabaño *et al.* (2007) emphasised a careful interpretation of genetic parameter estimates from RRM at the beginning and at the end of the time scale, being of importance especially for small datasets. Such an artefact of RRM, i.e. a deviation of genetic parameters at the extremes of the continuous scale, was described and proven in previous studies for female fertility traits (e.g. Yin *et al.*, 2012). A second explanation for increasing genetic variances and heritabilities at the ends of the environmental parameter space is given by Schierenbeck at al. (2011). They explained those findings with a pronounced genetic differentiation of functional traits in harsh environments.

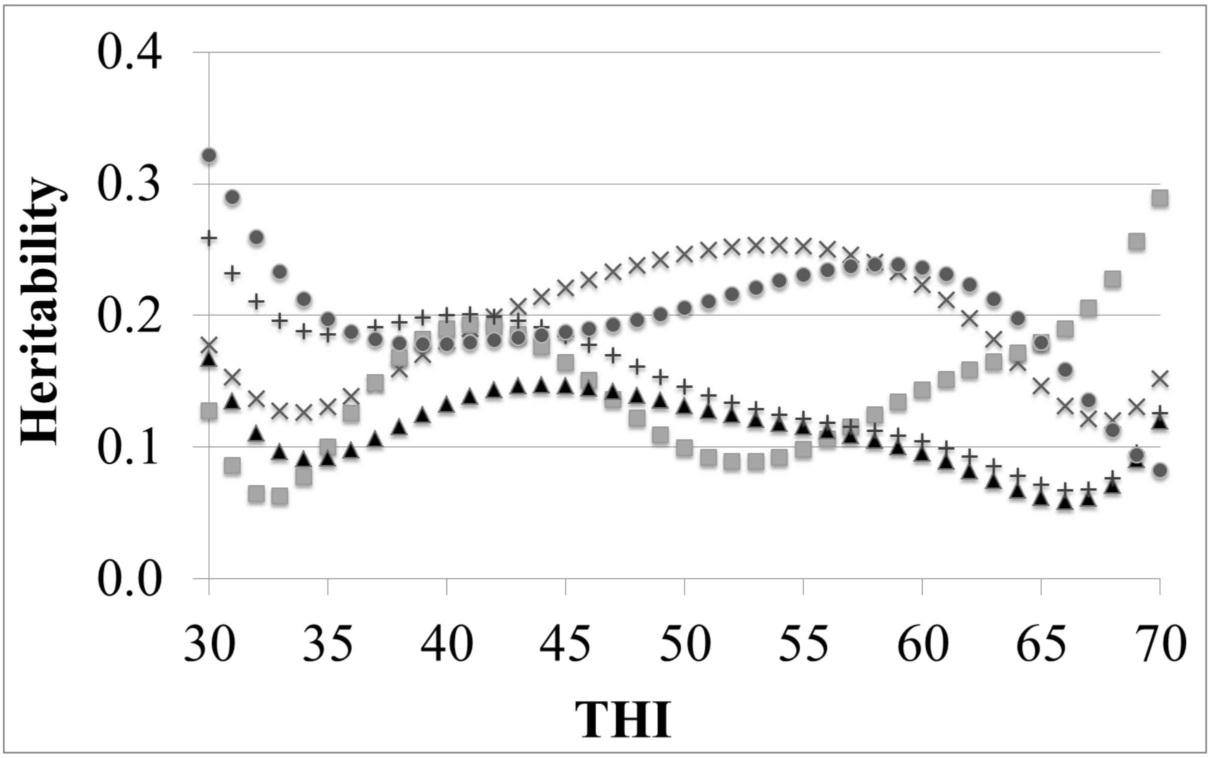

Figure 8. Heritabilities for semen volume (▲), semen motility (■), semen output (+), number of semen doses per ejaculate (●) and semen concentration (**x**) by THI measured at the ejaculation date.

To our knowledge, the present study is a first genetic study which aims at modelling the genetic trajectory of semen traits in dependency of an environmental descriptor (here: THI). For genetic analyses, only THI from the ejaculation date was used as continuous environmental descriptor by neglecting THI measurements from time intervals in greater distance. Changes of genetic parameters for semen traits by THI indicate alterations of gene expressions with changes of temperature and humidity, as proven on a molecular genetic level for heat stressed male mice (Cammack *et al.*, 2009). Heritabilities for semen quantity and semen quality traits in temperate zones (Figure 8) reflect estimates from previous genetic analyses using univariate or multiple trait models (as summarized in Table 1). For example, heritabilities for SV in our study were quite constant (0.10 to 0.15) in the comfort zone between THI 35 and THI 60. Similar results were found for large datasets from the US (Taylor *et al.*, 1985), from Austria (Gredler *et al.*, 2007), or from Spain (Karoui *et al.*, 2011). Pattern of heritability curves for SV and SO were almost identical (Figure 8), and illustrate the close relationship between both semen quantity traits. The semen trait in our study showing major fluctuations of heritabilities during THI was SM (Figure 8). For SM, maximal heritabilities were achieved at the extreme ends of the THI scale and at THI 43 ($h^2 = 0.20$), whereas minimal heritabilities ($h^2 < 0.10$) were identified between THI 33 and THI 54. Also across studies comparisons (Table 1) exhibit substantial variations of heritabilities for SM, e.g. from 0.01 (Mathevon *et al.*, 1998a) to 0.51 (Diarra *et al.* 1997). Interestingly, both studies were conducted using data from Canadian Holstein bulls. Gredler *et al.* (2007) stated that variation of genetic parameter estimates in the same traits from different studies are related to effects of the breed, to effects of the age, and to the statistical modeling. Mathevon *et al.* (1998b) also reported impact of bull age on heritabilities, on repeatabilities, and on variances for SV and SO of French Montbéliard bulls. In their study, lowest heritabilities were found for youngest bulls. Maxima of heritability curves for SC and NSD were found in close distance to the THI threshold (THI 60, Figure 8). The highest heritability was 0.27 for SC at THI 53, and 0.26 for NSD at THI 58. For both traits, genetic parameter estimates are in line with results from previous studies (Table 1).

Heritabilities for semen traits larger than 0.10 justify selection strategies with the overall aim to improve bull fertility. Inclusion of semen traits into breeding goals was suggested by e.g. Fitzpatrick *et al.* (2002) for Santa Gertrudis bulls in Australia. Semen traits are routinely recorded in laboratories of AI stations, and the existing infrastructure can be used for large-scale phenotyping. Also pedigrees of AI bulls are well and deep documented, whereas missing or wrong identifications are mostly relevant for cows from commercial production herds (e.g. Sanders *et al.*, 2006).

Nevertheless, inclusion of semen traits into overall breeding goals imply availability of genetic covariance components with other traits of interest (especially with production and functional traits of current breeding goals). Hence, we suggest an extension of univariate RRM to multiple trait RRM in ongoing studies. Furthermore (pre-conditioning high genetic correlations), estimated breeding values for semen traits of young bulls can be used as early predictors for genetic values of male fertility traits as used in official national and international genetic evaluations (e.g. paternal non-return rates). Gredler *et al.* (2007) estimated correlations between estimated breeding values for semen quality traits with estimated breeding values for official male fertility traits, but correlations were in a low to moderate range (0.08 – 0.17).

Nevertheless, also application of univariate RRM for semen quantity and semen quality traits allow optimization of genetic selection via selection of appropriate sires adapted to specific environments. As outlined by Yin *et al.* (2014), economic competitiveness of German dairy cattle breeding organizations strongly depends on exports of livestock and sire semen, especially in countries located in tropical climates in Asia, Africa, and South America. Availability of "THI specific" estimated breeding values for semen traits enables selection of superior genetics for specific climate conditions of the importing country.

3.2.2 Genetic correlations in same semen traits for different THI combinations

Classically, the proof of possible genotype by environment interactions in dairy cattle is based on genetic correlation estimates from multiple trait models, where observations for a given trait are defined as different traits in different environments. Such a “research design”, i.e. availability of progeny records from same sires in different environments, is given in dairy cattle through the widespread AI structure. Following Robertson (1959), genetic correlations in same traits between different environments lower than 0.80 indicate genotype by environment interactions, and suggest re-rankings of sires in different environments. König *et al.* (2005b) favored the application of multiple trait models for studies focusing on distinct environmental classes, e.g. different regions, countries, or production systems. In contrast, for continuous environmental descriptors (e.g. THI), applications of RRM to explain performances gradually over a range of environments, were suggested. Also fom a statistical point of view (a fewer number of parameters need to be estimated), the concept of reaction norms as applied by Kolmodin *et al.* (2002) for production traits is a promising alternative.

RRM applications allow to infer genetic correlations in same traits between all possible combinations of environmental descriptors. From the whole grid of pairwise estimates, we focused on genetic correlations between semen traits at THI 60 with identical semen traits from the whole THI range (Figure 9). For all semen traits, genetic correlations were $r_g \sim 0.80$ for THI levels in close distance, i.e. for THI ranging between 58 and 61. Genetic correlations continuously decreased with increasing distances between THI 60 and remaining THI. THI 60 was the identified THI threshold at the phenotypic scale. Apart from SM, genetic correlations between THI 60 with THI > 60 decreased substantially. Opposite genetic correlations for SM support findings on the phenotypic scale, where, in contrast to remaining semen traits, a stagnation or even slight increase of SM beyond THI 60 was observed (Figure 4). Generally, indications for genotype by environment interactions at high THI are larger for traits reflecting semen quantity when compared to sperm quality (SM). As a further particularity of SM in comparison to other traits, also additive genetic variances and heritabilities continuously increased beyond the heat stress threshold (Figure 8). With regard to genetic effects, also Karoui *et al.* (2011) found particularities for SM, i.e. substantially lower heritabilities compared to semen volume, concentration, and number of spermatozoa per ejaculate. In the present study, lowest genetic correlations were found for SM from THI 60 with SM from THI < 35. Especially the negative genetic correlation ($r_g = -0.18$) between SM from THI 60 with SM from THI 33 indicate genotype by environment interactions, and substantial re-rankings of sires in different climatic conditions. Again, interpretation of results should consider artefacts of the polynomial structure, and the reduced dataset at the extreme ends of the environmental scale.

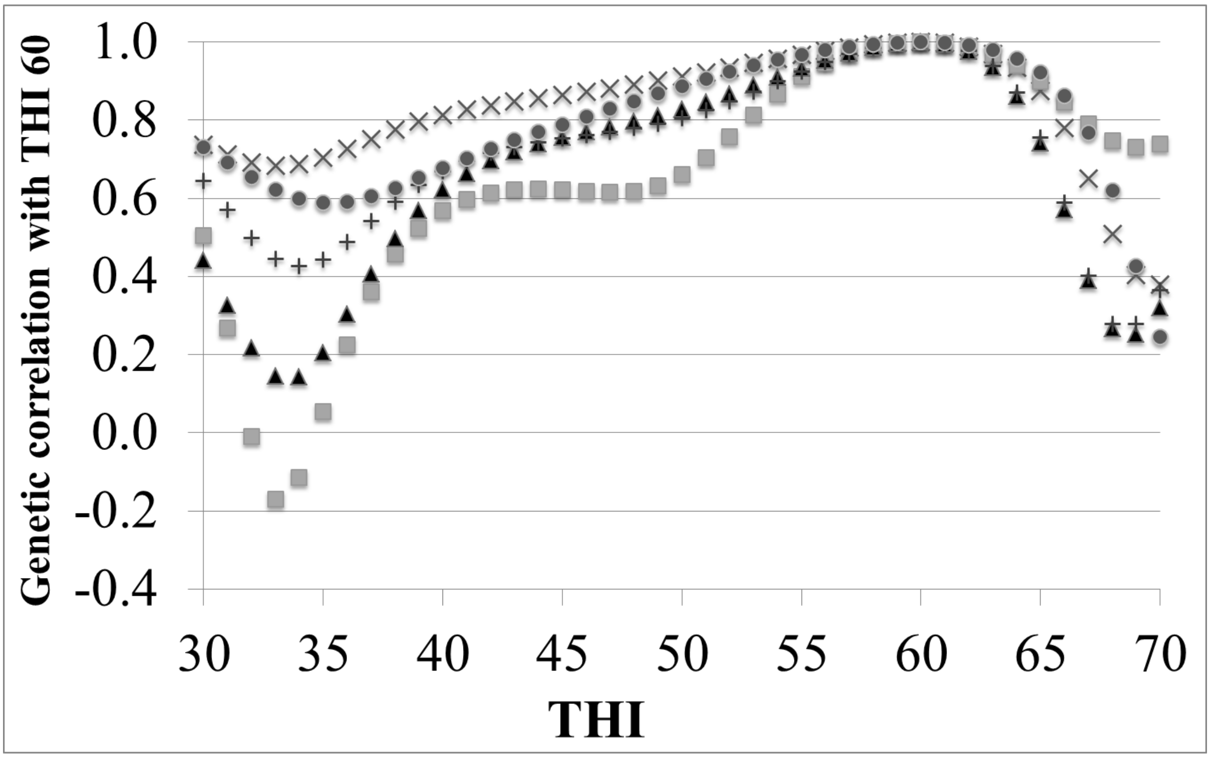

Figure 9. Genetic correlations for semen traits at THI 60 with corresponding semen traits in dependency of THI: Semen volume (▲), semen motility (■), semen output (+), number of semen doses per ejaculate (●) and semen concentration (**x**).

Indications for genotype by environment interactions for semen traits are in agreement with environmental sensitivity for female fertility traits (Brügemann *et al.*, 2013). However, for similar statistical RRM, same breeds and same regions, a focus on test-day production traits revealed genetic correlations throughout higher than 0.90 for all possible THI combinations (Brügemann *et al.*, 2011). Also for multiple trait models and production traits, limited evidence for genotype by environment interactions were found (e.g. König *et al.*, 2005b). Hence, strong environmental sensitivity for male and female fertility traits encourage ongoing studies addressing the topic of genotype by environment interactions on the genomic scale. Routinely large scale genotyping of AI sires allows application of genomic RRM (as introduced by Yin *et al.* (2014) for cow traits in dependency of THI) to semen traits. In contrast to traits expressed only in female progeny (e.g. protein yield), phenotypes of semen traits can be directly assigned to genetic markers of bulls, and directly related to their environmental descriptors.

4. Conclusions

Analyses of semen quantity traits SV, SO and NSD revealed environmental sensitivity for temperature x humidity combinations which indicate heat stress in dairy cattle. A general heat stress threshold of THI 60 showed a strong decline in semen productivity. Semen motility was partly affected by increasing THI, and different reactions were observed for different age classes of bulls. A change of additive genetic variances and heritabilities with alterations of THI was found for all traits. Strong indications for genotype by environment interactions were identified for semen quantity traits. RRM applications to semen traits allow to estimate THI specific breeding values, and to select sires adapted to harsh environments.

Acknowledgements

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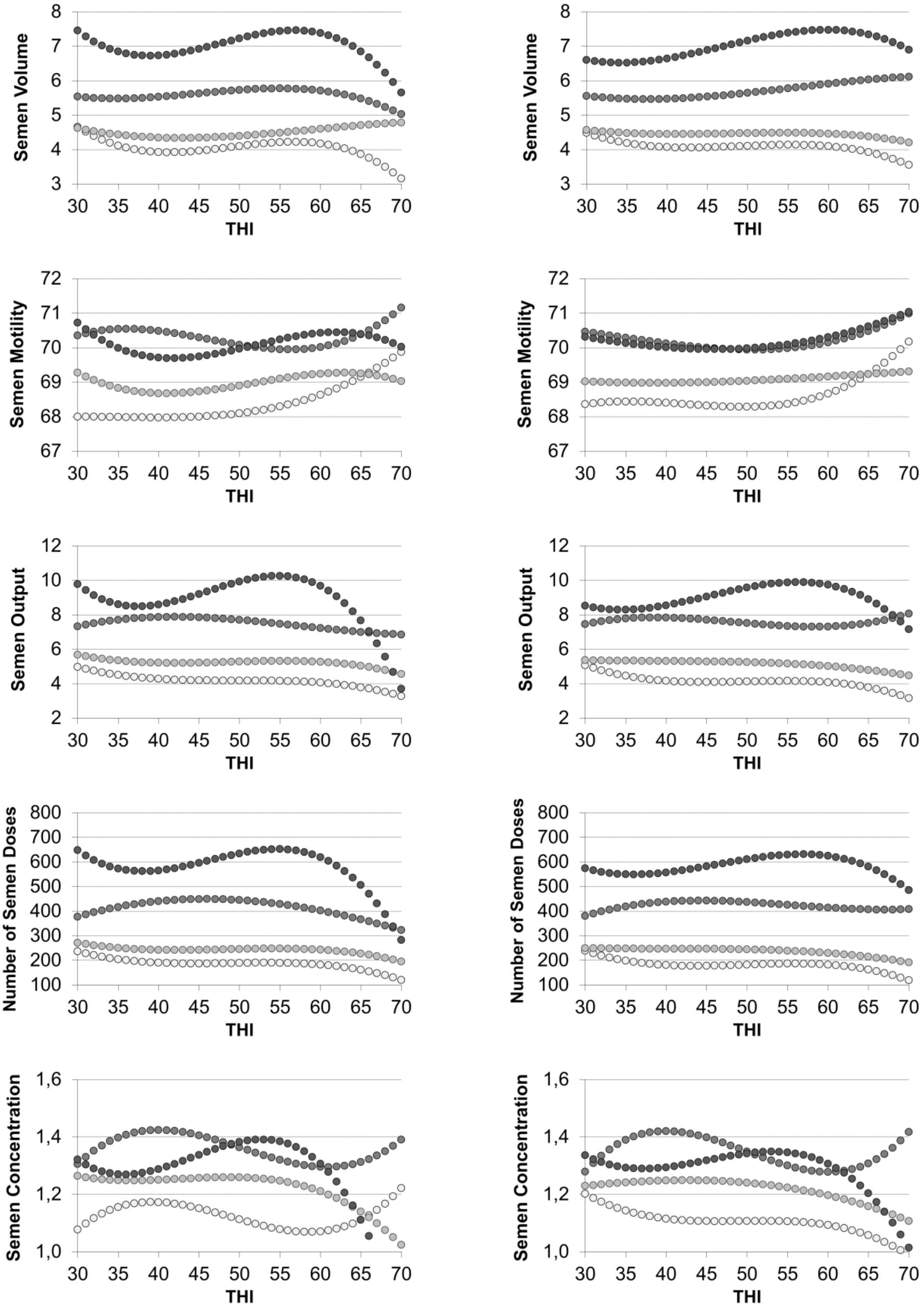
Supplementary **Figure S1**. Least squares means for semen volume (in ml), semen motility (in %), semen output (no. of sperms per ejaculate), no. of semen doses, and semen concentration (no. of sperms per ml ejaculate) in dependency of THI.

THI recording: 1-11 days before the ejaculation date	THI recording: 29-35 days before the ejaculation date



Chapter 3

Alterations of temperature x humidity indices effect physiological traits in dairy and dual purpose cows

A. Al-Kanaan[*, §], S. König[*] and K. Brügemann[*]

[*] Department of Animal Breeding, University of Kassel, 37213 Witzenhausen, Germany

[§] Department of Animal Production, college of Agriculture, University of Basrah, Basrah, Iraq

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ABSTRACT

Two experimental herds located in the northern part of Germany were the basis to study the effect of heat stress (**HS**) on physiological traits of Holstein Friesian (**HF**) and dual-purpose German black pied cattle (**DSN**). Physiological cow traits included rectal temperatures (**RT**), skin temperatures (**ST**), vaginal temperatures (**VT**), respiration rates (**RR**), and pulse rate (**PR**). Infrared thermography (**IRT**) was used to measure ST from four different body surface areas. Number of records was as follows: 2884 DSN and 550 HF observations for RT, 2747 DSN and 465 HF observations for ST measured from four different body surface areas, 1013 DSN observations for VT, 2037 DSN observations for RR, and 2192 DSN observations for PR. Meteorological data (ambient temperature and humidity) for calculating temperature-humidity indices (**THI**) were measured inside the barns**.** For statistical analyses, random regression models were applied by modelling the continuous environmental descriptor THI with Legendre polynomials of order 3. Statistical models further considered the fixed effects of the season and year of measurements, and further cow associated factors (milk yield, milk composition, days in milk, parity, and cow environmental sensitivity). For physiological traits, a general upper critical THI threshold of THI 65 was identified, with an optimal THI range from THI 50 to THI 65. Definition of the optimal THI range was based on only minor fluctuations of physiological response traits with increasing THI. An increasing in HS from THI 65 (threshold) to THI 86 (maximal THI) simultaneously increased RT by 0.6 °C (DSN) and 1 °C (HF), ST by 3.5 °C (HF) and 8 °C (DSN), VT by 0.3 °C (DSN), and RR by 47 breaths / minute (DSN), and decreased PR by 7 beats / minute (DSN). The increase of RT, ST, VT, and RR, and the decrease of PR with increasing THI beyond the THI threshold, was most pronounced for cows with high levels of milk yield and milk compositions, cows in later parity producing early in lactation, and during summer seasons in the year 2014. DSN cows respond earlier to HS compared to HF, which might be attributed to the lower phenotypic and genetic variation in the intensively selected HF breed. Physiological parameters were identified as valuable heat stress indicators in the temperate zone in middle Europe, and studying of their fluctuations might help to improve the well-being and health status of milking cows.

Key words: Heat stress, physiological traits, dairy and dual-purpose cows

1. INTRODUCTION

Exposure to warm summer climates depresses productivity, reproduction and welfare of dairy cows. The mostly used continuous environmental descriptor reflecting heat stress in farm animals is the temperature-humidity index (**THI**). THI combines air temperature with humidity, and a variety of THI formulas was evaluated by Bohmanova *et al.* (2007). The thermoneutral zone includes a broad THI range, mostly associated with an equal animal response. However, beyond this zone and if heat load increases, physiological and behavioral response substantially change in order to maintain an animals' body thermal balance. Such physiological responses to heat stress (**HS**) include decreasing heat production, which in turn causes a reduction in feed intake and milk yield, and also affects the endocrine and body physiological system (Hansen, 2004). Karimi *et al.* (2015) identified such detrimental HS effects in both high yielding cows in the peak phase of lactation and during the late gestation stage, with long-term effects observed in subsequent lactations.

Scientific studies addressing dairy and dual purpose cattle's HS sensitivity are of increasing importance, because of the expectations of global warming being relevant for a variety of production systems (Gauly *et al.*, 2013; Bernabucci *et al.*, 2014). Suitable indicators for heat stress sensitivity in cattle include increasing rectal temperatures (**RT**), vaginal temperatures (**VT**), skin temperatures (**ST**) and respiration rates (**RR**) (Dikmen *et al.*, 2014; Zimbelman *et al.*, 2009), as well as alterations in pulse rate (**PR**) (Muller and Botha, 1993; Singh *et al.*, 2012). Most of the field studies focused on high-yielding Holstein Friesian (**HF**) dairy cows, and neglecting the physiological adaptability of dual-purpose cattle which are mostly kept in low-input pasture based production systems. One important breed in this regard is the founder breed of the modern HF, i.e., the German black pied cattle (**DSN**). Especially for those breeds, it imperative to define proper selection criteria reflecting adaption to harsh environments.

Further indicator trait recording for health, stress, or physiology should utilize photography from infrared thermography (**IRT**): a suitable method for visualizing and analyzing local and temporal changes in surface temperatures. In dairy cattle, IRT identified ovulation and estrus time using photos from the vulva (Talukder *et al.*, 2014), and the emotional state using photos from the nose (Proctor and Carder, 2015). Further IRT applications included the detection of hoof lesions (Alsaaod and Büscher, 2012) and claw disorders (Alsaaod *et al.*, 2015).

Furthermore, IRT was used to assess the cutaneous evaporative rate (Silva and Maia, 2011), and to determine the udder surface temperature as early predictor for subclinical mastitis (Polat *et al.*, 2010). Generally, modern IRT technique represents a non-invasive diagnostic tool to indicate health, stress, or environmentally induced HS by measuring the skin temperature of cattle.

Many continuous, Gaussian distributed environmental descriptors, influence primary and functional traits in cattle. In this regard, environmental descriptors include the amount of specific fodder ingredients, but also values for temperature and humidity. From a statistical perspective, an alternative to stratification of environmental effects into a few distinct classes is the application of random regression models (**RRM**). Application of RRM allows to estimate genetic parameters and to study the phenotypic trajectory of physiological traits in dependency of THI. In previous heat stress studies, RRM were applied to test-day production traits (Brügemann *et al.*, 2011), female fertility (Brügemann *et al.*, 2013), and semen characteristics of bulls kept on AI stations (Al-Kanaan *et al.*, 2015). Those previous studies only indirectly addressed stress physiology by studying heat stress response in “conventional” indicator traits, i.e. such traits being available from official national recording schemes. Furthermore, previous studies used daily temperature and humidity as recorded in weather stations, which not depict detailed climatic farm conditions. As an alternative, specific experiments were designed, but only including a limited number of cattle from sometimes “artificial” environments.

In consequence, the objective of the current study was to assess the impact of HS defined as a continuous environmental descriptor (represented by THI) by applying RRM on physiological traits of dairy and dual-purpose cattle kept in experimental herds. All association studies between physiological cow traits and THI are based on indoor weather records from measurements in close intervals of one minute. Such an objective includes the identification of HS thresholds and comfortable zones for physiological traits. Furthermore, we studied the interplay between environment descriptors (THI, season and year of measurements) and further cow associated factors (milk yield, milk composition) to get deeper knowledge of cow adaptation in harsh environments.

2. MATERIALS AND METHODS

2.1. Data

2.1.1. Physiological cow traits

The two breeds reflect two DSN selection lines. Also the HF cows used in the present study are based on DSN genetics, but named in the following as “HF”. In this HF experimental herd, purebred DSN were kept until 1985, but strongly selected on increasing milk yield. Afterwards, original DSN genetics was replaced by using outstanding HF sires with high genetic values for milk yield. Currently, the genetic relationship between the original DSN cows from experimental herd A and from the HF cows in farm B is 0.01.

The two experimental herds used for this study are located close to the border of the states Hessen and Lower Saxony in the northern part Germany. The distance between the two farms

Table 1. Descriptive statistics for physiological traits of cows as used in the present study

Physiological traits	Breed	No.	Mean	SD	Min.	Max.
Rectal temperature (RT) °C	HF[1]	550	38.46	0.42	37.40	40.5
	DSN[2]	2884	38.29	0.38	37.20	41.0
	HF & DSN	3434	38.31	0.40	37.20	41.0
Upper skin temperature (STu) °C	HF	465	30.99	3.44	21.12	37.53
	DSN	2747	29.56	5.40	10.28	39.90
Back skin temperature (STb) °C	HF	465	30.03	3.77	17.97	37.18
	DSN	2747	28.96	5.81	8.54	39.67
Withers skin temperature (STw) °C	HF	465	29.97	3.83	18.13	36.44
	DSN	2747	29.18	5.58	8.82	43.20
Line skin temperature (STl) °C	HF	465	29.86	3.73	17.74	37.14
	DSN	2747	29.05	5.79	9.28	40.08
Vaginal temperature (VT) °C	DSN	1013	38.40	0.35	37.0	41.20
Respiration rate (RR) breath / min	DSN	2037	34.59	14.96	15	129
Pulse rate (PR) beats / min	DSN	2192	75.53	8.67	54	114

DSN[1]= *Deutsches Schwarzbuntes Niederungsrind* (German Black Pied cattle).

HF[2]= Holstein-Friesian cows.

was 36 km. Physiological cow traits included the following measurements: 2884 DSN and 550 HF observations for RT, 2747 DSN and 465 HF observations for ST measured from four different body surface areas, 1013 DSN observations for VT, 2037 DSN observations for RR, and 2192 DSN observations for PR. Descriptive statistical parameters for physiological traits are summarized in Table 1. Physiological measurements were recorded from one trained person between 10 am to 4 pm during the years 2012, 2013, and 2014. A digital veterinary thermometer (Scala SC 12) was used to measure RT and VT by inserting the thermometer into the rectum and into the vagina, respectively. PR and RR were recorded during a time period of 30 seconds with a stopwatch, and converted to a per minute basis. Pulse rate was defined as the number of beats per minute, and manual determined by pressing hand fingertips at the caudal artery. Respiration rate was defined as the number of flank movements per minute. For ST measurements, an infrared camera (Trotec IC120 LV) was used to capture infrared thermography of the upper part (without neck and head) from a cows' forward position. Application of this technique (Trotic IC-Report DuoVision 1.08.06S) allows the calculation of ST for desired distinct skin surfaces. In the present study, we distinguished between four different ST measurements representing four different areas (Figure 1): a) upper ST (**STu**) = a freeform polygon for the total upper cow body without neck and head, b) back ST (**STb**) = a polygon area of the cow rump and back between pin bones and shoulders, c) line ST (**STl**) = a curved line at the back line from tail to neck, and d) withers ST (**STw**) = a circle area around withers. For each cow, we calculated sensitivity values (**SENSI)**, being the within-cow standard deviation from repeated measurements for each physiological trait. SENSI levels were used to classify cows into four sensitivity groups: low, semi moderate, moderate, and high heat stress sensitivity.

The choice of the two breeds exhibits pronounced differences for production traits. Average test-day milk yield (TDM) of HF was 31.95 kg (±7.33 kg) with a fat percentage of 4.24 % (±0.74 %), while DSN cows showed substantially lower productivity (TDM: 17.14 kg ±5.46 kg, fat percentage: 4.15 % ±0.66 %). Means of milk urea nitrogen and somatic cell counts (**SCC**) were higher in DSN (249.09 ml/L ± 84.83 ml/L, and 298.20 52 x 10^3 cells/ml ± 23.52 x 10^3 cells/ml, respectively) than in HF cows (231.64 ml/L ± 59.66 ml/L, and 184.07 x 10^3 cells/ml ± 21.92 x 10^3 cells/ml, respectively). Minor differences were found for the average protein content between DSN (3.39 % ±0.38 %) and HF cows (3.39 % ±0.40 %).

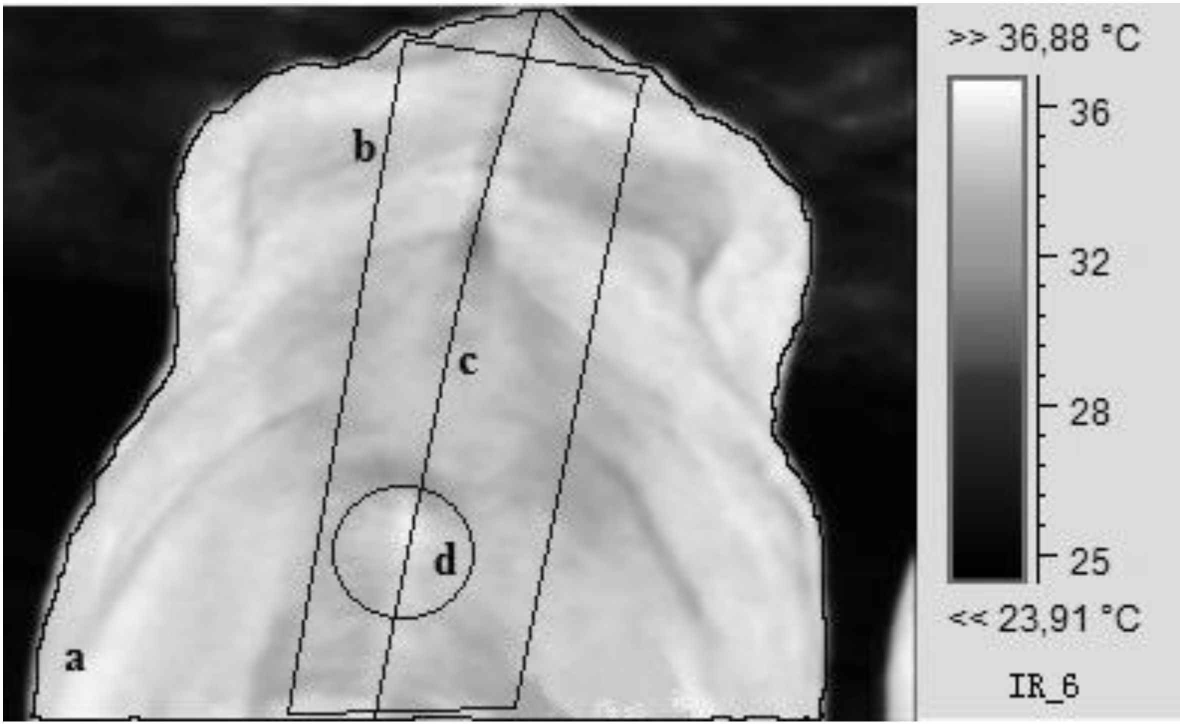

Figure 1. Skin temperature measurements from the forward-upper part of cow body from different areas: a) upper, b) back, c) line and d) withers using infrared thermography technique.

2.1.2. Meteorological data

Meteorological data (ambient temperature and humidity) were recorded inside the barn (1-minute intervals) with special data logger (Voltcraft DL-120 TH), which was installed at the level of the cow head. Recording of meteorological data in close distance to the cow, and at exactly the same time point where physiological traits were measured, is imperative for studies addressing both components cow physiology and heat stress sensitivity (Collier and Collier, 2012). THI was calculated by applying the formula of the National Research Council (1971), and considering the dry bulb temperature (**T °C**) and relative humidity (**RH %**) as follows:

$$THI = (1.8 * T\,°C + 32) – [(0.55 - 0.0055 * RH\,\%) * (1.8 * T\,°C - 26)]$$

Distribution of hourly THI reflects the different within-herd climatic conditions between both farms. With regard to the maximal daily THI (on the per hour basis) distribution of meteorological data was as follows: THI_{max} > 60 included 51.54 % of meteorological data from the DSN farm and 61.96 % of observations from the HF farm; THI_{max} > 65 included 37.51 % of meteorological data from the DSN farm and 47.82 % of observations from the HF farm; THI_{max} > 70 included 24.72 % of meteorological data from the DSN farm and 30.80 % of observations from the HF farm; THI_{max} > 75 included 11.93 % of meteorological data from the DSN farm and 12.85 % of observations from the HF farm; THI_{max} > 80 included 3.08 % of meteorological data from the DSN farm and 3.21 % of observations from the HF farm.

Highest average monthly THI and indicating longer periods of HS (THI > 65) in DSN and HF farms were observed during the summer months (June, July and August) (Figure 2). Apart from the early mornings in autumn in DSN farm, all maximal hourly THI on a daily scale during summer, spring and autumn were higher than a hypothesized THI threshold of THI > 65 (Figure 3). Inside barn measurements for average and maximum monthly THI and also THI on the daily scale were higher in the HF compared to the DSN farm (Figure 2 and Figure 3, respectively). Meteorological data from the nearest public weather station were almost identical for two farms, with generally lower THI, dry bulb temperature and humidity, but larger fluctuations, than corresponding indoor records. Differences of meteorological data between the two farms is attributed to the barn building type, including characteristics such as farm isolation, cooling technique, spaciousness, or general orientation of the building and especially of the roof .

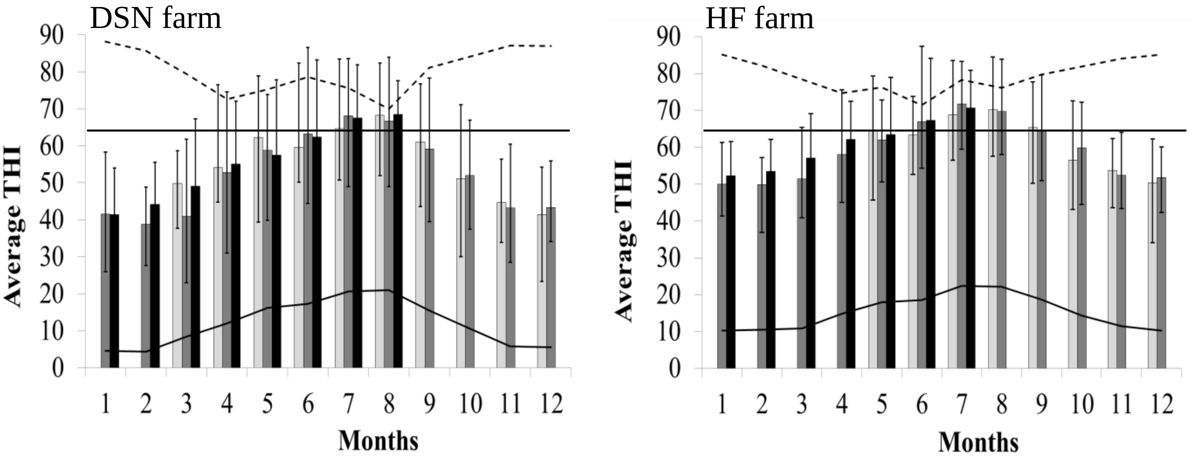

Figure 2. Average monthly (test-hour recorded) temperature-humidity indices (THI) for the years 2012 (light gray), 2013 (gray), and 2014 (black) inside barns of the DSN and HF farm. Stripes in the bars indicate monthly minimum and maximum for THI. Solid line: dry bulb temperature; dashed line: relative humidity; solid horizontal line: THI threshold.

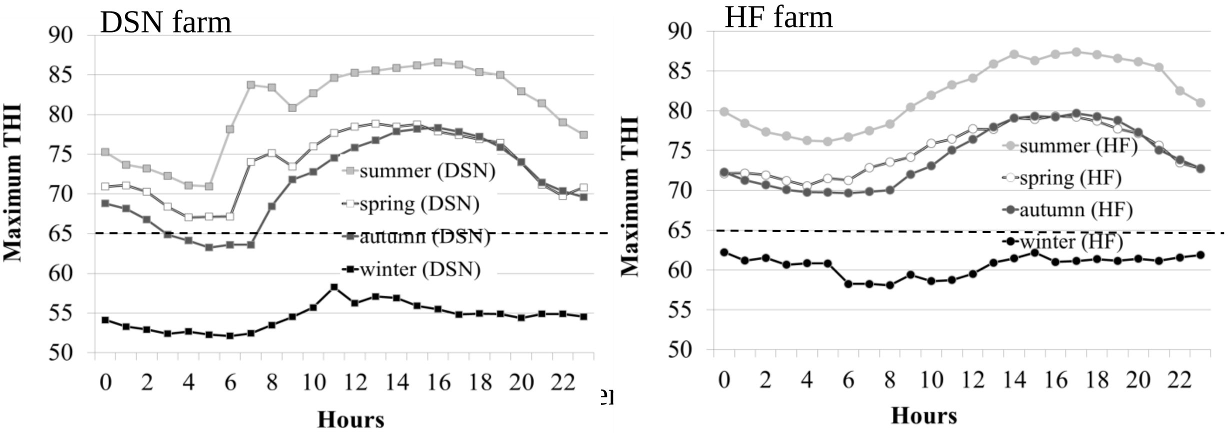

Figure 3. Maximum (test-hour recorded) temperature-humidity indices (THI) during 24 hours within seasons spring, summer, autumn and winter. The horizontal dashed line indicates the THI threshold.

2.2. Statistical analysis

A RRM was used to study alterations of physiological traits by THI on the phenotypic scale. For this purpose, a linear mixed model as implemented in the SAS 9.2 procedure PROC MIXED (SAS Institute, Cary, NC) was applied. The general statistical model in matrix notation was defined as follows:

$$\mathbf{y} = \mathbf{X}\text{ß} + \mathbf{Zu} + \mathbf{e}$$

where **y** = vector including physiological traits of cows, **b** = vector of fixed effects generally including parity, days in milk (**DIM**), year of the recording date, and season of the recording date, and regression for the covariate THI using third-order Legendre polynomials nested within fixed effect groups in consecutive runs (see models 1 to 8), **u** = vector of random cow effects, and **X**, **Z** and **W** are the associated incidence matrices, respectively.

Additional fixed effects in models 1 to 9 were defined as follows:

Model 1: additional fixed effect of test-day milk yield (**TDM**) from the official test-day closest to the physiological trait recording date including for levels for DSN cows (< 13 kg, 13-17 kg, 17-21 kg, and > 21 kg) and also four levels for HF cows (< 27 kg, 27-31 kg, 31-36 kg, and > 36 kg);

Model 2: additional fixed effect of SENSI including the four sensitivity levels low, semi moderate, moderate, and high heat stress sensitivity for the respective trait;

Model 3: additional fixed effect of test-day fat % from the official test-day closest to the physiological trait recording date including four levels (< 3.5 %, 3.5% - 4.0 %, 4.0 % - 4.5 %, and > 4.5 %);

Model 4: additional fixed effect of test-day protein % from the official test-day closest to the physiological trait recording date including four levels (< 3.0 %, 3.0 % -3.3 %, 3.3 % - 3.6 %, and > 3.6 %);

Model 5: additional fixed effect of test-day fat to protein ratio (f/p ratio) from the official test-day closest to the physiological trait recording date including four levels (< 1.1, 1.1 -1.2, 1.2 – 1.3, and > 1.3);

Model 6: additional fixed effect of test-day SCC from the official test-day closest to the physiological trait recording date including four levels (< 30 x10^3, 30 x10^3 - 80 x10^3, 80 x10^3 - 200 x10^3, and > 200 x10^3 somatic cells);

Model 7: additional fixed effect of test-day milk urea nitrogen from the official test-day closest to the physiological trait recording date including four levels (< 160 mg/l, 160 -220 mg/l, 220-270 mg/l, and > 270 mg/l);

Model 8: additional fixed effect of RT from the physiological trait recording date including four levels (< 38.1°C, 38.1 - 38.4°C, 38.4°C - 38.6°C, and > 38.6°C).

In the following, LSmeans for the basic fixed effects (levels of parity, DIM, year and season) are presented as results from model 1, but are in close agreement with solutions from remaining models 1 to 8.

3. RESULTS AND DISCUSSION

3.1. Significance of fixed effects and covariates on physiological traits

Analyses of variance (Table 2) revealed highly significant impact of THI (sum of squares type I tests) from all models for Legendre polynomials of order 1 ($P < 0.0001$) on all physiological cow traits. Also 2nd order Legendre polynomials for THI highly significantly influenced RT in both breeds, and STb, STl, VT and RR ($P < 0.0001$) in DSN cows. König *et al.* (2006) also used sum of square type I tests for regression coefficients, and they found decreasing significant impact with increasing polynomial order of a time dependent covariate on longitudinal cow behavior traits. As a consequence from the sequential analysis approach based on type I sums of squares, and as suggested by Littell *et al.* (1998), we removed regression coefficients of order 4 and higher from the model. Interestingly and depicting pronounced environmental sensitivity, STu, STb and RR recorded in DSN cows responded highly significant ($P < 0.0001$) to continuous alterations of THI modeled with Legendre polynomials of orders 1, 2 and 3. Generally, significant impact of cow-associated factors and environmental descriptors on physiological cow traits was more obvious in DSN than in HF cows. Those findings might be due to generally higher trait variation in DSN compared to intensively selected HF populations, which are characterized by identical founder sires and decreasing effective population size (König and Simianer, 2006). Furthermore, identical genealogy goes along with a worldwide identical breeding goal focus on productivity in worldwide HF populations in the past decades (Miglior *et al.*, 2005). As outlined from results in Table 2, we hypothesize reduced potential for adaptation to harsh environments, and in a further consequence, fewer possibilities to implement successful adaptive selection strategies, in HF than in DSN cows.

For fixed effects (= classification variables), type III tests for sum of squares were used to identify significant impact on physiological cow traits. Apart from RT ($P > 0.10$) in HF and VT ($P < 0.01$) in DSN cows, we identified highly significant seasonal impact ($P < 0.0001$). A similar tendency, i.e., a non-significant effect ($P > 0.10$) on RT and VT, was observed for the fixed effect of the year. Our statistical models 1 to 8 simultaneously considered the fixed effects of year and season, and regressions of THI. Generally, we assume strong confounding between seasons and meteorological data. Nevertheless, Al-Kanaan *et al.* (2015) referred to early physiological studies by Swanson and Herman (1944), and they emphasized further seasonal characteristics such as alterations in daylight length and fodder composites, contributing to the variation of male fertility traits.

Table 2. Analysis of variances: *P* values for the effects of factors in this study on the physiological traits of DSN and HF cows

Model no.	Effects	Rectal temperature		Skin Temperature								Vaginal temperature	Respiration rate	Pulse rate
		HF	DSN	Upper		Back		Withers		Line				
				HF	DSN	HF	DSN	HF	DSN	HF	DSN	DSN	DSN	DSN
1	TDM	ns	ns	ns	+	ns	ns	+	ns	+	ns	ns	ns	ns
2	Sensitivity	*	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	ns	<.0001	***
3	Fat %	*	ns	ns	**	ns	*	ns	ns	Ns	+	ns	*	*
4	Protein %	ns	ns	*	Ns	ns	ns	ns	ns	Ns	ns	ns	ns	*
5	f/p ratio	ns	ns	*	Ns	ns	ns	ns	ns	Ns	ns	**	<.0001	+
6	Urea	ns	***	ns	Ns	ns	ns	ns	ns	Ns	ns	ns	**	*
7	SCC	ns	ns	ns	Ns	ns	ns	ns	ns	Ns	ns	ns	**	ns
8	RT	-	-	ns	*	ns	*	ns	**	Ns	**	<.0001	<.0001	<.0001
9	Parity^1	*	<.0001	**	<.0001	**	<.0001	*	<.0001	*	**	**	<.0001	***
10	DIM^1	*	<.0001	*	<.0001	*	<.0001	*	<.0001	**	<.0001	<.0001	ns	ns
11	Season^1	ns	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	**	<.0001	<.0001
12	Year^1	ns	<.0001	***	**	***	***	*	ns	**	**	ns	*	<.0001
1	THI (LG1)	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001
1	THI (LG2)	<.0001	<.0001	*	<.0001	*	<.0001	**	**	*	<.0001	<.0001	<.0001	*
1	THI (LG3)	**	*	ns	<.0001	ns	<.0001	**	<.0001	*	<.0001	*	<.0001	<.0001

ns= not significant, += $P < 0.1$, *= $P < 0.05$, **= $P < 0.01$, ***= $P < 0.001$, and <.0001= $P < 0.0001$. DSN= *Deutsches Schwarzbuntes Niederungsrind* (German Black Pied cattle). HF= Holstein-Friesian cows. TDM= test-day milk yield. f/p ratio= Fat/ protein ratio. LG1, LG2 and LG3= Legendre Polynomials order 1, 2 and 3. The basic fixed effects of models 1-8 include: (parity + DIM + season + year) in addition to the tested factor.[1] LSmeans of parity, DIM, season and year calculated from model 1 (including TDM). LSmeans for rectal and skin temperature measured in separate runs for DSN and HF cows.

Among all "cow associated effects", SENSI explained a large amount of the phenotypic variation of physiological traits, with highly significant impact ($P < 0.0001$) on RT, RR and PR in DSN cows, and on all varieties of skin temperatures in both breeds. Environmental sensitivity was defined by Falconer (1990) as the difference between phenotypic values of animals in two environments, divided by the difference of the means of all individuals in both environments. In our study, SENSI directly measured intra-animal variations. These physiological variances of cows' responses to different climatic environments (during the three years of our study) may also reflect re-ranking of animals across environments, and may detect differences in environmental sensitivity (Calus and Veerkamp, 2003). Additionally, environmental sensitivity is related to differences in metabolic rates and hormones synthesis from the endocrine system, which is associated with the thermoregulation of heat production and heat loss from the animal's body. The requirement of constant thermal balance directly relates SENSI with physiological reactions, e.g. the alterations in core and skin temperatures, respiration rates, and pulse rates.

Only weak and mostly non-significant impact on physiological traits was identified for "cow factors" reflecting official performance test-day traits (TDM, fat %, protein %, fat to protein ratio, milk urea nitrogen). Only the fat to protein ratio significantly influenced response traits RR ($P < 0.0001$) and PR ($P < 0.10$) in DSN cows, and STu ($P < 0.05$) in HF cows. The fat to protein ratio especially early in lactation directly after calving is associated with energy efficiency, and was identified as a valuable predictor for involuntary culling (Bergk and Swalve, 2011). Santos *et al.* (2015) used structural equation models to infer physiological relationships between the fat to protein ratio with behavior and stress symptoms in dairy cows on genetic and phenotypic scales, but they only found weak and time lagged interactions between both traits. Significant relationships were also found between test-day milk urea nitrogen with RR ($P < 0.01$) and with PR ($P < 0.05$). Also milk urea nitrogen reflects energy efficiency in dairy cows, and was used as an early predictor for physiological female fertility traits (König *et al.*, 2008). Test-day SCC only had significant impact on RR ($P < 0.01$). Schrick *et al.* (2001) explained biological mechanisms between mastitis or mastitis indicators and a cow's reproductive physiology, but physiological heat stress response traits seem to be independent from those identified relationships.

In summary, routine test-day records insufficiently explain variations in novel physiological traits. In consequence, we recommend routinely recording of physiological traits to describe in detail a cow's response to environmental alterations. Such routinely recording schemes e.g.

RT, are recently implemented in large-scale dairy cattle contract herds (Gernand *et al.*, 2012) in the eastern part of Germany. The following Figures 4 to 9 study the physiological response by continuous alterations of THI. Here, we focus on such associations depicting pronounced detrimental heat stress effects.

3.2. Alteration of physiological traits in the course of THI

3.2.1. Rectal temperature

LSmeans for RT as a function of THI are presented on a continuous phenotypic scale of THI in Figure 4. Rectal temperature mainly showed minor variations during the low and moderate levels of THI, especially before exceeding the previously described environmental comfortable zones for cattle (Gauly *et al.*, 2013). Pronounced HS impact on increasing RT was detected for high THI values in both breeds. A substantial RT increase was observed for THI > 65. Such a critical THI threshold can be achieved if the dry bulb temperature is higher than 19 °C with a combination of relative humidity higher than 74%, or if the ambient temperature is higher than 24 °C in any combination with humidity values. The identified THI threshold for RT was slightly higher than reported HS thresholds of value THI = 60 for other functional or production traits of HF cows and bulls in the same region of Germany (Brügemann *et al.*, 2011; Brügemann *et al.*, 2012; Al-Kanaan *et al.*, 2015). The higher threshold in our present study might be attributed to meteorological data recording directly inside the barn. This explanation is supported by other studies in Germany which pointed out that the fitted THI threshold for conception rate of HF cows was 65 when using weather station information (Brügemann *et al.*, 2013), but increased to 73 when measuring THI inside the barn (Schüller *et al.*, 2014). Brügemann *et al.* (2012) found that only 17% and 4% of test-day records correspond to THI > 60 and THI > 65, respectively. Those proportions were substantially lower than results from our study with 44.94% and 26.38% of records above THI thresholds 60 and 65, respectively. Also Lambertz *et al.* (2014) used indoor meteorological data, and they found extremely high THI values exceeding the THI 60 threshold almost every day from June to September.

Phenotypic measurements of RT on the continuous THI scale showed different responses when comparing DSN with HF cows. RT of HF cows (Figure 4b) showed a stronger increase

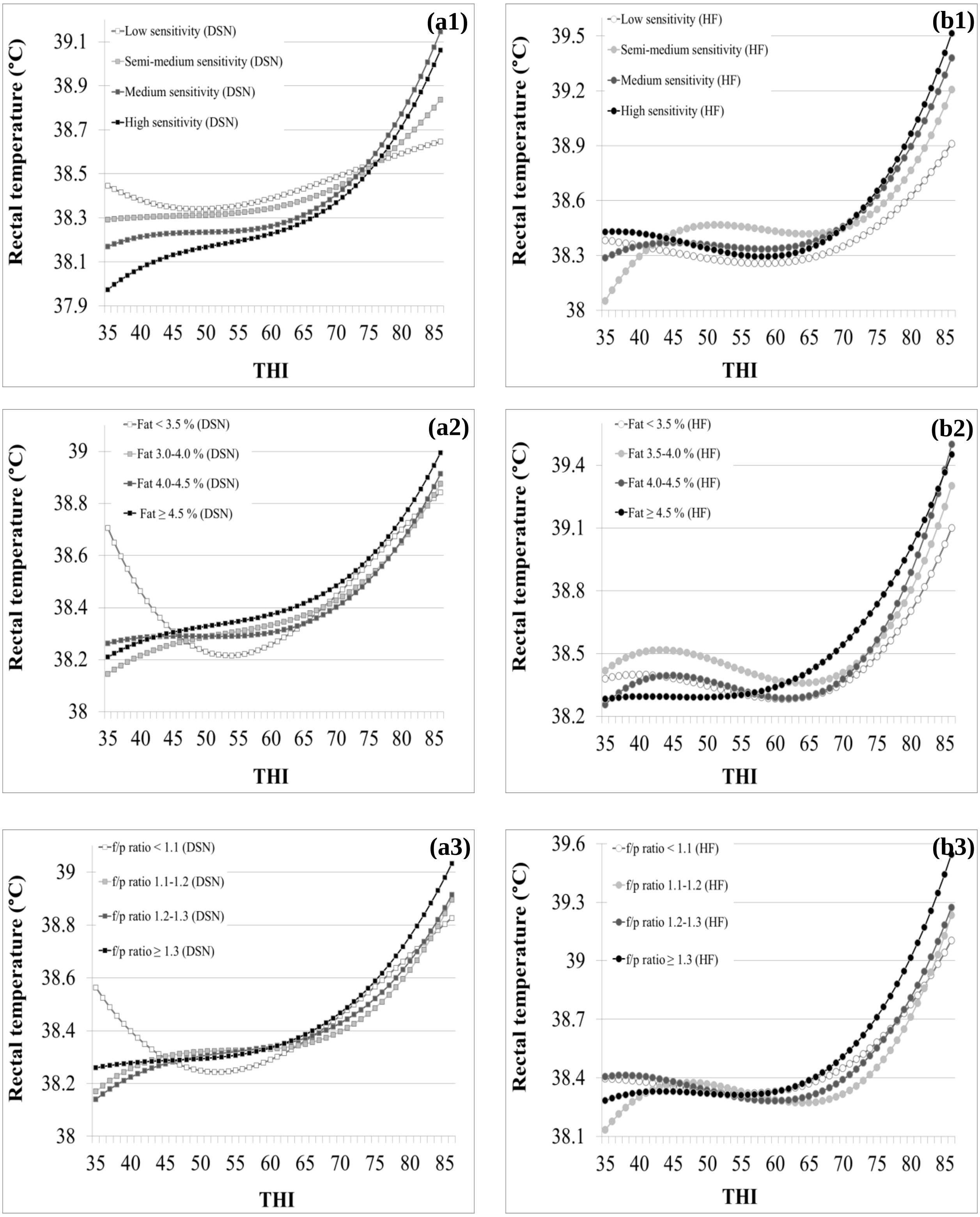

Figure 4. Effects of level heat stress sensitivity, fat %, and fat to protein ratio (f/p ratio) on rectal temperature (°C) of (a) DSN and (b) Holstein cows.

beyond the identified THI threshold associated with higher maximal RT values than DSN cows (Figure 4a). In contrast, DSN cows responded a bit earlier to increasing THI compared to HF, depicted by a slight RT increase of DSN cows within the thermo-neutral zone. The sudden elevation in physiological parameter curves of HF beyond the HS threshold may reflect high environmental sensitivity and low climatic adaptation. Also the generally higher average RT of HF (38.46 °C) compared to DSN cows (38.29 °C) (Table 1) illustrates high metabolic heat production of high yielding cattle breeds with large body size (Collier *et al.*, 2006; Lamp *et al.*, 2015). The optimal THI range for RT in this study, characterized by minor RT changes by alterations of THI, was identified from THI 50 to THI 65. This optimal THI range is in agreement with results for physiological fertility traits from HF bulls (Al-Kanaan *et al.*, 2015).

In both breeds, cows classified as high SENSI group showed highest RT during all heat stress level (= THI threshold > 65), whereas lowest RT were identified for the SENSI group characterized by lowest within-cow standard deviations (Figures 4a1 and 4b1). Lower RT of low SENSI groups within the “heat stress zone” reflects better adaptation of those cows showing quite equal physiological response during aging. Nevertheless, reasons for adaptation, here expressed by similar RT response in a time frame of 3 years, need to be identified in ongoing studies. Collier and Collier (2012) reported the long-term potential for adaptation, and referred to a research study where animals with generally high environmental sensitivity realized metabolic and physiological adjustments only after continuous confrontation to harsh environments. In this adaptive process, also modifications on the genetic scale might play an important role.

Cows with highest values for test-day fat % (fat % > 4.5 %) (Figures 4a2 and 4b2), and with highest fat to protein ratio (f/p ratio > 1.3) (Figures 4a3 and 4b3) tended to be the most susceptible group to heat stress. This finding is illustrated by highest RT values of these cow groups for THI > 65. Nevertheless, there is a general antagonistic relationship between milk yield and fat %, and detrimental effect of THI on milk yield (Brügemann *et al.*, 2012) in turn increases milk percentage traits. An increasing fat to protein ratio in milk is an indicator of negative energy balance in milking cows. A negative energy balance was observed especially for stressed cows with low levels of oxytocin (Santos *et al.*, 2015). Hence, results from the present study support those identified relationships between stress components (here: HS), body fat mobilization, and physiological body reaction expressed by increasing RT. Strong relationships between HS and high milk percentage traits and increasing RT were identified by (Bouraoui *et al.*, 2002), especially during the hot summer period in Tunisia.

Suthar *et al.* (2012) pointed out that body temperature in cattle is a more sensitive indicator for HS identification compared to, e.g., increasing sweating or reduced dry matter intake. Hence, longitudinal RT measurements can be used to monitor or to predict a cow's health status which is current practice in large-scale contract herds in the eastern part of Germany. RT reflects the degree of adaptability of livestock to the particular environment, since elevation of this parameter strongly indicates hyperthermia. An increase in 1 °C as a response of HS, is sufficient to significantly reduce cattle performance (Maurya *et al.*, 2015). RT was also highly significant ($P < 0.0001$) correlated with other physiological traits used in this study. The largest phenotypic correlation was found between RT with VT (0.75), and correlations were moderate with RR (0.32), STu (0.24), STb (0.24), STw (0.25), and STl (0.25). The only low correlation was found between RT and PR (0.12) (Table 3).

Table 3. Phenotypic correlations (number of observations) among physiological traits in the present study.

	RT	Upper	Back	Withers	Line	VT	RR
Upper	0.24**** (3036)						
Back	0.24**** (3036)	0.99**** (3036)					
Withers	0.25**** (3036)	0.96**** (3036)	0.97**** (3036)				
Line	0.25**** (3036)	0.98**** (3036)	0.99**** (3036)	0.98**** (3036)			
VT	0.75**** (1006)	0.19**** (837)	0.20**** (837)	0.19**** (837)	0.22**** (837)		
RR	0.32**** (1904)	0.60**** (1680)	0.60**** (1680)	0.59**** (1680)	0.60**** (1680)	0.18**** (974)	
PR	0.12**** (2022)	-0.15**** (1849)	-0.15**** (1849)	-0.14**** (1849)	-0.14**** (1849)	0.07* (977)	-0.07** (1809)

*= $P < 0.05$, **= $P < 0.01$, and ****= $P < 0.0001$

3.2.2. Skin temperature

Skin temperature measured from the four different areas (upper, back, withers and line) showed strong similarities regarding least square means in the course of increasing THI. Similarity is also expressed by highly phenotypic correlation among the four different ST traits in the range from 0.95 to 0.99) (Table 3). With regard to significance values (Table 2), STu was the most sensitive ST trait in response to environmental HS. Also the average of STu was higher than other skin areas (Table 1), and LSmeans least square mean curves are characterized by highest values in high yielding cows (Figure 5).

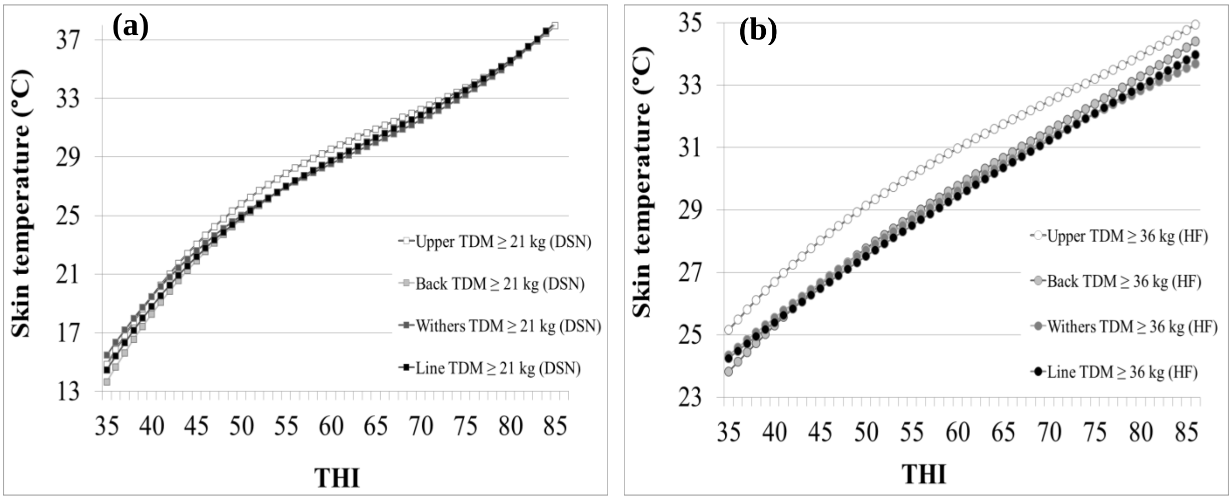

Figure 5. Effect of test-day milk yield (TDM) for high yielding (a) DSN (TDM > 21 kg) and (b) Holstein (TDM > 36 kg) cows on skin temperature (°C) measured from upper, back, withers, and line in the course of THI.

As additionally outlined in Table 1, skin temperatures of HF cows (STu = 29.65 °C, STb = 29.05°C, STw = 29.27 °C, and STl = 29.15 °C) were larger than corresponding traits in DSN (31.06 °C, 30.10 °C, 30.03 °C, and 29.93 °C, respectively). Regarding breed comparisons, also least square means of STu from high-yielding HF cows (TDM > 36 kg; Figure 5b) were higher than STu of the high-yielding DSN group (TDM > 21 kg; Figure 5a). Interestingly, STu of DSN cows was characterized by a wider range of temperatures (8.54 to 43.20 °C) compared to the limited but higher temperature range of HF (17.74 to 37.53 °C). Larger values of STu for HF may be due to the higher milk production and larger body size, being associated with extra heat production and advanced difficulties in thermoregulation of body and surface temperatures in harsh environments (Alfonzo *et al.*, 2015). In detail, Alfonzo *et al.* (2015) identified that HF cows had more difficulties in dissipating heat than Brazilian Girolando due to their larger body size, as well as due to their thicker and longer hair.

We observed a very interesting continuously increase of STu with increasing THI to a ceiling value (Figure 6). This remarkable gradual increase of ST with increasing THI complicates the identification of an obvious heat stress threshold. By trend, first parity cows are characterized by slightly lower STu (Figures 6a2 and 6b2) compared to multiparous cows. As an explanations, first parity cows produce less metabolic heat, have a less body size, and produce less milk than cows in remaining parity groups (West, 2003; Collier *et al.*, 2006; Novak *et al.*, 2009; Gauly *et al.*, 2013; Bernabucci *et al.*, 2014). Also increasing levels of protein % in milk led to a significant increase ($P < 0.05$) of upper ST of Holstein cows at the extreme end of the THI scale (Figures 6a3 and 6b3). In Australian HF, Cowley *et al.* (2015) also simultaneously studied physiological body responses and protein % due to HS, and found pronounced detrimental impact of HS on physiological traits and on the milk protein composition. They concluded that the decrease in protein % caused by reduced protein synthesis is a result of changes in physiology and metabolism of the mammary gland, and not related to the general reduction in milk yield.

In livestock, skin is considered as an important pathway for heat exchange, and ST is the result of regulation of this exchange between the body core and skin by blood flow. Thus, measuring surface body temperature by using infrared thermography, was considered as a suitable method for stress identification in cattle (Collier *et al.*, 2006). IRT as a technique that is based on a non-invasive, simply handled method, with specific analyzing software, represents a promising tool for recognizing HS in large numbers of farm animals within an acceptable time span. Modern IRT technology is also characterized by a low cost approach and quite easy to handle by trained persons from an observer to cow distance that may avoid additional stress symptoms.

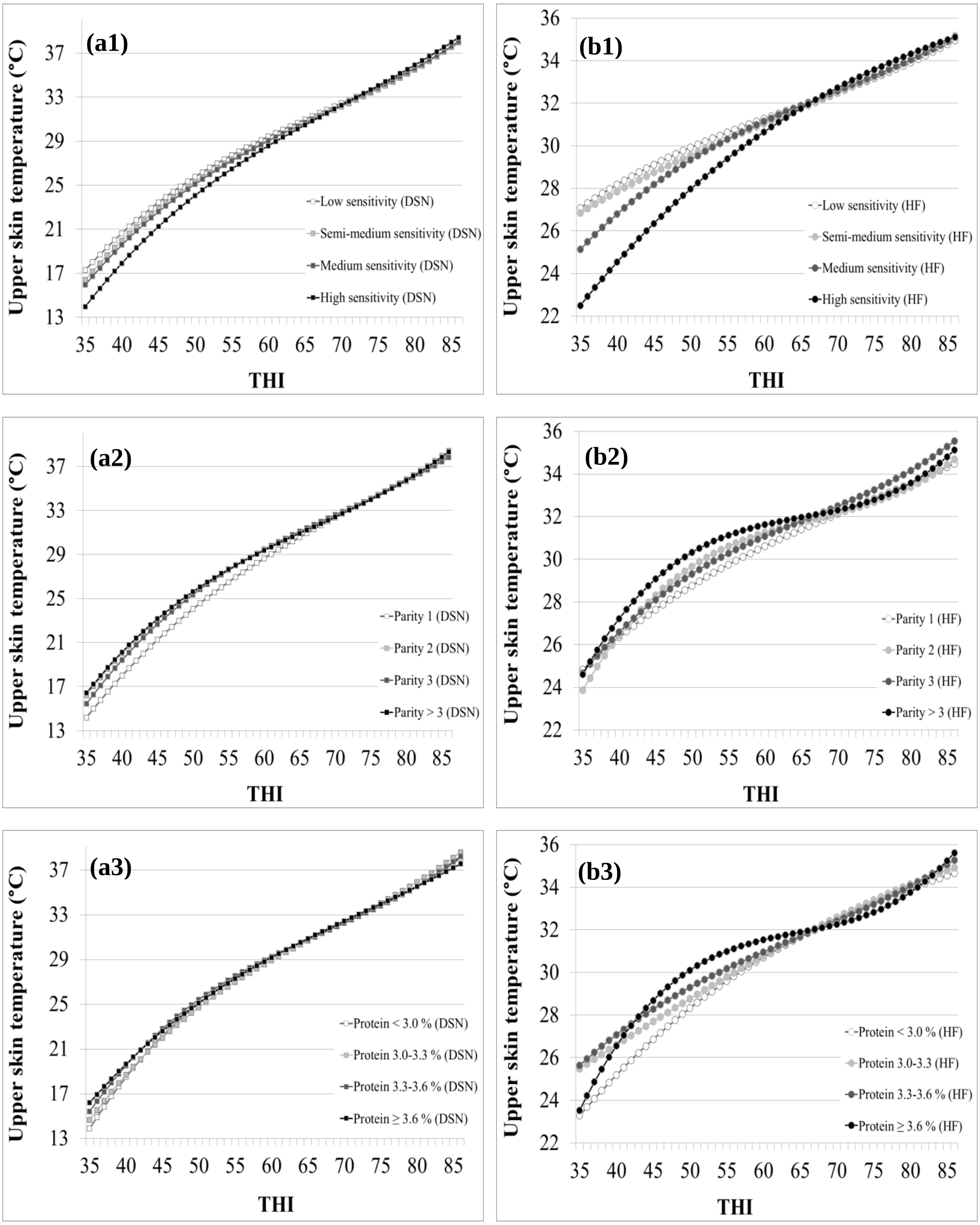

Figure 6. Effects of level heat stress sensitivity, parity, and protein % on upper skin temperature (°C) of (a) DSN and (b) HF cows.

3.2.3. Vaginal temperature

Least square means of vaginal temperature tends to increase in dependency of THI during the high level of thermal stress (Figure 7). At the lower end of the THI scale, VT was quite high, and afterwards decreased in the "cow comfort zone". The progressive VT decrease was observed up to THI 55. Results of this study indicate that VT is more sensitive to HS than RT, because the VT heat stress threshold (THI = 55) is lower than the visual threshold for RT (THI = 65). Pronounced heat stress sensitivity for VT compared to other body temperatures was also outlined by (Dikmen *et al.*, 2014), and suggests the use of VT for genetic selection.

When evaluation the effect of days in milk in the course of THI, VT of cows at an early stage of lactation (DIM < 70 days) were higher than for the late lactation period (Figure 7b). Mid-lactating dairy cows (DIM 150 to 240) only showed minor VT reactions with increasing HS. Similar response curves were observed for RT and RR. Own results are in agreement with findings by Novak *et al.* (2009), who inferred that cows from the first stage of lactation (0 to 50 days) were the most sensitive group, and were significantly affected by HS. In opposite, Lambertz *et al.* (2014) identified most obvious HS effects during late lactation (201 to 305 DIM). Conversely, Gauly *et al.* (2013) reviewed that the mid-lactating cows are the most heat sensitive individuals due to a high body heat production associated with high milk yield. Therefore, to alleviate effects of HS on dairy cows well-being, Gauly *et al.* (2013) suggested inducing of calving seasons, so that the cow spends the late lactation period or the dry period during warm summer seasons.

In analogy to RT, DSN cows with a high fat to protein ratio strongly responded with increasing VT on increasing heat stress for THI > 65 (Figure 7c). Again, this finding also for the physiological trait VT indicates that a high metabolic rate (= mobilization of body fat depots) is associated with higher heat production (Collier and Collier, 2012), especially when additional environmental heat stress occurs (Bouraoui *et al.*, 2002). Modelling RT as an additional fixed effect significantly influenced all physiological traits of DSN cows in the current study. Basically, cows allocated to the highest RT group had highest VT values during THI, especially beyond the identified THI threshold (Figure 7d). Only minor alterations of VT with increasing THI were identified for the cow group with lowest RT. In analogy to VT, same trends were observed for STu and RR. Singh *et al.* (2012) also focused on the positive correlation between RT with the other physiological traits RR and PR, with environmental heat stress indicators, and with biochemical parameters of local Indian cattle.

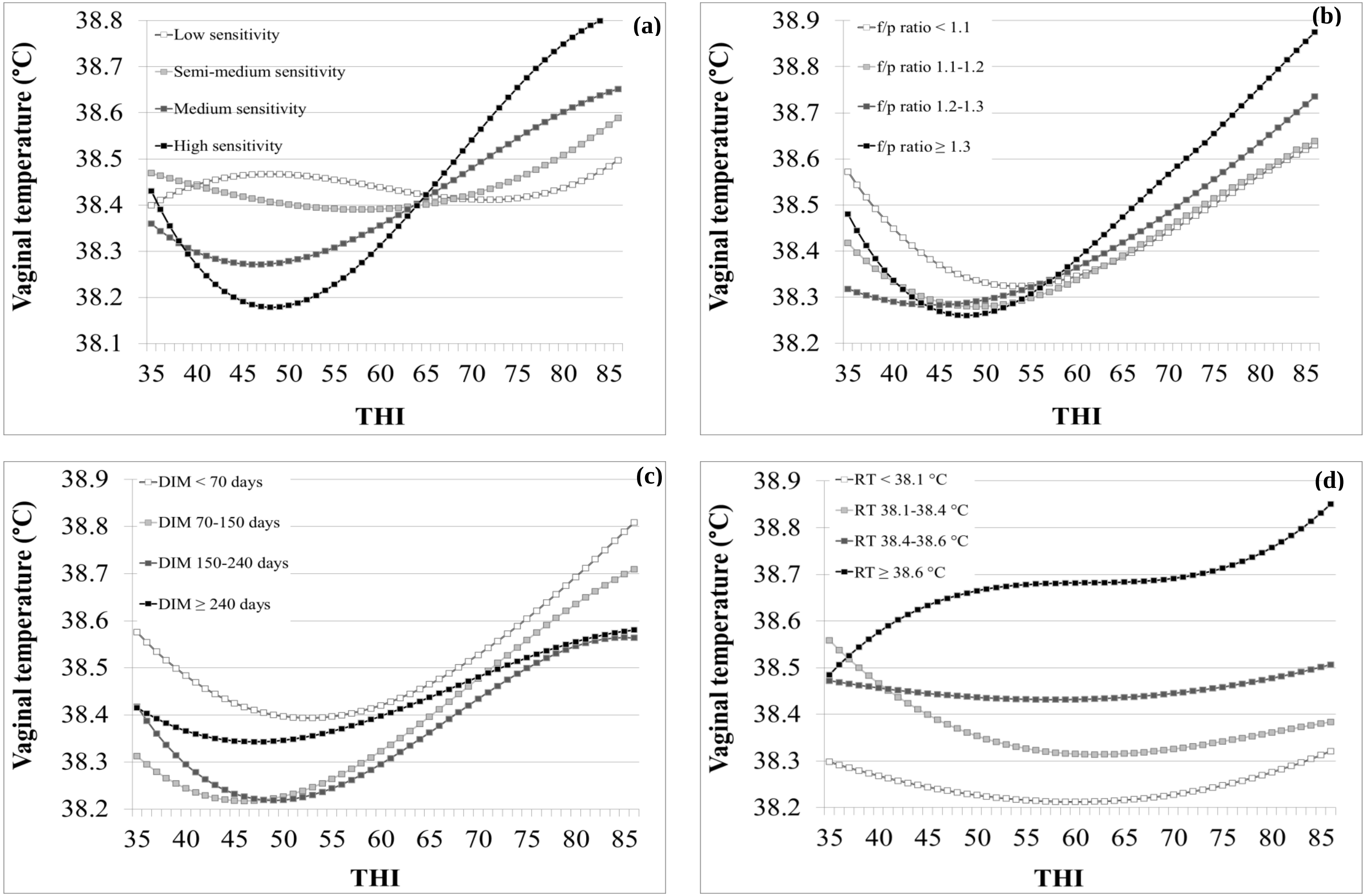

Figure 7. Effects of level of a) heat stress sensitivity, b) fat to protein ratio, c) days in milk and d) RT (°C) on vaginal temperature of DSN cows.

In mammalian, the basic thermoregulatory strategy is to maintain a higher body temperature that is higher than the ambient temperature to allow heat flow from the core body towards the surrounding environment, with the ultimate goal to keep body temperature in constant balance (Collier *et al.*, 2006). If primary behavioral and physiological mechanisms are not sufficient to control thermal balance, first animal signs are increasing RT, VT and ST, as well as a substantial reduction of dry matter intake (Dikmen *et al.*, 2014). Hence, those body temperature indicators also have been used in harsh environments for an early detection of infectious diseases (Suthar *et al.*, 2012). VT of dairy cattle generally shows pronounced susceptibility to thermal load, and it has considered as a sensitive indicator of thermal stress (Kadzere *et al.*, 2002; Suthar *et al.*, 2012; Gauly *et al.*, 2013). Recently, models have been developed in cattle to predict physiological responses of RT, VT, and RR with regard to specific environmental thermal conditions, based on measurements for ambient temperature and humidity (Hernánde-Julio *et al.*, 2015).

3.2.4. Respiration rate

With increasing THI, we observed a simultaneous increase of RR, especially for THI > 65 (Figure 8). As a response of high ambient temperature, homoeothermic animals enhance RR in order to dissipate additional body heat via vaporization in the surrounding environment (Singh *et al.*, 2012). This increasing loss of water from the respiratory tract by panting accounts for about 60% of the total heat loss, whereas the remaining body heat dissipates from the skin surface by sweating (Cwynar *et al.*, 2014). On the other hand, with increasing RR during HS, respiratory alkalosis and respiratory acidosis are important ruminal disorders due to fluctuations of rumen pH and bicarbonate (Kadzere *et al.*, 2002). Exposing cattle for a long time to HS, increasing RR may also lead to deteriorations in health status, e.g. lameness (Kadzere *et al.*, 2002).

For RR, the identified visual THI threshold was THI 65, with an optimal THI range from THI 50 to 65. This threshold value is in close agreement with the viewpoint of Gauly *et al.* (2013), who emphasized that THI 68 is a reliable HS indicator for dairy production in the center of Europe. Accordingly, Zimbelman *et al.* (2009) illustrated that RR was negatively affected starting from THI 68, especially for high yielding dairy cows. Previous studies indicated that RR is positively correlated with ambient temperature and THI. In consequence, also RR was suggested as a valuable indicator for thermal stress in cattle (Zimbelman *et al.*, 2009). In the latter study, HS was defined as an increase in RR by 2 breaths / min per increase in one THI unit. Nevertheless, this increase varied from 2.8 to 3.3 breaths / min per 1°C increase in ambient temperature in the study by (Gaughan *et al.*, 2000), and was additional five inspirations per minute when THI increased from 68 to 78 (Bouraoui *et al.*, 2002). In our present study, the least square mean for RR was about 34 breaths / min at THI 65, and increased to a maximal value of 82 breaths / min at the maximal THI (= THI 86). A simultaneous increase in RR was reported by (Berman, 2005), who defined the stress threshold for RR with 60 breaths / min. Also RR is a valuable and sensitive HS indicator, because RR is quite easy to measure and to monitor, without the need for any costly equipment or causing further cow stress (Scharf *et al.*, 2012).

Generally, RR significantly increased with increasing THI, and most pronounced for increasing levels of SENSI, parity, fat %, and RT (Figure 8). Also increasing levels of milk urea nitrogen and SCC, especially in warm summer months (results not shown) were associated with increasing RR. The highest significant effect on RR ($P < 0.0001$) was due to SENSI, which is also illustrated by curve developments for THI > 65 (Figure 8a). Relationships between the potential for adaptation and physiological response traits were also discussed by Al-Kanaan *et al.* (2015) and Hammami *et al.* (2013). Generally, higher sensitivity of European cattle might be explained by the intensive selection for high milk yield during the last decades by neglecting adaptive aspects (Kadzere *et al.*, 2002; Hammami *et al.*, 2009). First parity cows had lowest RR for extremely high THI, while highest RR was identified for cows assigned to parity > 3 (Figure 8b). This is in analogy with results for RT, STu and VT, where also multi-lactating cows (parity > 3) increased their RR with increasing THI during the “heat stress zone” (results are not shown). Again, we see the causality of high metabolic heat production associated with large body size and high milk production, especially for cows in higher parities as discussed in previous studies, e.g. by (West, 2003; Collier *et al.*, 2006; Novak *et al.*, 2009; Gauly *et al.*, 2013; Bernabucci *et al.*, 2014). Furthermore, Bernabucci *et al.* (2014) additionally identified larger variations for heat tolerance of third parity compared to first parity cows, also on genetic scales. Fat % significantly affected RR, so that the detrimental impact of HS on RR was most obvious for the high fat % group (Figure 8c). The same trend, i.e. increasing RR with increasing milk composition traits, was confirmed by Karimi *et al.* (2015). The highest RR response to HS was identified for cows with the highest RT level (RT > 38.6 °C), followed by the medium

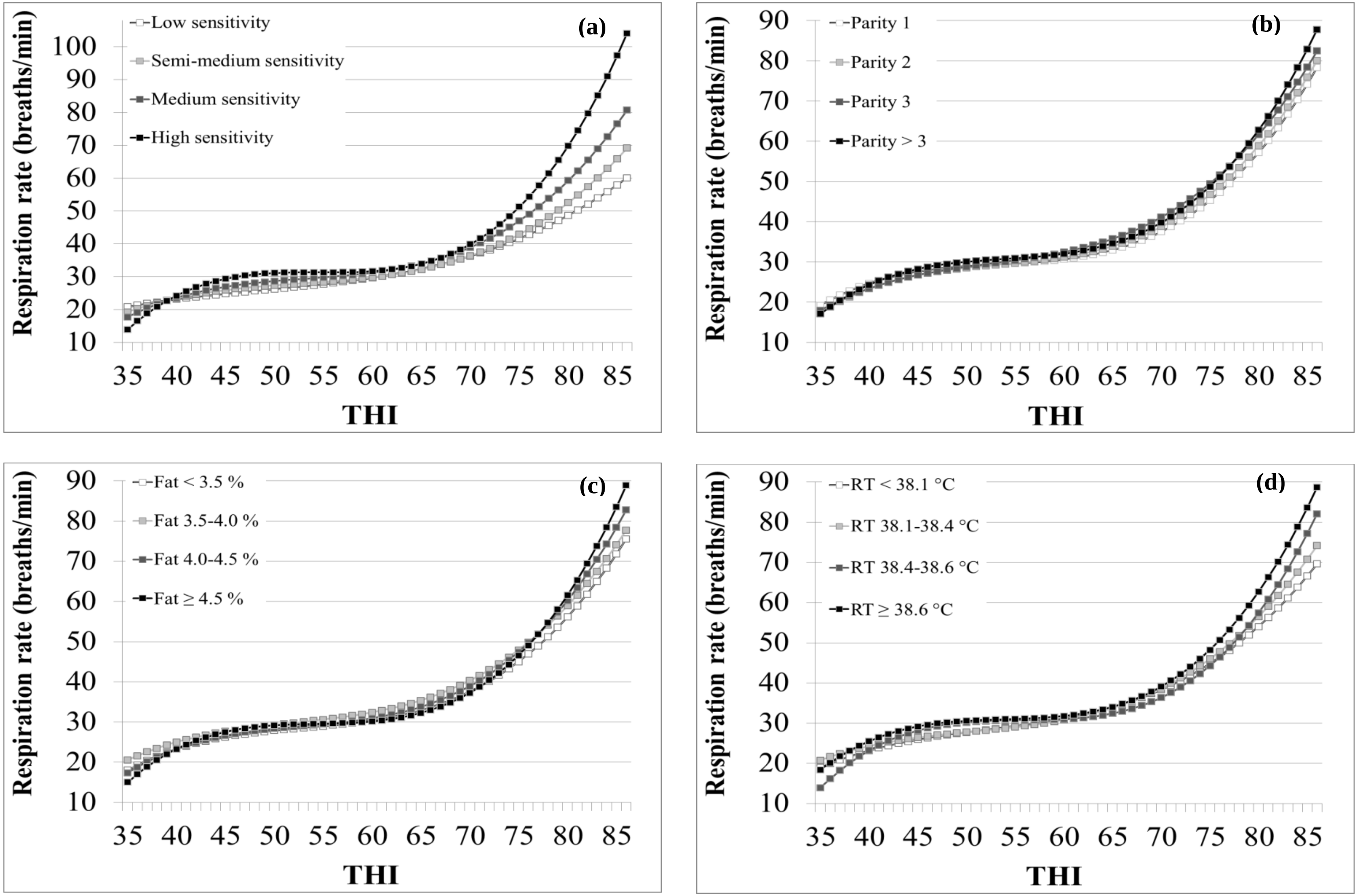

Figure 8. Effects of level of a) heat stress sensitivity, b) parity, c) fat %, and d) RT (°C) on respiration rate of DSN cows.

RT groups. Cows with lowest RT (RT < 38.1 °C) showed significantly lowest RR (*P < 0.001*) (Figure 8d).

3.2.5. Pulse rate

Interestingly, and in contrast to other physiological traits in this study, PR showed a decline during high THI levels (Figure 9). Decreasing PR might be due to the fact that cows were not acclimatized to HS conditions, and PR reduction is an attempt to reduce activities and heart rate frequencies. In this context, Kadzere *et al.* (2002) mentioned that a decreasing heart rate is typical in cattle, because a decrease in heart rate is associated with a reduced heat production. Least square means for PR curves started with high values during the "cold period", and then dropped to intermediate values in the comfort zone. The substantial decline in PR occurred after THI > 70, suggesting that the suitable threshold for PR is THI 70. The optimal THI range showing only minor PR fluctuations was in the range from THI 50 to THI 70. The delayed PR response due to HS, as illustrated by the high THI threshold, suggests to prefer the other physiological traits RR, ST or RT as indicators for HS, especially in the temperate climate in middle Europe. The less pronounced reaction of PR on increasing HS compared to RT, ST, VT, and RR was previously described by Muller and Botha (1993). Those authors summarized that European cattle, exposed to moderate heat, showed a decrease in PR by utilizing mechanisms of peripheral vasodilation. In contrast, in extreme HS environments, animals responded with increasing PR in the course of increasing temperatures. In a related research design, Cwynar *et al.* (2014) found an increase in heart rate during moderate HS from THI 61 to TH 82, and heart rate decrease in extremely hot climates from THI 82 to THI 112. They explained heart rate elevation during moderate HS by an increase of blood flow from the core to the skin surface, in order to induce heat emission via conduction, convection and radiation, and also through insensible reactions, e.g. sweating. Increasing PR was also observed by Singh *et al.* (2012) during the hot summer months in Sahiwal cattle, while only minor PR responses were reported during the moderate spring season. Kadzere *et al.* (2002) remarked that heart rate and associated PR increase is only a reaction to HS in a short-term perspectives, and again decreases for lasting HS periods.

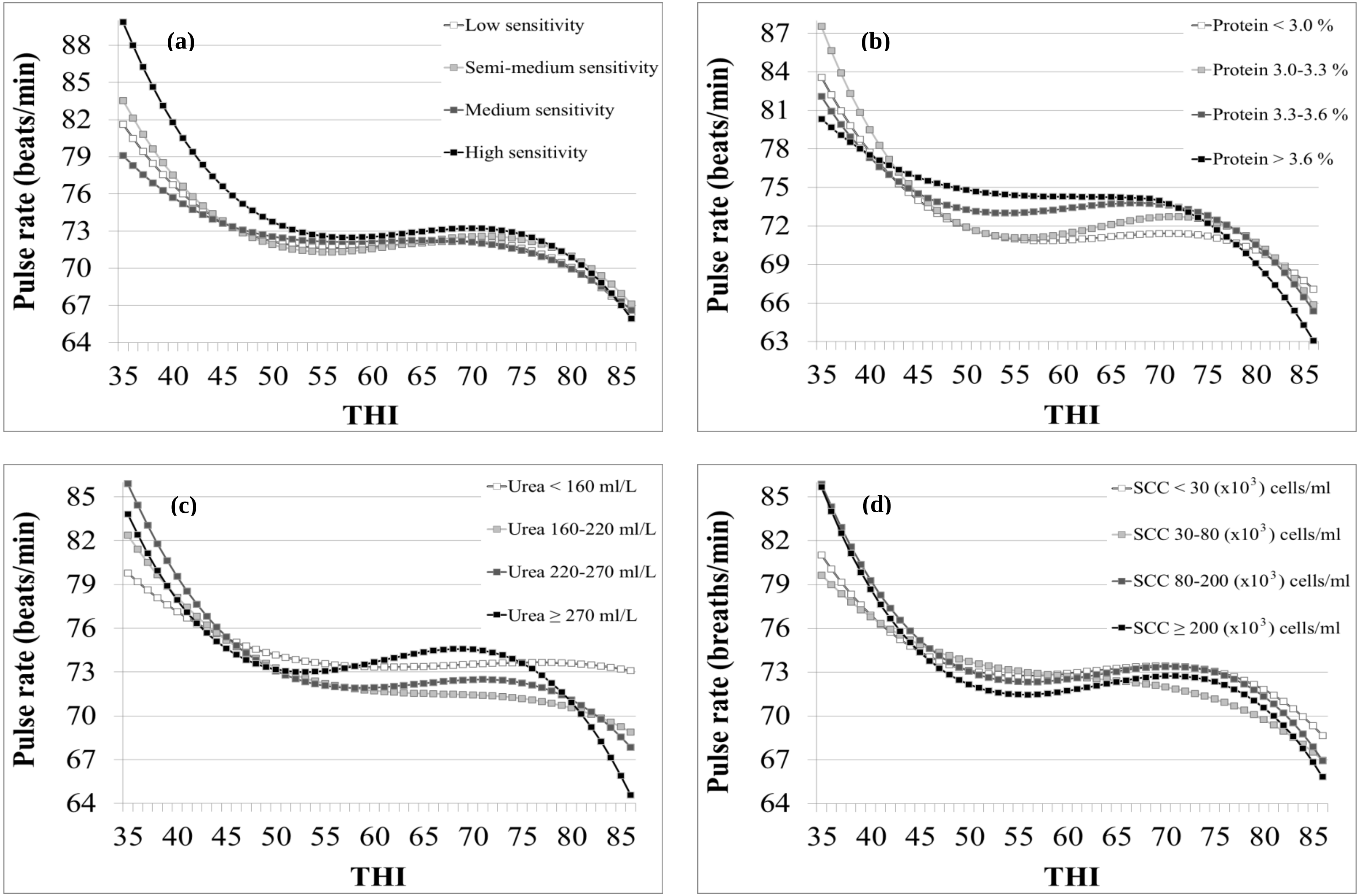

Figure 9. Effects of level of a) heat stress sensitivity, b) protein %, c) milk urea nitrogen and d) SCC in milk on pulse rate of DSN cows.

For increasing THI beyond the identified threshold value, the decline in PR was most obvious for cows allocated to the highest levels of SENSI, protein %, milk urea nitrogen and SCC (Figures 9a, 9b, 9c, and 9d, respectively). Cows characterized by high protein % (> 3.6 %) showed highest PR during the THI comfort zone, but their PR substantially declined for THI > 70. (Figure 9b). Cowley *et al.* (2015) also pointed out that heart rate decrease during periods of HS was accompanied with a reduction in milk protein and casein fractions. These authors hypothesized that the decline of heart rate in response to HS was due to reduced dry matter intake, causing deceleration of an animals' metabolism.

Although the effect of SCC on PR was not significant ($P > 0.05$), the high SCC groups showed more pronounced PR depressions for extremely high THI values compared to the low SCC group (Figure 9d). Bouraoui *et al.* (2002) indicated an undesired effect of HS on increasing SCC (from 4.1 to 8.6 x 10^5 cells/ml), which was associated with detrimental impact on further physiological parameters during the summer season. A low SCC value is also an indicator reflecting improved farm management. In the present study, especially the DSN cows from the organic low input herd with high SCC reacted with decreasing PR (Figure 9d), but only minor reactions were found for intensively managed HF cows (results not shown). The average SCC of HF cows was substantially lower compared to DSN (184.07±21.92 x 10^3 cells/ml vs 298.20±23.52 x 10^3 cells/ml). According to Smith *et al.* (2013) and Lambertz *et al.* (2014), SCC and SCS is one of the most important indicators for udder health and milk quality, and this indicator is strongly influenced by climatic changes. Further, somatic count was defined as a valuable trait to study a cow's immune response to general stress situations (Brügemann *et al.*, 2012). It is noteworthy that HS impaired the mammary defense mechanisms of high SCC cattle (Bouraoui *et al.*, 2002), and increased the prevalence and incidence of udder inflammation (Lambertz *et al.*, 2014). An increasing number of leukocytes in milk might stimulate cows to change physiological mechanisms when HS occurs.

4. Conclusion

The physiological traits RT, ST, VT, RR, and PR were identified as valuable HS indicators. Beyond an identified HS threshold, cows responded with abnormal physiological reactions, e.g. increasing body and skin temperatures, or increasing breaths per minute. For most of the physiological traits, THI 65 was identified as a general HS threshold. Only for PR, we found delayed reactions, and we identified the threshold THI 70 for abnormal reactions. Abnormal physiological reactions were additionally observed for extremely low THI values, whereas the general "comfort zone" characterized by quite constant physiological responses, ranged from THI 50 to THI 65. The environmental stress increase from THI 65 (threshold) to THI 86 (maximal THI) simultaneously increased RT by 0.6 °C (DSN) and 1 °C (HF), ST by 3.5 °C (HF) and 8 °C (DSN), VT by 0.3 °C (DSN), RR by 47 breaths / minute (DSN), and decreased PR by 7 beats / minute (DSN). The increase of RT, ST, VT, and RR, and the decrease in PR, was more pronounced for cows with high level of milk yields and milk percentage traits, for cows in later parities, for cows in an early lactation stage, and during summer seasons in the year 2014. Interestingly, DSN cows responded earlier to HS compared to HF, presumably due to the effect of selection. Intensive selection in the HF population is associated with reduced phenotypic and genetic variances for production and functional traits. In conclusion, we identified HS sensitivity in dual-purpose DSN as well as in HF cows through alterations of physiological traits beyond THI thresholds. Hence, we suggest routinely monitoring of those physiological parameters in order to assess environmental stress, and to improve health and welfare of milking cows.

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Chapter 4

Exploring the genetic background of physiological traits in harsh environments using two selected lines of native black and white cattle

Al-Kanaan, A.[*,§], K. Brügemann[*], M. Mahmoud[*], E. Gernand[§], and S. König[*]

[*]Department of Animal Breeding, University of Kassel, 37213 Witzenhausen, Germany

[§]Department of Animal Production, College of Agriculture, University of Basrah, Basrah, Iraq

[§] Thuringian State Institute of Agriculture, 07743 Jena, Germany

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ABSTRACT

Physiological traits including 3679 observations for rectal temperature (RT), 3211 observations for skin temperature (ST) measured from four different body areas, 1012 observations for vaginal temperature (VT), 2036 observations for respiration rate (RR), and 2191 observations for pulse rate (PR) were recorded on 238 native dual-purpose black and white cows (DSN), and their genetically upgraded crosses with Holstein Friesian sires (genetic group DSN_HF). The coefficient for average genetic relationships between both lines was 0.01. Cows were kept on two experimental herds in pasture based production systems reflecting a harsh environment. The DSN herd followed organic guidelines with low input feeding strategies and without feeding any concentrates, whereas the selection line DSN_HF strongly focused on productivity improvements by considering concentrate supplements. The aim of this study was to infer genetic (co)variance components of physiological traits in relation to environmental alterations. The environmental descriptor used in this study was a temperature humidity index (THI), calculated on the basis of meteorological data which were directly recorded in the farm environment. For the estimation of genetic (co)variance components of the longitudinal physiological trait data structure, two statistical models were applied: First, a repeatability model (RM), and second a random regression model (RRM) with THI as a continuous covariate. Heritability estimates from the RM were as follows: 0.066 for RT, 0.018 to 0.035 for ST based on different areas of body surfaces, 0.037 for VT, 0.048 for RR, and 0.072 for PR, with acceptable SE in the range from 0.022 to 0.044. Taking into account the permanent environmental variance component, repeatabilities were about 10% for all traits. Afterwards, we separated the dataset into two subsets by defining a THI 65 threshold. Apart from VT and RR, additive genetic variances, permanent environmental variances, heritabilities and repeatabilities were smaller in the dataset reflect a harsh heat stress environment (THI > 65). Correlations between EBV in the same trait for cows with records across both environments were 0.399 for RT, 0.166 to 0.294 for different ST definitions, 0.304 for VT, 0.537 for RR, and 0.542 for PR. Taking into account the EBV reliability, genetic correlations throughout lower than 0.80 indicated genotype by environment interactions. Highest additive genetic variances at a cows’ comfort zone (THI < 60) and genotype by environment interactions were confirmed by applying RRM. Nevertheless, the RRM estimated increased variances at both extreme ends of the continuous THI scale, probably due to the limited number of data for extreme levels of THI. Moderate EBV correlations were identified between different trait combinations, meaning that selection for a physiological trait A simultaneously genetically improves a physiological trait B. In conclusion, it might be possible to implement genetic evaluations for physiological traits. Accuracy of predictions will increase when setting up a data structure for repeated measurements. For example, gain in prediction accuracy for RR increased by 34% when using two observations per cow instead of one single record.

Key words: Physiological cow traits, heat stress, genetic parameters, genotype by environment interactions

1. INTRODUCTION

The breeding history of German black and white cattle is described in detail by Mügge *et al.* (1999). In brief, a nucleus including ~8,0000 native dual-purpose cattle of the breed *Deutsche Schwarzbunte Niederungsrind* (**DSN**) was exported from the grassland regions in North-West Germany towards North-America more than 130 years ago. With the availability of specific beef cattle breeds in the US and Canada, North American farmers were encouraged focusing DSN breeding only on milk by totally neglecting the beef component. As a result within a breeding period of less than fifty years, a modern Holstein Friesian (**HF**) dairy cow was developed. Availability of artificial insemination and the possibility of sire semen transfer without time and country border restrictions, also initiated HF progeny testing programs (Skjervold and Langholz, 1964), and in further consequence rigorous replacement breeding of DSN with HF starting in the 1970s in Germany. Such a breeding strategy on milk yield was justified due to the economic pressure, resulting in breeding goals or selection indices with a strong emphasis on productivity, especially in the eighties and nineties of the 20th century (Miglior *et al.*, 2005). Only a few German DSN breeders consequently relied on the original breeding strategy by still considering the meat component via traits like muscling score or daily gain. Those original DSN farms are mostly located in pasture based systems at the coast in the region of East Friesland, but also to a lower extent in other parts of Germany (Jaeger and König, 2015). Common DSN farm characteristic is the focus on grazing systems, mostly integrated in the general framework of low input or organic production systems. Adaptation to harsh environments in outdoor systems was reflected by an improved health and welfare status (Brügemann *et al.*, 2015), but also by improved parasite (May *et al.*, 2016) and heat stress (**HS**) resistance (Al-Kanaan *et al.*, 2013) of DSN cows in comparison to high yielding Holstein cows.

It is especially the HS component addressing increasing attention when keeping cows in harsh environments, e.g. pasture based production systems. Importance is due to detrimental impact of HS on cow productivity and on cow wellbeing, both contributing to increasing economic loss. The temperature humidity index (**THI**) is the most world-wide used indicator to depict HS in dairy cattle, as it measures the combined effects of ambient temperature and relative humidity. Even under the temperate climatic conditions in Germany, increasing THI was associated with a decline in milk yield (Gauly *et al.,* 2013), with impaired female fertility (Brügemann *et al.,* 2013), and with negative impact on male fertility including both components semen quality and quantity traits (Al-Kanaan *et al.,* 2015) as well as reduction in the conception rate from 31% to 12% (Schüller *et al.,* 2014). All these German HS studies were conducted in HF populations which have been selected for indoor systems since decades, and all of the authors emphasised the rarely developed adaption mechanisms of HF cows to overcome climatic changes. Poor robustness of HF dairy cows implies a high degree of environmental sensitivity, meaning that the adverse effects of HS in Germany were identified at quite low THI thresholds (THI 60 to THI 65). For the same cattle breed kept in and adapted to tropical or subtropical countries, but exhibiting substantially lower milk productivity than German HF cows, Kadzere *et al.* (2002) reported a substantially higher threshold of THI 72.

Aguilar *et al.* (2009) stated that selection for increasing milk yield is the major reason for HS sensitivity. The heritable component of HS was determined by evaluating genetic parameters of production and functional traits being available from official recording systems in dependency of the continuous environmental descriptors THI (Aguilar *et al.*, 2010). The heritable HS component also differed according to the stage of lactation (Aguilar *et al.,* 2009; Brügemann *et al.,* 2011; Carabaño *et al.,* 2014), and according to parity, calving season and calving year (Aguilar *et al.,* 2009), and also depended on the production level and on percentages of milk components (Brügemann *et al.,* 2013; Bernabucci *et al.*, 2014;; Carabaño *et al.*, 2014; Hammami *et al.*, 2015). Different results on genetic scales for identical traits recorded in different climatic zones furthermore address the topic of genotype by environment (**GxE**) interactions (Hammami *et al.*, 2009). To overcome this problem, several authors suggested modifications of genetic evaluations, i.e. modelling genetic parameters and estimated breeding values (**EBV**) as a function of THI. So far, HS breeding strategies focused on genetic model improvements by considering THI thresholds for the conventional pattern of traits, i.e. mostly longitudinal test-day records from official milk recording schemes. A further alternative might be the implementation of direct breeding strategies on HS resistance, requiring databases and genetic evaluations for novel physiological traits.

Physiological traits including rectal temperature (**RT**), skin temperature (**ST**), vaginal temperature (**VT**), respiration rate (**RR**), and pulse rate (**PR**), were already used as an indicator for HS in studies addressing feeding strategies and feeding efficiency (Umphrey *et al.*, 2001; Ominski *et al.*, 2002; Martello *et al.*, 2015), evaluating cooling technique (Smith *et al.*, 2006; Legrand *et al.*, 2011; Ortiz *et al.*, 2015) and shade systems (Brown-Brandl *et al.*, 2005; Schütz *et al.*, 2011; Van Laer *et al.*, 2015b), and exploring mechanisms of cellular thermotolerance (Deb *et al.*, 2015; Sajjanar *et al.*, 2015). Most of these studies also analyzed associations between physiological traits with production, reproduction and animal welfare. Hence, these physiological traits play an important role in thermoregulation in mammalian, but their genetic background and their value for genetic selection towards improved HS resistance is widely unknown. Studies proved that regulation of body temperature in dairy cattle is heritable in cattle (Dikmen and Hansen, 2009; Dikmen *et al.*, 2012; Dikmen *et al.*, 2013; Dikmen *et al.*, 2014), with moderate heritability (0.17) for rectal temperature during heat stress (Dikmen *et al.*, 2012). In addition to the moderate quantitative genetic background of body temperature, Dikmen *et al.* (2014) transferred a specific single gene (*SLICK*) from Senepol cattle to US Holstein and evaluated with RT, ST and RR, and with impact on body temperature regulation during heat stress. Furthermore, using data from high throughput genotyping, single-nucleotide polymorphisms (**SNP**) significantly related with body temperature and RR were identified (Deb *et al.*, 2015; Sajjanar *et al.*, 2015; Verma *et al.*, 2015). Some SNP also play a role as functional mutations, i.e. directly affecting syntheses of heat shock proteins (Verma *et al.*, 2015; Sajjanar *et al.*, 2015; Deb *et al.*, 2015).

Genetic parameter estimation for physiological traits only considered one single record per animal (Dikmen *et al.*, 2012; Dikmen *et al.*, 2013). However, physiological traits showed substantial fluctuations on phenotypic scales with an animals aging, and with environmental changes (Al-Kanaan *et al.*, 2013). Hence, it might be imperative also for genetic studies to consider repeated measurements for physiological traits via a longitudinal data structure. Repeated measurement analysis is essential if the repeatability of the trait of interest is low, and for such cases, accuracy of prediction will substantially increase with increasing measures (Mrode, 2005).

Al-Kanaan *et al.* (2013) compared different selection lines of DSN cows, i.e. a purebred DSN low input selection line versus a high input DSN_HF line, and they reported breed specific HS responses with regard to productivity and udder health on phenotypic scales. The original DSN line was more robust, and show less HS sensitivity. The objective of the present study is to combine data from both lines in order to estimate genetic parameters for physiological traits. Genetic studies in the context of HS also imply to address the topic of GxE.

2. MATERIALS AND METHODS

2.1. Physiological cow traits and meteorological data

Physiological traits included 3679 observations for RT, 3211 observations for ST measured from four different body areas, 1012 observations for VT, 2036 observations for RR, and 2191 observations for PR. Data recording spanned a period of three years, including the measuring years 2012, 2013, and 2014. Repeated measurements were from 155 purebred DSN cows kept on an organic farm, and from 83 cows reflecting a mating strategy DSN cow with HF sire (**DSN_HF**) since 1985 kept on a high input farm. Also milk production of the conventional high input farm is based on a grazing system from April until October, and allowing seven hours per day access to pasture (from 6 am – 1 pm). The 238 DSN and DSN_HF cows had 85 different sires. The average coefficient of relationship was 0.08 within the DSN cows, 0.06 within the DSN_HF cows, and 0.01 between both groups. The current average production level of the low input DSN cows is 5177 kg milk per year, with a fat content of 4.25%, and a protein content of 3.42%. The high yielding DSN_HF cows produced 11456 kg milk per year, with a fat content of 4.19%, and a protein content of 3.39%.

Table 1 summarizes the descriptive statistical parameters for physiological cow traits stratified according to pre-defined THI thresholds, i.e. below and above THI 65. This means that cow trait recording and recording of meteorological data were from exactly the same time point. Generally, one trained person recorded the physiological traits in the afternoons inside the barn after the daily grazing period. For this purpose, we recorded meteorological data (ambient temperature and humidity) in 1-minute intervals using special data logger (Voltcraft DL-120 TH), which were installed at the level of the cow head.

Table 1. Descriptive statistics of physiological cow traits considering the whole dataset (all), and for reduced datasets according to a defined THI threshold (THI < 65 and THI > 65).

		Statistical parameters					
Traits		N	Mean THI	Mean	SD	Min.	Max.
Rectal temperature (°C)	All	3679	62.14	38.22	0.52	37.00	41.00
	THI < 65	1964	54.31	38.20	0.43	37.00	41.00
	THI > 65	3211	72.6	38.44	0.39	37.90	40.50
Upper skin temperature (°C)	All	1784	62.74	29.77	5.18	10.28	39.90
	THI < 65	1427	54.51	26.70	4.68	10.28	37.93
	THI > 65	3211	73.01	33.61	2.52	25.29	39.90
Back skin temperature (°C)	All	1784	62.74	29.12	5.58	8.54	39.67
	THI < 65	1427	54.52	25.77	4.96	8.54	39.53
	THI > 65	3211	73.01	33.29	2.80	24.07	39.67
Withers skin temperature (°C)	All	3211	62.74	29.30	5.37	8.82	43.20
	THI < 65	1784	54.52	26.13	4.69	8.82	43.11
	THI > 65	1427	73.01	33.25	3.04	22.96	43.20
Line skin temperature (°C)	All	3211	62.74	29.17	5.55	9.28	40.58
	THI < 65	1784	54.52	25.86	4.89	9.28	40.58
	THI > 65	1427	73.01	33.31	2.91	23.82	40.08
Vaginal temperature (°C)	All	1012	66.78	38.39	0.36	37.00	41.20
	THI < 65	403	54.76	38.33	0.42	37.00	41.20
	THI > 65	609	74.74	38.44	0.30	37.80	39.80
Respiration rate (breaths/min)	All	2036	63.98	34.58	14.95	15.00	129
	THI < 65	1204	57.02	28.59	8.89	15.00	69
	THI > 65	832	74.06	43.24	17.49	15.00	129
Pulse rate (beats/min)	All	2191	63.31	75.53	8.67	54.00	114
	THI < 65	1281	56.26	77.04	8.82	54.00	114
	THI > 65	910	73.24	73.40	7.99	54.00	102

A digital veterinary thermometer (Scala SC 12) was used to measure RT and VT by inserting the thermometer into the rectum and the vagina for a time period of exactly 10 seconds. Pulse rate and RR were recorded during a period of 30 seconds with a stopwatch, and afterwards both traits were converted to a per minute basis. Pulse rate reflects the number of beats, which were counted by pressing in hand fingertips on the caudal artery. Respiration rate was measured by counting the number of flank movements of the cow.

Skin temperature was recorded using an infrared camera (Trotec IC120 LV) to capture infrared thermography of the upper body part (without neck and head) from a cows' forward position. Software of this technique (Trotic IC-Report DuoVision 1.08.06S) allows the calculation of ST for desired distinct skin surfaces from different areas: a) upper ST (**STu**) = a freeform polygon for the total upper cow body without neck and head, b) back ST (**STb**) = a polygon area of the cow rump and back between pin bones and shoulders, c) line ST (**STl**) = a curved line at the back line from tail to neck, and d) withers ST (**STw**) = a circle area around withers. Areas used for the measurements of skin temperature are illustrated by Al-Kanaan *et al.* (2013).

The THI was calculated by applying the formula of the National Research Council (1971), which considers dry bulb temperature (**T °C**) and relative humidity (**RH %**) as follows:

$$\mathrm{THI} = (1.8 * \mathrm{T}\,^{\circ}\mathrm{C} + 32) – [(0.55 - 0.0055 * \mathrm{RH}\,\%) * (1.8 * \mathrm{T}\,^{\circ}\mathrm{C} - 26)]$$

This equation was also considered and evaluated in a comprehensive HS study by Bohmanova *et al.* (2007). By comparing different THI formulas, Bohmanova *et al.* (2007) concluded that dry bulb temperature was the most important parameter when assessing heat tolerance of cattle in dry climates, whereas humidity was more valuable for the identification of HS in humid climates. During the time of data recording, we observed different climatic conditions in the two farms. With regard to maximum daily THI, a substantially higher percentage of cow records was identified in the DSN_HF farm beyond pre-defined THI thresholds 60, 65, 70, 75, and 80 (Table 2). Highest monthly THI and indicating periods of general HS in DSN and HF farms (assuming THI > 65 from a previous study as an obvious indicator for HS ; Brügemann *et al.* 2012) were observed during the summer month June, July, and August (Figure 1). According to the percentage distributions of observations above defined HS thresholds (Table 2), values for average monthly THI, maximal monthly THI, minimal monthly THI, average monthly relative humidity, and dry bulb temperature were substantially higher in the DSN_HF farm compared to measurements inside the DSN farm

Table 2. Percentages of observations for physiological traits recorded beyond defined heat stress thresholds.

Threshold	Farm 1 (DSN cows)	Farm 2 (DSN_HF cows)
THI > 60	51.42 %	61.96 %
THI > 65	37.51 %	47.82 %
THI > 70	24.72 %	30.80 %
THI > 75	11.93 %	12.85 %
THI > 80	03.08 %	03.21 %

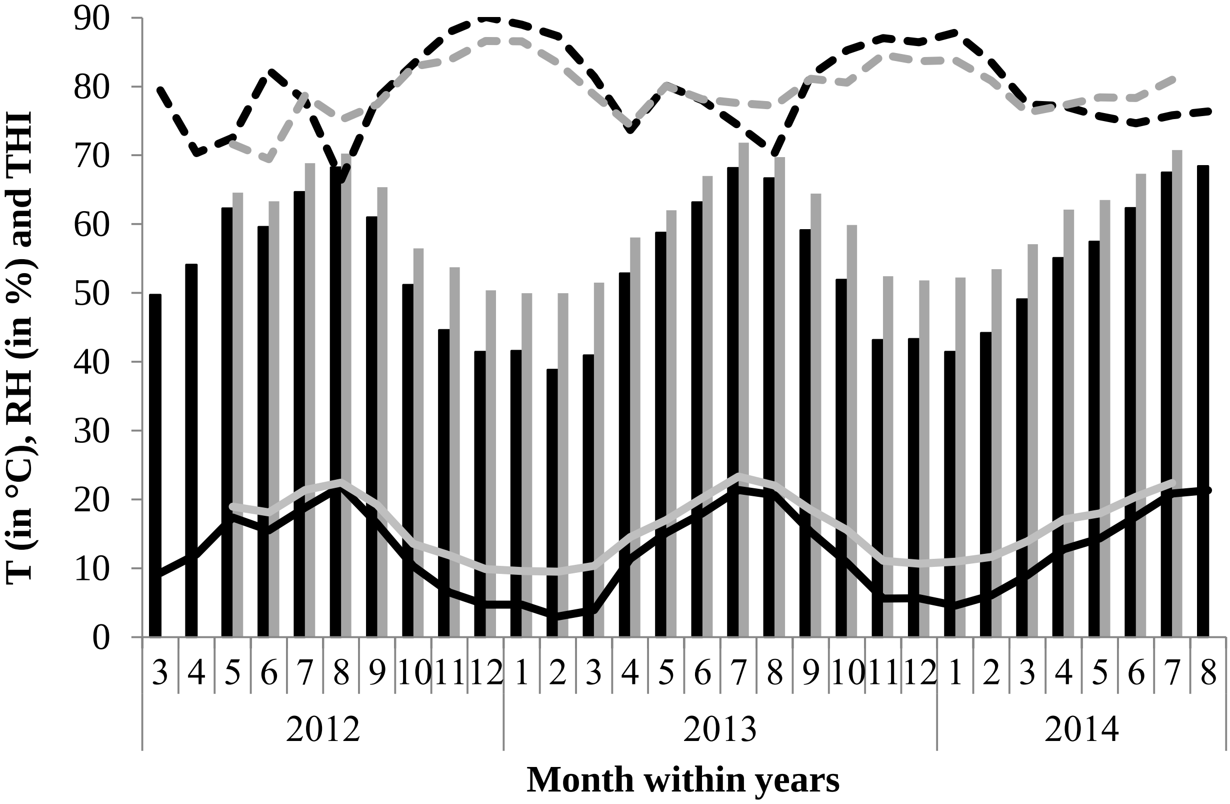

Figure 1. Monthly averages for dry bulb temperature (in T °C; solid line), relative humidity (in RH %; dashed line), and temperature-humidity indices (bars) for the years 2012, 2013, and 2014 for the DSN farm (grey) and the DSN_HF farm (black).

(Figure 1). Both farms are located in the same climatic zone in a distance of only 35 km. Hence, differences of meteorological measurements between the DSN_HF and the DSN farm are due to characteristics of the barn building type, especially with regard to the isolation of the walls and the roof, and the orientation of the farm building. Generally, the quite modern only 10 years old DSN farm considers a variety of cooling equipment, such as space boards, fans, and sprinkling technique. We also compared meteorological data from inside barn recordings with corresponding daily measurements from the nearest weather station. Dry bulb temperatures recorded inside the barns were throughout higher than outdoor measurements. Also relative humidity inside the barns were higher, but also more stable in comparison to weather stations data.

1.1. Genetic statistical analyses

1.1.1. Repeatability model

Genetic parameters of physiological traits were estimated by applying the software package DMU (Madsen and Jensen, 2000), and using the AI-REML algorithm. Model [1] for the genetic analyses of longitudinal physiological traits was a univariate repeatability animal model (**RM**) as applied by Gernand *et al.* (2011) for repeated measurements of health data. In matrix notation, the RM was defined as follows:

$$\mathbf{y} = \mathbf{X\text{ß}} + \mathbf{Zu} + \mathbf{Wpe} + \mathbf{e}\ \mathbf{[1]}$$

where **y** = vector of observations for physiological cow traits; **ß =** vector of fixed effects including the effect of the farm (DSN or DSN_HF), the effect of parity (parity 1, 2, or greater and equal than 3), the effects days in milk (**DIM**) considering the three lactation stages < 100 days, 100 – 200 days or > 200 days after calving, the effect of the measuring year (2012, 2013 or 2014), the effect of the measuring season (March – May, June – August, September to November, or December to February), and regressions on THI using second order Legendre polynomials; **u** = vector of random additive genetic effects; **pe** = vector of random permanent environmental effects for cows; and **e** = vector of random residual effects; and **X**, **Z**, and **W** are incidence matrices for **b**, **u** and **p**, respectively. Statistical modelling considered the following (co)variance structure for random effects:

$$\mathrm{var}\begin{bmatrix} u \\ pe \\ e \end{bmatrix} = \begin{pmatrix} \sigma_g^2 A_u & 0 & 0 \\ 0 & \sigma_{pe}^2 & 0 \\ 0 & 0 & \sigma_e^2 \end{pmatrix}$$

with σ_g^2, σ_{pe}^2, σ_e^2 denoting variances of additive genetic, permanent environmental, and residual effects, respectively; and $\mathbf{A}_u$ is an additive genetic relationship matrix for u animals in the whole pedigree traced back to found animals born in 1926. Heritability (**h^2**) and repeatability (**t**) from model [1] were calculated as follows:

$$h^2 = \frac{\sigma^2 g}{(\sigma^2 g + \sigma^2 pe + \sigma^2 e)}$$

$$t = \frac{\sigma^2 g + \sigma^2 pe}{(\sigma^2 g + \sigma^2 pe + \sigma^2 e)}$$

In a second step, physiological traits were assigned into two groups according to the corresponding value for THI by defining a threshold of THI 65. Both datasets were analysed separately utilizing the same model [1]. Afterwards, we correlated the estimated breeding values (**EBV**) for cows with records between all physiological traits combinations. Correlation between EBV for a trait 1 and a trait 2 were transformed into genetic correlations (r_g) by applying the equation as suggested by Calo *et al.* (1973) and Blanchard *et al.* (1983):

$$\tilde{r}_{g_{1,2}} = \frac{\sqrt{(\sum_i R_{i_1}) * (\sum_i R_{i_2})}}{\sum_i (R_{i_1} * R_{i_2})} * r(EBV_1, EBV_2),$$

where R_{i_j} is the reliability of the EBV of a cow i in trait j. This equation suggests that correlations between EBV are always an underestimation of genetic correlations, because especially cow EBV have quite small reliabilities. The average reliability of cow EBV for physiological traits was 0.24 (implying an EBV accuracy of 0.49).

1.1.2. Random regression model

For studying quantitative genetic parameters of physiological traits on a continuous THI scale, model [2] was a single trait random regression model (**RRM**). The general form of this RRM in matrix notation also was:

$$\mathbf{y = X\beta + Zu + Wpe + e\ [2]}$$

vector **ß** included the same fixed effects as specified for model [1]. The difference between the RM and RRM addresses the modeling of random effects. Specifically for model [2], **u** = vector of additive genetic effects for random regression coefficients on THI using second-order Legendre polynomials, and **pe** = vector of random permanent environmental effects using second-order Legendre polynomials. The decision for the use of Legendre polynomials of order 2 for both random effects additive genetic and permanent environmental reflects our previous experiences regarding applications of RRM to estimate genetic parameters on continuous environmental and time scales (Brügemann *et al.*, 2011, 2013; Al-Kanaan *et al.*, 2015). Furthermore, model comparisons revealed smaller values for AIC and BIC when using Legendre polynomials of order 2 in comparison to models basing on Legendre polynomials of order 3 or 4.

As explained by Gernand *et al.* (2013), the chosen model [2] implies the following (co)variance structure of random effects:

$$\mathbf{var}\begin{bmatrix}\mathbf{u}\\ \mathbf{pe}\\ \mathbf{e}\end{bmatrix}=\begin{bmatrix}\mathbf{K}_u\otimes\mathbf{A} & \mathbf{0} & \mathbf{0}\\ \mathbf{0} & \mathbf{K}_{pe}\otimes\mathbf{I}_n & \\ & & \mathbf{K}_e\end{bmatrix}$$

where $\mathbf{K}_\mathrm{u}$ = (co)variance matrix of dimension 3 x 3 of random regression coefficients (i.e., intercept, Legendre polynomial of order 1, Legendre polynomial of order 2) for direct genetic effects by THI; **A** = additive genetic relationship matrix which was build using a depth pedigree traced back to founder animals born in 1926; $\mathbf{K}_\mathrm{pe}$ = (co)variance matrix of dimension 3 x 3 of random regression coefficients for permanent environmental effects by THI; $\mathbf{I}_\mathrm{n}$ = an identity matrix for *n* cows with physiological trait records; and ⊗ = Kronecker product. For residual variances $\mathbf{K}_\mathrm{e}$, a diagonal matrix was modeled by assuming constant residuals for all traits in the course of THI. The application of an RRM and availability of random regression coefficients allows the estimation of genetic correlations between same traits for all levels of THI. This RRM strategy also implies availability of cow and sire EBV for all THI. Accordingly to a lactation EBV when using a DIM time scale and summing up all daily EBV, an overall EBV for physiological traits might be the sum of the THI EBV. We standardized Legendre polynomials between THI 35 and THI 80, implying THI EBV for all THI within this defined range.

3. RESULTS AND DISCUSSION

3.1. Accuracy of data recording

Repeated measurements for physiological cow traits represent a novel functional trait category, substantially expanding the list of functional traits as categorized by Mark (2004). Hence, it is imperative for a first analysis to make assessments for the quantity and quality of data recording.

Repeatability (*t*) from model [1] is a parameter including genetic and environmental sources of variation, and therefore represents the degree of association between observations from the same animal for traits that are measured more than once. Estimations of *t* or further equations based on *t*, can be used when determining the optimal number of serial records per animal. Also with a research focus on HS under climatic conditions in the US combined with physiological traits of Holstein cows, Seath (1947) and Umphrey *et al.* (2001) predicted the required number of records using *t* or equations based on *t*. According to Falconer (1990), the percentage within-animal variance reduction (**VR**) for repeated measures is:

$$\mathrm{VR} = 100\left[1 - \left\{\frac{1 + (t)(n-1)}{n}\right\}\right]$$

As a further evaluation criterion for longitudinal data, the accuracy of prediction (*r*) with repeated records compared with single records (Mrode 2005) is:

$$r = \sqrt{\frac{1}{t + \frac{(1+t)}{n}}}$$

again, with *t* = repeatability and *n* = the number of repeated measures per animal. Hence, gains for VR and *r* depend on the repeatability and on the number of records. Because of low repeatabilities of physiological traits from the present study (Table 3), there will be substantial gain in variance reduction and in accuracy of prediction when using repeated measurements in comparison to the use of only one single record. The trait with the lowest repeatability (0.064) is STu, resulting in highest values for VR and *r* among all physiological traits. Also for all other physiological traits, Table 3 shows the substantial increase in VR and *r* from repeated data recording. Highest gain in VR and *r* will be achieved when increasing the number of records per animal from one to two, and implying only marginal gain in prediction accuracy when extending data recording for an already established longitudinal

Table 3. Gain in variance reduction (VR, in %) and gain in prediction accuracy (*r,* in %) when using repeated records in comparison to a single observation for physiological trait recording.

Traits		t ± SE	Number of repeated records									
			2		4		6		8		10	
			VR	*r*	VR	*r*	VR	*r*	VR	*r*	VR	*r*
Rectal temperature		0.111	44	34	67	73	74	96	78	112	80	124
Skin temperature	Upper	0.064	47	37	70	83	78	113	82	135	84	152
	Back	0.071	46	37	70	82	77	110	81	131	84	147
	Withers	0.100	45	35	68	75	75	100	79	117	81	129
	Line	0.078	46	36	69	80	77	108	81	127	83	142
Vaginal temperature		0.087	46	36	68	78	76	104	80	123	82	137
Respiration rate		0.115	44	34	66	72	74	95	77	111	80	122
Pulse rate		0.117	44	34	66	72	74	95	77	110	79	121

t= repeatability and SE = standard error.

data structure. As one example, VR for RT increased from 44% to 67% when increasing the number of repeated measurements from two to four, but the increase was only 12% when considering eight instead of six repeated measurements. For the trait STu with the lowest repeatability, VR was 47% and accuracy of prediction increased by 37% when using two measures in comparison to selection based on a single record. The additional gain was 23% for VR and to 46% for *r* when considering the fourth measurement. According to the low repeatability and high gain in VR, it is imperative to implement recording systems allowing repeated measurements (at least two) for novel physiological traits of dairy cattle. The desired impact of a longitudinal data structure on the prediction accuracy of genetic parameters was identified for other novel trait categories, i.e. dairy cow health (Gernand *et al.,* 2011), and dairy cow behavior (König *et al.,* 2006). Especially automatic systems, e.g. automatic milking or sensor technology (Jaeger and König, 2015), allow data recording in close intervals.

Availability of repeated measurements for those novel traits is especially valuable when studying their alterations in dependency of environmental descriptors exhibiting pronounced fluctuations, e.g. temperature or humidity. When aiming to identify robust cows, i.e. cows with quite stable phenotypes across environments, it is also imperative to generate longitudinal data structure. On such a basis, reaction norm models can be applied (Streit *et al.,* 2013). Also further model developments focusing on recursive relationships or “feed back” mechanisms among traits (Rehbein *et al.,* 2013) require a repeated measurement data basis.

1.1. Genetic parameters for physiological traits from the repeatability model

1.1.1. Variance components and variance ratios

Variance components for physiological traits along with corresponding heritablities and repeatabilities as obtained from model [1] are given in Table 4. Additive genetic variances were lowest for RT (0.001) and for VT (0.003). For both traits, also permanent environmental and residual components were quite small, resulting in heritabilities of value 0.066 (RT) and 0.037 (VT). Due to the extremely small residual variance component (0.129), RT was the trait with the highest heritability among all physiological cow “temperature traits”. Lowest heritabilities were estimated for the skin temperature traits, ranging from 0.018 (STl) to 0.035 (Stu). Also heritability for VT was of a similar magnitude (0.037). Variance components and variance ratios for the physiological traits RT and VT, especially the low additive genetic variances, are in agreement with estimates for female fertility traits from official recording systems (König *et al.,* 2008). Nevertheless, female fertility traits are widely considered in breeding goals or selection indices (Miglior *et al.,* 2005), but genetic gain or realized selection intensity for those traits was quite small (König *et al.,* 2007). The additive genetic variance and heritability for RT in the merged dataset of dual-purpose DSN and DSN_HF cows were substantially smaller than the heritability of value 0.17 for US Holstein (Dikmen *et al.,* 2012). Seath (1947) determined heritabilities for physiological traits on the basis of sire-progeny differences using 99 lactating Holstein and Jersey cows. Heritability estimates for RT (0.151) and RR (0.766) were also larger compared to results from our study in an endangered dual-purpose population. Following selection theory, the intensively selection in dairy breeds during decades, and the realized pronounced phenotypic trends in many traits, we hypothesize decreasing genetic variances and also smaller heritabilities in HF populations. (Hill 2014) and Piepho *et al.* (2014) attributed more than 50% of the phenotypic trait improvements to genetics and selection. As outlined by König and Simianer (2006), such intensive selection strategies will exploit genetic diversity, and lowering genetic variances in a long-term perspective. However, past HF dairy cattle breeding mainly focused on intensive selection towards improvements of production traits (Powell *et al.,* 2003). No targeted selection on novel physiological traits might be an explanation for the currently higher genetic variances for physiological traits in HF compared to the DSN population. The highest heritability was found for PR (0.072). This trait is quite simple to measure, without requiring installation of any recording technique. Traits with the highest heritability, i.e. RT and PR, are already routinely recorded in large-scale dairy herds during the first week after calving to detect diseases and stress symptoms early in lactation.

Table 4. Variance components ($\boldsymbol{\sigma^2 a}$= additive variance, $\boldsymbol{\sigma^2 pe}$= permanent environmental variance, $\boldsymbol{\sigma^2 e}$= residual variance, and $\boldsymbol{\sigma^2 p}$= total variance), and resulting heritabilities (h^2±SE) and repeatabilities (t±SE) for physiological traits estimated with a repeatability model.

		Variance components / variance ratios					
Traits		σ^2a	σ^2pe	σ^2e	σ^2p	h^2±SE	t±SE
Rectal temperature		0.010	0.007	0.129	0.145	0.066±0.032	0.111±0.017
Skin temperature	Upper	0.156	0.130	4.166	4.452	0.035±0.023	0.064±0.013
	Back	0.148	0.204	4.637	4.988	0.030±0.023	0.071±0.013
	Withers	0.197	0.463	5.976	6.637	0.030±0.029	0.100±0.016
	Line	0.103	0.333	5.147	5.582	0.018±0.022	0.078±0.014
Vaginal temperature		0.003	0.004	0.074	0.080	0.037±0.044	0.087±0.025
Respiration rate		3.947	5.543	73.034	82.523	0.048±0.044	0.115±0.021
Pulse rate		4.177	2.562	50.984	57.723	0.072±0.038	0.117±0.021

As outlined in Table 4, these physiological traits RT and PR with the highest heritability also displayed highest repeatabilities (0.111 and 0.117, respectively). In spite of the smaller heritability, repeatability for RT was in agreement with results (0.152) by Seath (1947). Hence, this is due to a substantially higher permanent environmental variance component in our study. Also repeatabilities for RT (0.111), RR (0.115), and the four body areas used for ST measurements (from 0.064 to 0.100) were slightly higher than corresponding estimates (0.055, 0.058, and 0.001, respectively) as reported by Umphrey *et al.* (2001). The present genetic study is based on a comparatively small number of cows. However, in spite of the small number of animals, both variance ratios heritabilities and repeatabilities are characterized by quite small standard errors in the range from 0.013 to 0.087 for repeatabilities, and from 0.018 to 0.072 for heritabilities (Table 4). Small standard errors also reflect the accuracy of data recording, which was accomplished by only one trained person. Also in their pilot study exploring the genetic background of claw disorders (König *et al.*, 2005b), the datasets from routinely claw trimming were generated from only one trained claw trimming team. Genetic statistical modeling considered more complicated threshold models for a binary data structure, but also in this study, standard errors of genetic parameters and EBV were small. A second explanation for the quite small standard errors might be due to herd selection, which was focussed on only two experimental herds. Generally, genetic parameter estimates based on on-station tests or on designed experiments are characterized by higher heritabilities with small standard errors in comparison to population wide recording schemes (e.g. Swalve *et al.*, 1999).

1.1.2. Correlations among physiological cow traits

The correlations among EBV of physiological traits are shown in Table 5. For all trait combinations, correlations were positive, except the low negative relationship between RT and RR (-0.055). However, this correlation was close to zero, and therefore not significantly different from zero ($P > 0.05$). Highest correlations in the range from 0.688 to 0.937 were identified among the different body parts used for the measurements of skin temperature. Within regard to the skin temperature trait category, this smallest correlation of 0.688 was the estimate between STu with STw. Taking into account the low reliability on cow EBV, genetic correlations among ST traits were close to one. Hence, different ST measurements are genetically the same trait. Phenotypic correlations among ST traits were throughout higher than 0.96. Apart from STu, remaining skin temperatures moderately correlated with RT (0.165 to 0.221). The EBV correlation between STu with RT was only 0.095. When transforming EBV correlation into genetic correlations using the approximation by Calo *et al.* (1972), r_g was 0.132 when considering only the cows with phenotype. Additionally, STu was the body area with the lowest repeatability when measuring skin temperatures. Altogether, we cannot recommend STu recording in ongoing studies for the identification of stress, behavior or welfare in dairy cattle. Jaeger and König (2015) emphasized the importance and possibilities of novel trait recording with infrared cameras, but they also mentioned practical problems with regard to STu recording, e.g. due to a cows' movement. Interestingly, on the other hand, STu reflected the highest correlation with VT (0.315).

We found a heritable component of physiological traits, and desired genetic correlations between all trait combinations (apart from the slightly negative value between RT and RR). Those findings suggest inclusion of physiological traits into overall breeding goals for dairy cattle, because selection on a physiological trait A is without detrimental impact on selection response for a physiological trait B. Inclusion of traits into overall breeding goals requires economic weights. Derivation of economic weights for physiological traits might be an unsolved challenge, because for traits without direct costs and revenues, it might be difficult to setting up a profit function. Alternative strategies focus on surveys to identify a farmers or breeders willingness to pay for the improvement of a specific trait, a so called "contingent valuation technique" (von Rohr *et al.,* 1999; Edel and Dempfle 2004). Furthermore, it is also

Table 5. Correlations between estimated breeding values of physiological traits for cows with records along with a statistical test if the correlations are significantly different from zero.

	Physiological traits						
	PR	RR	VT	STl	STw	STb	STu
RT	0.121^{ns}	-0.055^{ns}	0.312^{***}	0.212^{***}	0.22^{***}	0.165^{**}	0.095^{ns}
STu	0.091^{ns}	0.253^{****}	0.315^{****}	0.778^{****}	0.686^{****}	0.910^{****}	
STb	0.131^{*}	0.234^{***}	0.237^{***}	0.937^{****}	0.879^{****}		
STw	0.132^{*}	0.097^{ns}	0.122^{*}	0.923^{****}			
STl	0.166^{**}	0.187^{**}	0.151^{*}				
VT	0.076^{ns}	0.001^{ns}					
RR	0.138^{*}						

RT= rectal temperature, STu= upper skin temperature, STb= back skin temperature, STw= withers skin temperature, STl= line skin temperature, VT= vaginal temperature, RR= respiration rate, PR= pulse rate. ^{ns}= not significant, *= $P < 0.05$, **= $P < 0.01$, ***= $P < 0.001$, and ****= $P < 0.0001$.

mandatory to estimate genetic correlations between physiological traits and all the other traits considered in the breeding goal, i.e. traits of the categories production, conformation, longevity, fertility, and milking speed. For this purpose, substantially larger datasets are required. First approximations via correlations between cow EBV revealed an antagonistic relationship between physiological traits and milk yield, e.g. 0.23 between RT with 305-d lactation milk yield, or 0.19 between RR with 305-d lactation milk yield. The positive correlation indicates obvious physiological reactions of high yielding cows. The loss in adaptation, high environmental sensitivity and obvious HS symptoms of modern cattle breeds might be due to the intensively selection for milk yield during the last decades (Kadzere *et al.*, 2002; West, 2003; Gauly *et al.*, 2013). Identified genetic variation within and genetic covariances among physiological traits in German dual-purpose DSN cattle allows for selection to simultaneously decreasing RR, PR, and temperature traits, contributing to heat tolerance during HS periods. As shown for health traits (König *et al.,* 2005b), highest response for selection will be achieved when implementing direct selection strategies including all of the physiological traits. However, due to the identified genetic and phenotypic correlations, also correlated selection response can be used, especially for such ST traits which are difficult to measure under practical farm conditions. Also Dikmen *et al.* (2012) recommended inclusion of RT into selection indices or overall breeding goals for US Holstein cattle, but they also focussed on the importance of genetic correlations between RT with other breeding traits having strong economic relevance.

1.1.3. Genetic parameters below and above a defined THI threshold

In our previous HS study for physiological traits on phenotypic scales, Al-Kanaan *et al.* (2013) identified THI 65 as a “THI threshold”. Above this threshold, RT, RR, VT and ST substantially increased with increasing THI, with pronounced effects for cows with high levels of test-day milk yield in the early stage of lactation, and especially during the summer seasons. Accordingly, we decided to use the identified THI 65 threshold to separate our data for genetic analyses: data recorded at THI < 65 represents the comfortable environment A, and data recorded at THI > 65 include only records from a HS environment B.

For all of the temperature traits, i.e. RT and the ST measurements, and also for PR, additive genetic variances and heritabilities were smaller in the HS environment B (comparison of results in Table 6 with results in Table 7). Such results are in agreement with early findings

Table 6. Variance components ($\boldsymbol{\sigma^2 a}$ =additive variance, $\boldsymbol{\sigma^2 pe}$= permanent environmental variance, $\boldsymbol{\sigma^2 e}$= residual variance, and $\boldsymbol{\sigma^2 p}$= total variance), and resulting heritabilities (h^2±SE) and repeatabilities (t±SE) for physiological traits measured at THI < 65 (environment A).

		Variance components / variance ratios					
Traits		σ^2a	σ^2pe	σ^2e	σ^2p	h^2±SE	t±SE
Rectal temperature		0.014	0.007	0.139	0.160	0.085±0.042	0.131±0.022
Skin temperature	Upper	0.412	0.270	5.280	5.962	0.069±0.042	0.114±0.022
	Back	0.460	0.288	5.897	6.645	0.069±0.042	0.113±0.022
	Withers	0.747	0.557	7.645	8.950	0.083±0.052	0.146±0.025
	Line	0.495	0.405	6.620	7.520	0.066±0.045	0.120±0.023
Vaginal temperature		6.7x 10^{-7}	0.014	0.068	0.083	8.2 x 10^{-6}±0.099	0.175±0.057
Respiration rate		1.206	5.352	35.083	41.642	0.029±0.051	0.158±0.028
Pulse rate		4.985	2.511	52.305	59.800	0.083±0.046	0.125±0.027

Table 7. Variance components ($\boldsymbol{\sigma^2 a}$ =additive variance, $\boldsymbol{\sigma^2 pe}$= permanent environmental variance, $\boldsymbol{\sigma^2 e}$= residual variance, and $\boldsymbol{\sigma^2 p}$= total variance), and resulting heritabilities (h2±SE) and repeatabilities (t±SE) for physiological traits measured at THI > 65 (environment B).

		Variance components / variance ratios					
Traits		σ^2a	σ^2pe	σ^2e	σ^2p	h^2±SE	t±SE
Rectal temperature		0.005	0.003	0.110	0.119	0.044±0.036	0.072±0.021
Skin temperature	Upper	0.010	1.2 x 10^{-5}	2.459	2.469	0.004±0.015	0.004±0.014
	Back	0.022	5.4 x 10^{-6}	2.834	2.856	0.008±0.017	0.008±0.014
	Withers	0.047	0.087	3.635	3.770	0.013±0.022	0.036±0.016
	Line	0.018	0.023	3.102	3.143	0.006±0.017	0.013±0.013
Vaginal temperature		0.007	5.7 x 10^{-8}	0.073	0.079	0.082±0.061	0.082±0.034
Respiration rate		5.326	10.939	116.501	132.766	0.040±0.068	0.123±0.032
Pulse rate		2.718	5.561	41.266	49.545	0.055±0.054	0.167±0.035

by Hammond (1947), who identified a smaller range of genetic values in harsh environments. Generally, the whole dataset from cows in pasture based production systems represents a “harsh environment”, but in addition, dataset B includes the harsh HS component. Accordingly, additive genetic variances and heritabilities for RT, ST traits and PR were larger when using the whole dataset (Table 4) compared to respective estimates from dataset B (Table 7). Accordingly, highest additive genetic variances and heritabilities for these traits were found in dataset A (Table 6). Based on his findings, Hammond (1947) also recommended to test and to select animals in superior environments, which simplifies detection of their true genetic potential. Such breeding aspects were considered when implementing on-station tests for potential bull dams , or nowadays when selecting co-operator herds for progeny testing schemes (Schierenbeck *et al.*, 2011). Schierenbeck *et al.* (2011) based their selection strategies to identify co-operator herds on intra-herd variances for protein yield deviations. On the other hand, with regard to somatic cell count, the same authors suggested to select harsh environments with challenging impact on a cows’ immune system. This might be an explanation for the larger heritabilities for VT and RR when basing genetic parameter estimation only on dataset B depicting the harsh environment (Table 7). We assume larger differences in genetic parameter estimates when defining a dataset B based on field data from commercial herds located in harsh grassland systems. In the present study, both herds are well-managed research farms, and the only major characteristic depicting a “harsh environment” is due to the grazing system. In contrast to the remaining physiological traits, VT only was recorded during a short time period in the hot summer month. This might be a further explanation for opposite results for VT when comparing datasets A and B.

For production traits, several studies compared genetic parameters in two distinct environments, with and without HS, or on a continuous HS or THI scale. In a sample of the German HF population, Brügemann *et al.* (2011) identified decreasing additive genetic variances and heritabilities for test-day protein yield with increasing THI. In contrast in US Holsteins, Aguilar (2008) reported an increase in heritabilities and variance components of production traits with increasing THI. Nevertheless, identified pronounced effects of THI on variance component estimates suggest consideration of THI in statistical models used for genetic evaluations. THI as environmental descriptor explained a high proportion of the phenotypic variation of a trait, and might also be a valuable characteristic for international genetic evaluations in the sense of a “borderless herd clustering” (Weigel and Rekaya, 1999). Such ideas also formulated by Bernabucci *et al.* (2014) and Hammami *et al.* (2015). Also

Bohlouli *et al.* (2013) focussed in their study on ideas for improvements of genetic statistical modelling for traits recorded in different climatic zones.

However, previous studies mostly used meteorological data from weather stations to characterize a specific herd environment, which also was primary information in their first “borderless clustering idea” (Weigel and Rekaya, 1999). Based on the results by Al-Kanaan *et al.* (2015) and based on the results from this present study, we suggest utilization of weather data recording in close distance to a cow. Such a strategy allows an optimal match between phenotypic and environmental data with regard to space and time scales.

Correlations between EBV in the same physiological trait measured in the two different environments were positive, but generally lower than 0.55. The between environment EBV correlations were as follows: 0.399 for RT, 0.166 for STu, 0.277 for STb, 0.294 for STw, 0.249 for STl, 0.304 for VT, 0.537 for RR, and 0.542 for PR. Applying the Calo *et al.* (1973) equation, genetic correlations between physiological traits recorded in both environments A and B were throughout lower than 0.80. Hence, results of genetic correlations indicate GxE, because Robertson (1959) defined a threshold of $r_g = 0.8$ for an assessment of GxE. König *et al.* (2005a) summarized genetic correlations for production traits recorded in different environments or production systems, i.e. results from classical bivariate or multiple trait modelling by neglecting residual covariances among traits (Falconer 1990). Genetic correlations were only lower than 0.80 for obvious production system differences, e.g. small family versus large-scale farms, low input versus high input feeding strategies, or data from different climatic zones. For functional traits with low heritability reflecting fertility, health and animal welfare, genetic correlations were below this threshold even for very similar environments A and B (Brügemann *et al.*, 2015)

1.2. Genetic parameters for physiological traits from the random regression model

The variance ratios from the RRM (model [2]) on the continuous THI scale are shown for heritabilities (Figure 2) and repeatabilites (Figure 3). According to the results from the RM, additive-genetic variances (not shown), heritabilities and repeatabilities were largest for small THI values from THI 35 to THI 45. For RR, RT and STu, we observed a pronounced

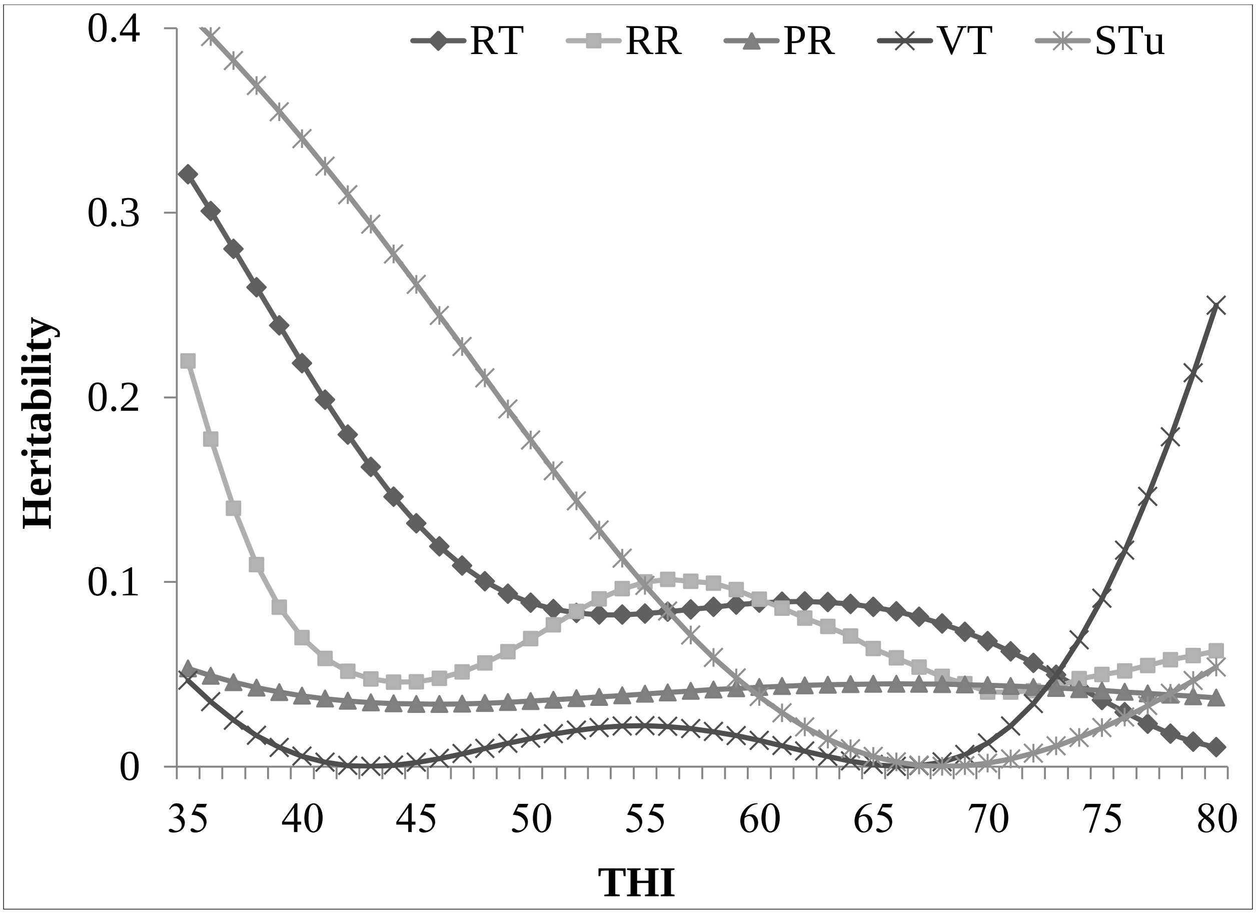

Figure 2. Heritabilities of physiological traits in dependency of a continuous THI scale.

heritability and repeatability decline up to THI 45. Alterations of additive-genetic variances and heritabilities indicate that different genes are affected at different levels for THI (Hammami *et al.,* 2015). Only for PR, heritabilities and repeatabilities for PR were quite stable along the continuous THI scale. An unexpected strong increase in heritabilities and repeatabilities for THI > 70 was observed for VT. However, VT is the physiological trait with the smallest number of observations. Also Yin *et al.* (2012) and Yin *et al.* (2014) applied RRM, and they reported an increase of genetic variances at the extreme ends of the continuous scale for production and functional health traits. This increase especially was obvious for small datasets and small contemporary groups. For binary health traits, additionally they identified an effect of disease incidences on genetic parameter estimates. In accordance with those previous RRM studies, we suggest the application or at least verification of RRM results by applying more robust RM. A further alternative might be the application of sire instead of animal models, in order to avoid 'extreme data category problem' , meaning that some sub-classes for fixed or random effects only include a small number of observations. Gernand *et al.* (2013) studied the effects of the polynomial structure on genetic variances and heritabilities at extreme levels for the covariate days in milk. However, both chosen polynomials Legendre of order 3 and order 4, contributed to a similar shape of genetic parameter curves.

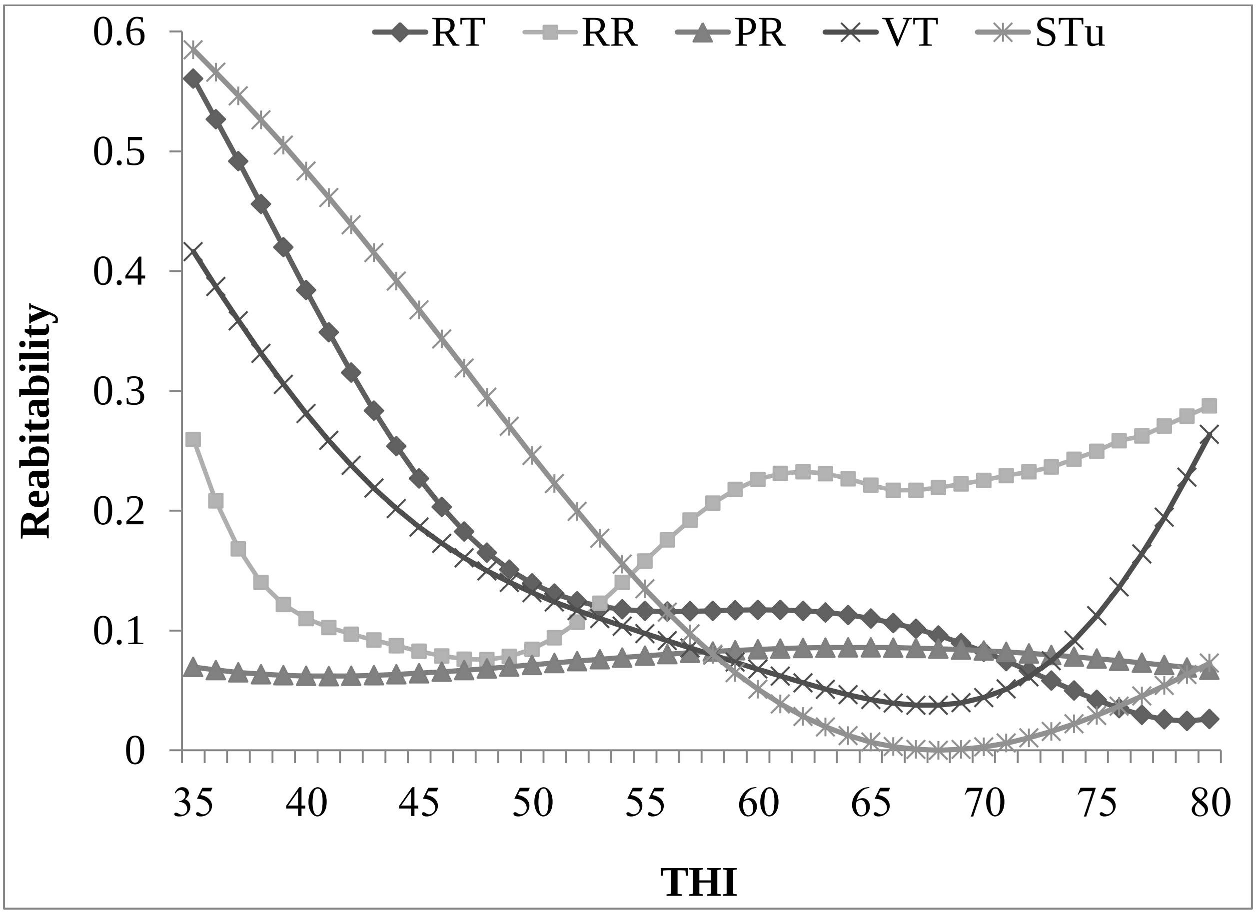

Figure 3. Repeatabilities of physiological traits in dependency of a continuous THI scale.

From the whole set of estimated genetic correlations across THI combinations, we illustrated GxE by depicting r_g recorded at THI 65 with all remaining THI (Figure 4). For all physiological traits, r_g between neighbouring THI were close to 1, but substantially dropped for THI at a greater distance. For RR and RT, genetic associations were even negative when correlating the same trait recorded in the HS environment (THI 65) and in the comfort zone (THI 35). Genetic correlations were -0.38 for RT, and -0.36 for RR. Those negative genetic correlations suggest a substantial re-ranking of animals for the same trait in different climatic zones.

The curves for genetic correlations as depicted in Figure 4 show substantial alterations for different THI pairs. Nevertheless, also the more sophisticated RRM support our results from EBV correlations based on the RM estimates for distinct environments, i.e. strong proves for GxE. Genotype by environment interaction previously have been reported in RRM or reaction norm studies concerning genomic (Streit *et al.*, 2013) or genetic (Hammami *et al.*, 2009) environmental sensitivity of dairy cows. According to Dikmen *et al.* (2013), utilization of genome-wide associations based on high density genomic marker data will contribute to a deeper understanding of environmental sensitivity. Subsequently, future research also focuses on novel biomarkers involved in physiological processes in different climatic zones (Deb *et al.*, 2015; Sajjanar *et al.*, 2015; Verma *et al.*, 2015).

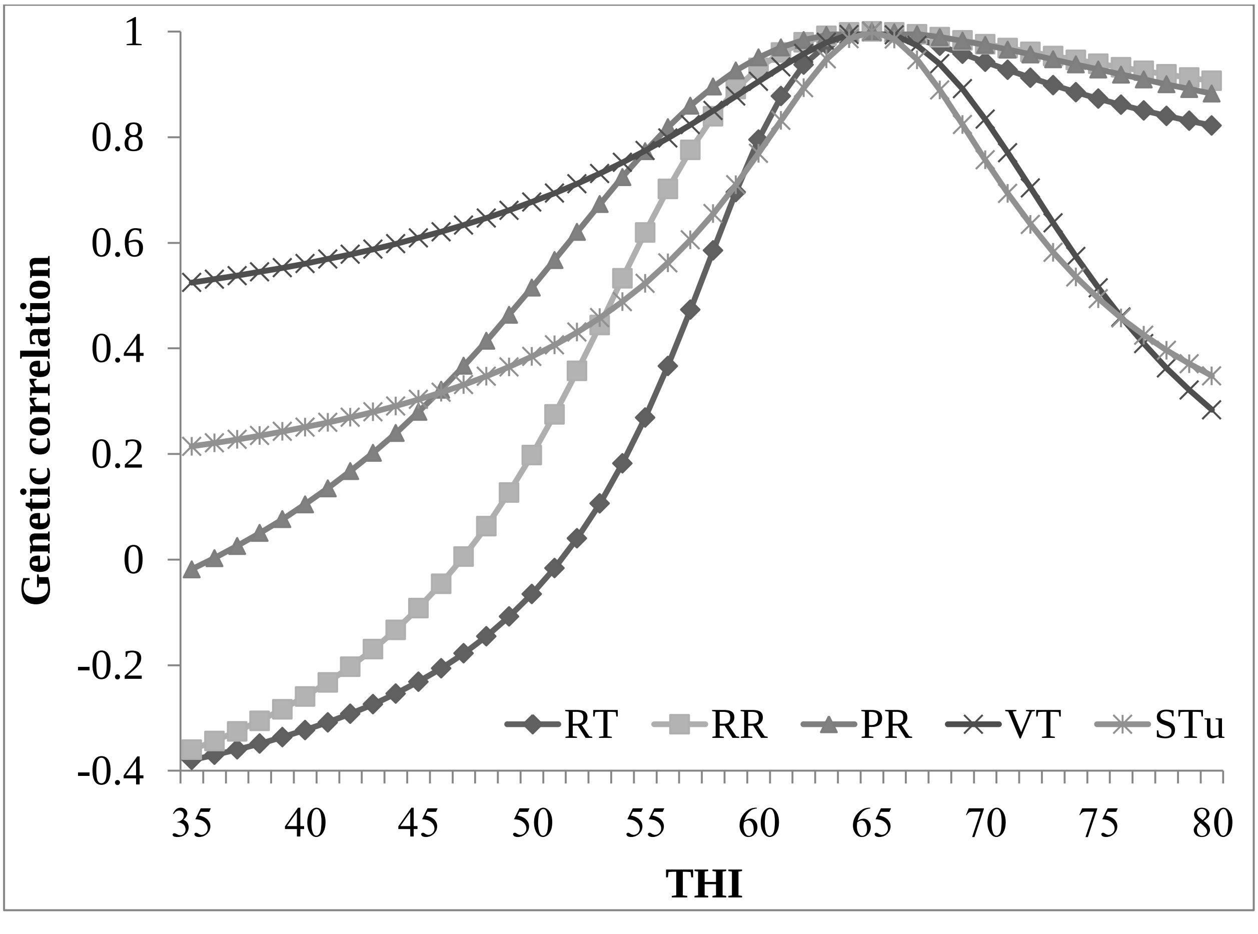

Figure 4. Genetic correlations for physiological traits measured at THI 65 with corresponding physiological traits at remaining THI.

CONCLUSION

To our knowledge, the present study is a first approach to identify genetic variations in farm animals in relation to environmental challenges, defined as HS from cows kept in outdoor pasture based systems. Heat stress was considered by either considering THI classes or continuous THI scales. We found a heritable component for all physiological traits RT, VT, RR, ST and PR, with a maximal heritability of 0.072 for PR estimated with a robust RM. Heritabilities were lower in a data subset only including observations recorded in a "HS environment" with THI > 65. Genetic correlations between same traits measured in two different environments A (THI > 65) and B (THI < 0.65) were throughout lower than 0.80, indicating GxE. Same trends for additive genetic variances, heritabilities and genetic correlations between same traits recorded in different environments were observed when applying a more sophisticated RRM. However, variances and variance ratios from the RRM were quite large at the extreme ends of the continuous environmental scale. We identified moderate desired genetic correlations among the different physiological traits, meaning that genetic selection for a functional trait A simultaneously genetically improves a functional trait B. Ongoing genetic studies have to infer genetic associations among physiological traits and remaining traits used in dairy cattle breeding goals.

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Chapter 5

General discussion

Preface

Thermal stress in dairy cattle results in substantial economic losses represented in deterioration of milk production, impairment of reproductive ability and alteration in health status (Collier *et al.*, 2006). In 2010, the annual loss for the entire dairy sector caused by HS was approximately 1.2 billion dollars in the US (Key *et al.*, 2014). While expecting a continuous average THI increase of 1.67-3.95 until 2030, the potential decline in milk production will be between 6 to 17 percent (i.e. the additional HS will lower the milk production for the average US dairy about 0.60 to 1.35 percent per year in 2030) (Key *et al.*, 2014). Although many breakthroughs in technology and management systems concerning HS abatement have been developed to reduce the total economic losses in the US from 2.4 billion to 1.7 billion dollars (St-Pierre *et al.*, 2003), major productions losses still exist.

In the temperate climate of middle Europe, the impact of HS on traditional production as well as functional traits during warm summer is potentially underestimated. Furthermore, most of the studies concerning this topic have been conducted in the tropical or subtropical countries, where animals are more adapted to hot climates. Thus, so far, less information is available regarding the environmental sensitivity of lactating cows kept in temperate climates in Europe. Also little is known about possibilities of cattle adaptation (Gauly *et al.*, 2013), especially when assuming the expected pronounced climatic changes during the next decades (IPCC, 2014). The few number of studies investigating HS in the moderate climate mostly focused on the commercial aspects (production and reproduction) neglecting physiological attributes of cattle. Hence, the motivation of this investigation was attempting to fill the gap of studies in this field.

Physiological traits of semen characteristics

Physiological traits of semen characteristics were discussed in chapter 2. Semen traits of 562 Holstein sires were collected during the years 2009, 2010, and 2011. 10,341 observations from 10 to 136 months old bulls kept in an AI center in northern Germany were analyzed in the first study. Longitudinal semen traits were merged with meteorological data from the nearest weather station to assess the effect of heat stress on phenotypic and genotypic physiological aspects in dependency on the continuous environmental descriptor THI. On the phenotypic scale, a fixed regression model was used to study the impact of heat stress on physiological semen traits. The direct effect of the covariate THI on semen traits was measured as a regression from the ejaculation date nested within age classes of bulls, and modelled using Legendre Polynomials of order 3. In addition to the effect of THI, fixed effects included the age of bull, the year-season of semen collection, and the intervals between consecutive semen collection dates.

Analysis of variance revealed a significant impact of THI on physiological traits of Holstein semen during periods of 1-11 days, 12-65 days, 22-28 days, and 29-35 days before ejaculation. On the day of ejaculation, results showed a highly significant effect ($P < 0.001$) of THI using Legendre Polynomials of order 3 on SV, SO, and NSD. During the period 1-11 days before ejaculation, the effect of THI using third Legendre polynomials showed a significant effect on all semen traits included in this study (Table 1). Thus, we identified the period of epididymal maturation (1-11 days before semen collection) as the most effective period on semen production. Additionally, the decline in semen traits curves during the of period 1-11 days before insemination was more obvious and even starts earlier compared to the day of ejaculation (chapter 2; Figure 3, 4, 5, 6, 7 and S1) and in the period of 12-65 days before ejaculation (Figure 1).

Hence, the results corroborate that the effect of hot environmental conditions on semen quality is not limited to the day of ejaculation, but extends to a period of about two months before semen collection. However, the period of spermatogenesis (days 12-65 before semen collection) turned to be less significantly affected by HS in comparison to the day of ejaculation and other periods (Table 1 and Figure 1).

A further analysis was carried out to explore the starting period of HS as affect on semen production. In this examination, a fixed effects model was applied to determine the effect of HS on semen traits up to 14 weeks before ejaculation. The same data set of semen traits and meteorological parameters was used. THI in the fixed model was classified into 3 classes: THI 20-39, THI 40-59, and THI 60-79 to examine the impact of HS on semen traits and to identify earlier periods with a potential effect (Figure 2). Taking into account all the three classes of THI, the period of 7-9 weeks (49-63 days) before ejaculation seems to be the most effective period to identify heat stress starting for semen traits (Figure 2). This finding is in agreement with Fuerst-Waltl *et al.* (2006), who identified the period of the 12th to 65th day before collection of semen as starting period for heat stress.

Table 1. Analysis of variances: *P* values of THI; as Legendre polynomials order 1 (LG1), 2 (LG2), and order 3 (LG3), age of bull, intervals between collection dates, and year-season of the collection on semen traits on the day of ejaculation, 1-11 days before ejaculation, and 12-56 days before ejaculation.

Dates	Effect	*P*-values				
		SV	SM	SO	NSD	SC
Day of ejaculation	THI (LG1)	0.2576	<.0001	0.1153	0.1367	0.0385
	THI (LG2)	0.1249	0.058	0.002	0.0021	0.0387
	THI (LG3)	<.0001	0.3497	<.0001	0.0004	0.258
	Age of bull	<.0001	<.0001	<.0001	<.0001	<.0001
	Interval	<.0001	0.1559	<.0001	<.0001	<.0001
	Year-season	<.0001	<.0001	<.0001	<.0001	<.0001
1-11 days before ejaculation	THI (LG1)	0.289	0.1465	0.1176	0.0523	0.0309
	THI (LG2)	0.0252	0.4138	<.0001	0.0002	0.0009
	THI (LG3)	<.0001	0.00907	<.0001	<.0001	0.0006
	Age of bull	<.0001	<.0001	<.0001	<.0001	<.0001
	Interval	<.0001	0.2278	<.0001	<.0001	<.0001
	Year-season	<.0001	<.0001	<.0001	<.0001	<.0001
12-65 days before ejaculation	THI (LG1)	<.0001	0.2111	<.0001	<.0001	0.0372
	THI (LG2)	0.202	0.0302	0.0042	0.1504	0.0897
	THI (LG3)	0.8016	0.5477	0.119	0.1323	0.0173
	Age of bull	<.0001	<.0001	<.0001	<.0001	<.0001
	Interval	<.0001	0.0127	<.0001	<.0001	<.0001
	Year-season	<.0001	<.0001	<.0001	<.0001	<.0001

Interval: intervals between collection dates= < 3 days and ≥ 3 days. Age of bull= < 12 months, 12–18 months, 18–48 months, and > 48 months.

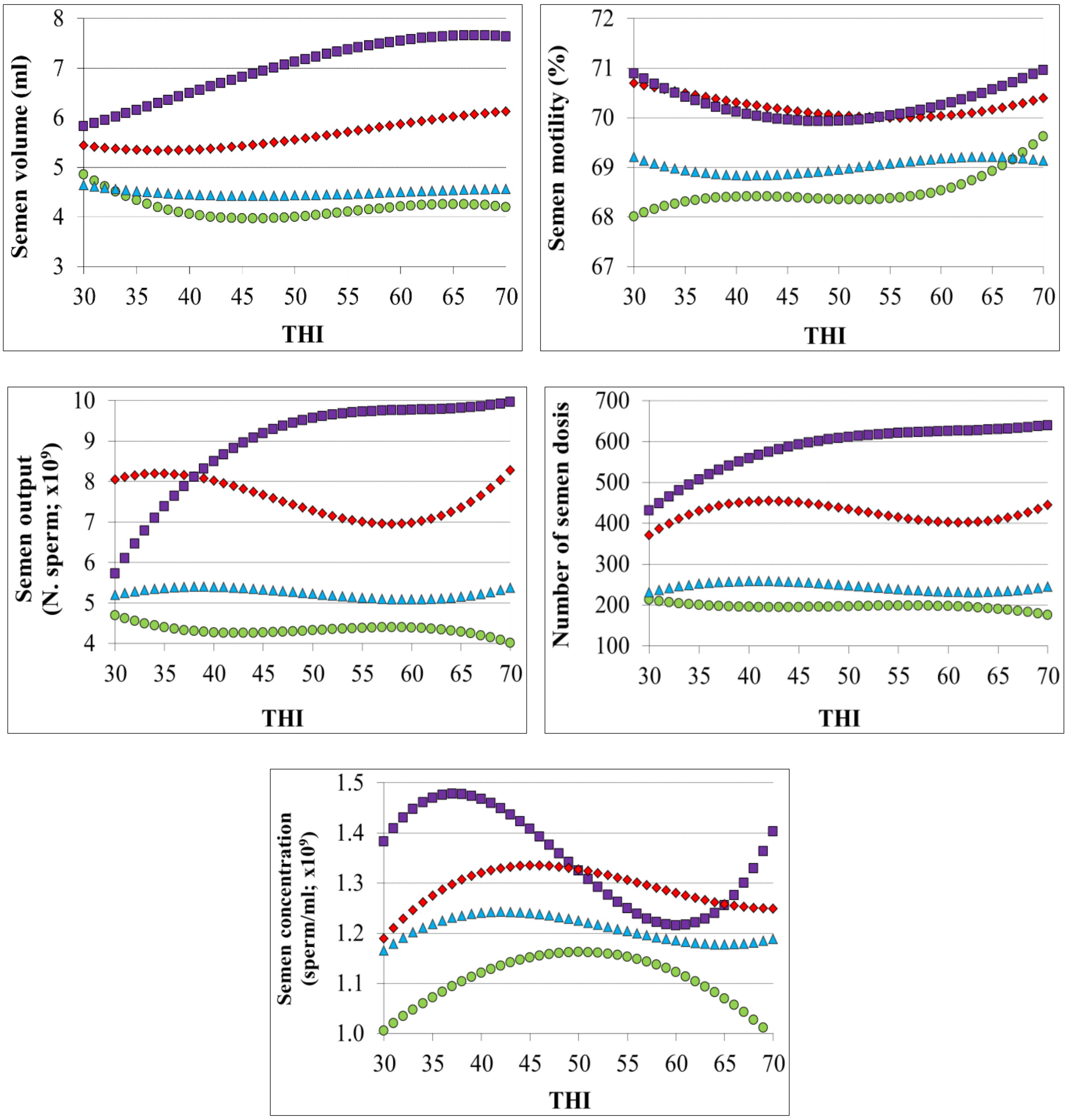

Figure 1. Least squares means for semen volume, semen output, semen motility, number of semen doses per ejaculate, and semen concentration of AI sires by THI measured at 12-65 days before the ejaculation date for different age classes of bulls. Age classes of bulls: < 12 months (●), 12–18 months (▲), 18–48 months (◆), and > 48 months (■).

The THI threshold (=THI 60) and the comfortable zone (THI range from 50 to 60) for semen production (both identified by visual evaluation) were lower than in other studies conducted in tropical and subtropical countries. Detrimental effects of heat stress on semen traits were identified beyond that threshold. The lower range of the optimal environment reflect the lower adaptability of AI bulls for hot conditions in Germany.

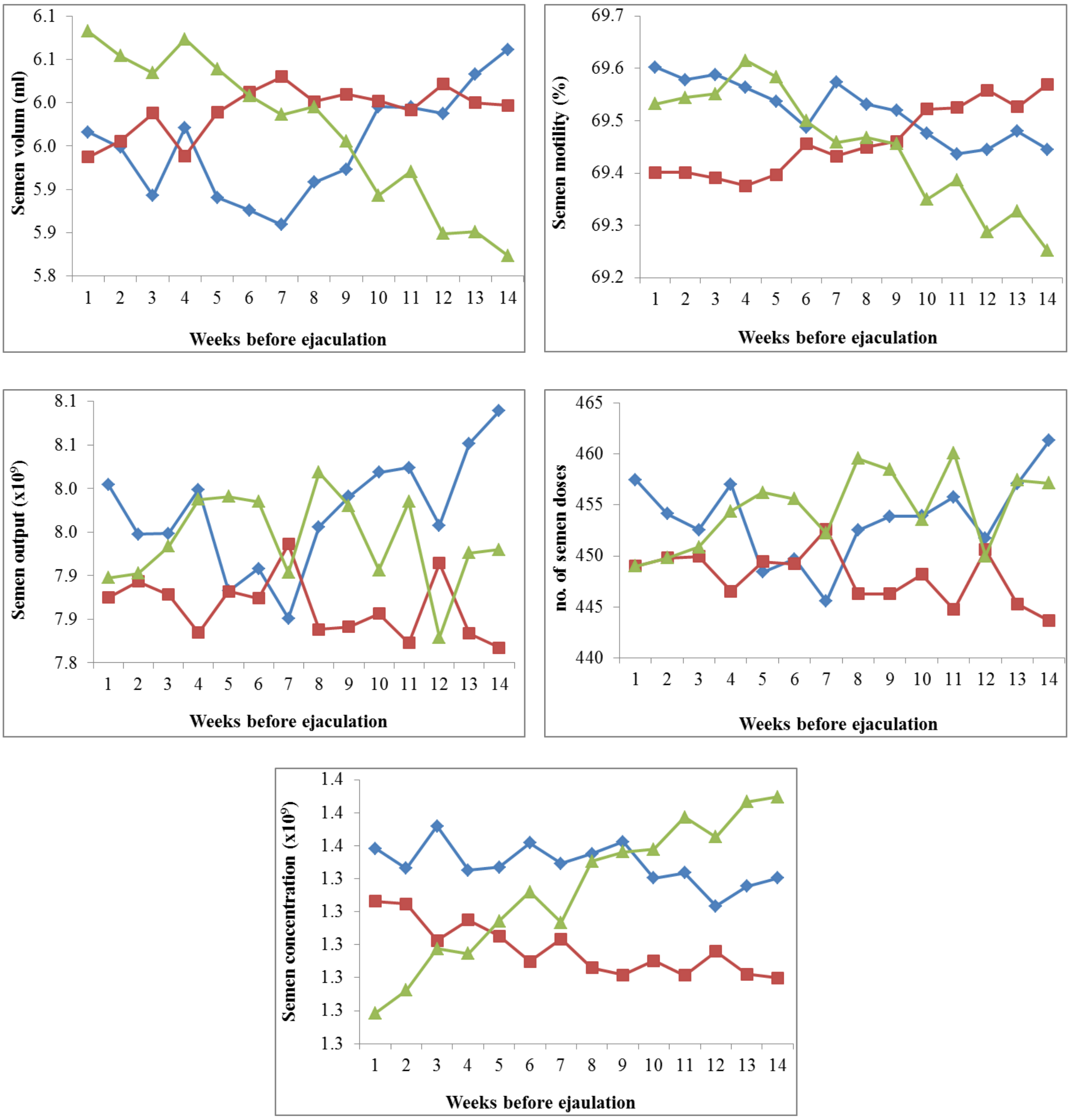

Figure 2. Least squares means for semen volume, semen output, semen motility, number of semen doses per ejaculate, and semen concentration of AI bulls by 14 weeks before ejaculation for different THI classes. THI classes: THI 20-39 (◆), THI 40-59 (■), and THI 60-79 (▲).

From a genetic perspective, the results of the current study showed a decline in genetic parameters of semen characteristics in harsh environments. This decrease in heritability and genetic correlations in the same trait were in line with the reduced additive genetic variance of semen traits with increasing THI above the threshold (Figure 3). Thus, the heritability reduction might be the outcome of a decrease in the additive genetic variance of semen traits during the period from the THI threshold to the extreme hot THI (Figure 3). Low to moderate heritabilities for all examined semen traits (max. values ranged from 0.18 to 0.29 associated with different THI values) demonstrate the possibilities of genetic selection for heat tolerance of superior animals. Additionally, it is assumed that including semen traits affected by the surrounding environments into the breeding goals, could be useful to improve heat tolerance of dairy cattle.

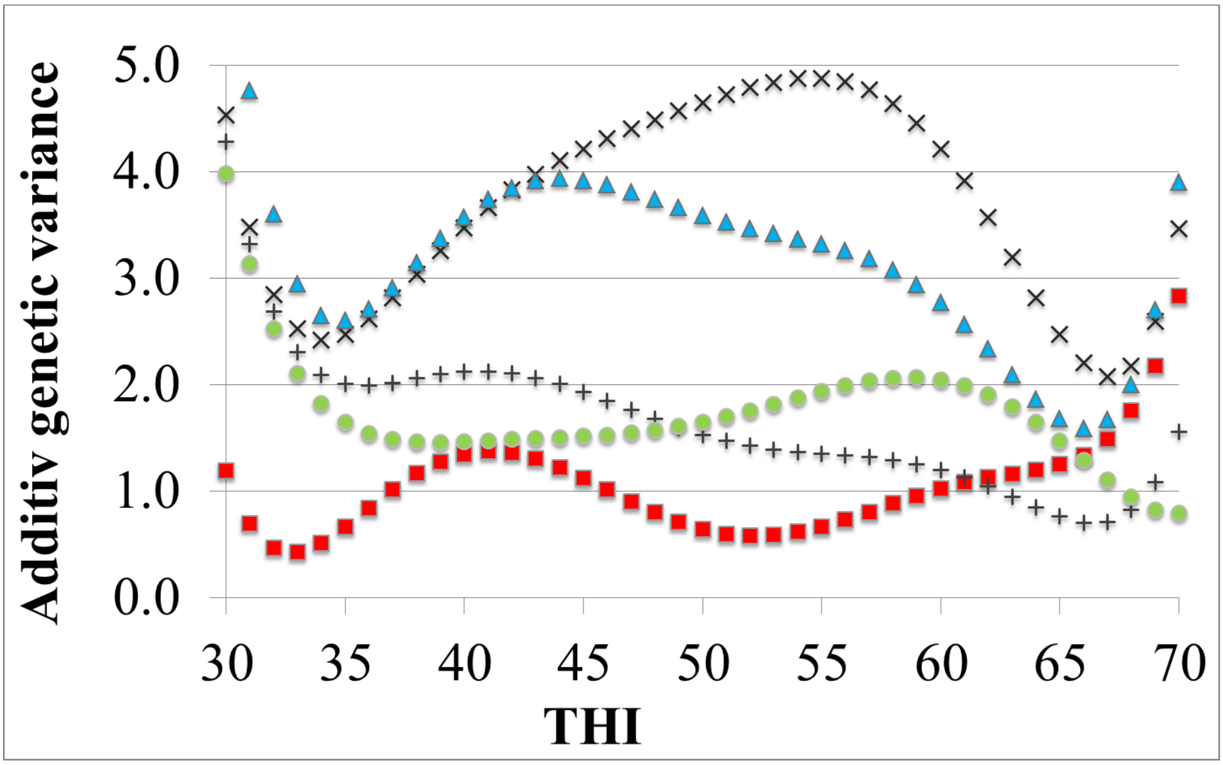

Figure 3. Additive genetic variances for semen volume (▲; /ml x10), semen motility % (■), semen output (+; x10^9), number of semen doses per ejaculate (●; sperm /5000) and semen concentration (x; x10^{11}) of AI sires by course of THI on day of ejaculation.

Physiological traits of dairy and dual purpose cows

Effects of heat stress on physiological traits of lactating cows were discussed in chapter 3. The main objective of this study was to assess the effect of HS on RT, ST, VT, RR, and PR measured from dairy (HF) and dual purpose (DSN) lactating cows in dependency of a continuous THI scale. Ambient temperature and humidity data used to calculate THI in this investigation were recorded in close distance to the cow (indoor weather), and exactly at the same minute when measuring physiological traits. In this present study, some indicator effects were used: Test-day milk yield and days in milk were indicators of productivity, parity was used as an indicator of cow age, milk fat% and milk protein% as indicators of milk composition, fat to protein ratio and milk urea nitrogen reflect energy efficiency and energy balance, somatic cell counts represent mastitis and udder health, season and year of measurements are indicators of environmental differences and feeding availability during the study, sensitivity as an indicator of level of HS susceptibility for individual cows.

Analyses of variance showed a highly significant effect of THI across the models for Legendre polynomials of order 1 ($P < 0.0001$) in all physiological cow traits. For Legendre polynomials order 2, THI highly significant influenced RT in both HF and DSN cows, and skin temperature measured from back (STb) and line (STl) areas, VT and RR ($P < 0.0001$) in DSN cows, but were generally significant ($P < 0.05$) for remaining trait and breed combinations (Table 2). Non-significant ($P > 0.10$) regression coefficients partly were identified for Legendre polynomials of order 3 for THI, i.e. for the analyses of STu and STb in HF cows. The non-significant relationship between THI and physiological traits was predominant for further higher polynomials orders. Results in Table 2 reveal the considerable influence of lactating German cattle during summer heat stress. Subsequently, this high environmental sensitivity in cattle reflects the need for efforts that should take place to alleviate the HS effect on cattle adapted to the moderate climate in the centre of Europe. Mitigation of undesired HS effects on European cattle performance could be achieved first by using cooling systems and changing management during hot days in addition to changes in the breeding goal.

Table 2. Analysis of variances: *P*-values of the effect of THI on the physiological traits of DSN and Holstein cows nested in different models within Legendre Polynomials order 1 (LG1), order 2 (LG2), and order 3 (LG3).

Traits	Factors	DSN cows			Holstein cows		
		P-value THI(LG1)	*P*-value THI(LG2)	*P*-value THI(LG3)	*P*-value THI(LG1)	*P*-value THI(LG2)	*P*-value THI(LG3)
Rectal temperature	Model 1 (TDM)	<.0001	<.0001	0.0788	<.0001	<.0001	0.0033
	Model 2 (sensitivity)	<.0001	<.0001	0.0043	<.0001	<.0001	0.0008
	Model 3 (Fat %)	<.0001	<.0001	0.0053	<.0001	<.0001	0.0003
	Model 4 (Protein %)	<.0001	<.0001	0.0647	<.0001	<.0001	0.0003
	Model 5 (F/P ratio)	<.0001	<.0001	0.0018	<.0001	<.0001	0.0018
	Model 6 (Urea)	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001
	Model 7 (SCC)	<.0001	<.0001	0.0181	<.0001	<.0001	0.0014
	Model 8 (Parity)	<.0001	<.0001	<.0001	<.0001	<.0001	0.0003
	Model 9 (DIM)	<.0001	<.0001	0.0758	<.0001	<.0001	0.0014
Upper skin temperature	Model 1 (TDM)	<.0001	<.0001	<.0001	<.0001	0.0293	0.5605
	Model 2 (sensitivity)	<.0001	<.0001	<.0001	<.0001	0.0163	0.913
	Model 3 (Fat %)	<.0001	<.0001	<.0001	<.0001	<.0001	0.0031
	Model 4 (Protein %)	<.0001	<.0001	<.0001	<.0001	0.0024	0.0161
	Model 5 (F/P ratio)	<.0001	<.0001	<.0001	<.0001	<.0001	0.1992
	Model 6 (Urea)	<.0001	<.0001	<.0001	<.0001	0.0141	0.1129
	Model 7 (SCC)	<.0001	<.0001	<.0001	<.0001	0.0247	0.3397
	Model 8 (RT)	<.0001	<.0001	<.0001	<.0001	0.0156	0.1117
	Model 9 (Parity)	<.0001	<.0001	<.0001	<.0001	0.0057	0.0281
	Model 10 (DIM)	<.0001	<.0001	<.0001	<.0001	0.0014	0.0611
Respiration rate	Model 1 (TDM)	<.0001	<.0001	<.0001			
	Model 2 (sensitivity)	<.0001	<.0001	<.0001			
	Model 3 (Fat %)	<.0001	<.0001	<.0001			
	Model 4 (Protein %)	<.0001	<.0001	<.0001			
	Model 5 (F/P ratio)	<.0001	<.0001	<.0001			
	Model 6 (Urea)	<.0001	<.0001	<.0001			
	Model 7 (SCC)	<.0001	<.0001	<.0001			
	Model 8 (RT)	<.0001	<.0001	<.0001			
	Model 9 (Parity)	<.0001	<.0001	<.0001			
	Model 10 (DIM)	<.0001	<.0001	<.0001			
Pulse rate	Model 1 (TDM)	<.0001	0.0321	<.0001			
	Model 2 (sensitivity)	<.0001	0.0043	<.0001			
	Model 3 (Fat %)	<.0001	0.0104	<.0001			
	Model 4 (Protein %)	<.0001	0.0017	<.0001			
	Model 5 (F/P ratio)	<.0001	0.0089	<.0001			
	Model 6 (Urea)	<.0001	0.0068	<.0001			
	Model 7 (SCC)	<.0001	0.0016	<.0001			
	Model 8 (RT)	<.0001	0.0824	<.0001			
	Model 9 (Parity)	<.0001	0.0059	<.0001			
	Model 10 (DIM)	<.0001	0.0305	<.0001			
Vaginal temperature	Model 1 (TDM)	<.0001	<.0001	0.0451			
	Model 2 (sensitivity)	<.0001	0.0004	0.0619			
	Model 3 (Fat %)	<.0001	0.002	0.0013			
	Model 4 (Protein %)	<.0001	0.0011	0.2686			
	Model 5 (F/P ratio)	<.0001	0.0015	0.1558			
	Model 6 (Urea)	<.0001	<.0001	0.1613			
	Model 7 (SCC)	<.0001	0.0013	0.1907			

Impact of year and season on physiological traits

Effect of season and year were significant for all physiological traits in this study (excluding RT of HF cows; Table 3). Physiological traits recorded during summer seasons showed the highest skin temperature measured from upper, back, withers, and line in DSN and HF cows. However, autumn season showed the highest rectal and vaginal temperatures, as well as the highest respiration and pulse rates in DSN cattle (Table 3). Results from literature studies emphasize that the highest physiological traits values were in the hot seasons (Abdelatif and Alameen, 2012; Singh *et al.*, 2012; Costa *et al.*, 2015; Deb *et al.*, 2015). In studies of HS in Brazil, Costa *et al.* (2015) found that the percentage of cows which exceeded the threshold of RT (≥ 39.3 °C) and RR (≥ 36 breaths/ min) was higher in the dry than rainy season in crossbred Girolando cattle. In the tropical environment, hot summer was associated with high RT, RR, and hematological profile for different pregnancy stages in Sudan cattle (Abdelatif and Alameen, 2012). The same result also confirmed in Brazilian Holstein (Garcia *et al.*, 2015). Further, for the highly adapted growing and adult Sahiwal cattle in the semi-arid climate of India, Singh *et al.* (2012) reported that RT, ST, RR, and PR were higher during summer and hot humid seasons than in spring and winter, using morning as well as afternoon measurements. Seasonal variations, in addition to the ambient climatic factors (i.e. temperature, humidity, wind, precipitation, solar radiation and daylight), include also differentiations in forage-based diets. Further effects of seasonal photoperiod on cattle physiology, growth, lactation, and immune functions are clearly discussed by Collier *et al.* (2006).

In the year 2014, the highest values for RT and ST measured from four areas (in HF and DSN cows), VT, and RR were found. However, in the same year, the lowest pulse rate was identified (Table 3). These annual variations might be due to the quality and quantity of feeding (particularly grass silage) and managements for cattle in pasture-based systems. The highly significant effect of season and year on physiological traits of lactating cows in this experiment prove the findings of our previous study (chapter 2), concerning the impact of those factors on physiological semen quality and quantity traits of Holstein sires kept in the AI station.

The current investigation proved that the application of random regression methodology is imperative for studies addressing the effect of heat stress on longitudinal physiological traits. It is concluded that RRM is the best alternative to the classification of environmental effects

Table 3. Effects of season and year on rectal temperature, skin temperature, respiration rate, pulse rate and vaginal temperature (±SE) of Holstein (HF) and German black pied (DSN) cattle.

Factors		Rectal temperature ±SE		Skin temperature								Vaginal temperature ± SE	Respiration rate ± SE	Pulse rate ±SE
		HF	DSN	Upper ± SE		Back ± SE		Withers ± SE		Line ± SE				
				HF	DSN	HF	DSN	HF	DSN	HF	DSN	DSN	DSN	DSN
Season	Spring	38.48 ±0.04	38.34 ^{c} ±0.01	31.65 ^{a} ±0.21	30.15 ^{a} ±0.13	30.79 ^{a} ±0.32	29.81 ^{a} ±0.14	30.58 ^{a} ±0.28	29.67 ^{a} ±0.15	30.54 ^{a} ±0.24	29.89 ^{a} ±0.14	38.46 ^{a} ±0.02	30.17 ^{c} ±0.62	73.90 ^{b} ±0.53
	Summer	38.41 ±0.05	38.38 ^{b} ±0.02	31.95 ^{a} ±0.28	30.35 ^{a} ±0.13	30.84 ^{a} ±0.23	29.83 ^{a} ±0.13	31.04 ^{a} ±0.38	29.70 ^{a} ±0.15	30.70 ^{a} ±0.33	30.02 ^{a} ±0.14	38.34 ^{b} ±0.01	33.75 ^{b} ±0.57	71.13 ^{c} ±0.59
	Autumn	38.41 ±0.04	38.44 ^{a} ±0.02	29.47 ^{b} ±0.35	29.48 ^{b} ±0.16	28.55 ^{b} ±0.39	28.4 3 ^{b} ±0.16	28.24 ^{b} ±0.46	28.37 ^{b} ±0.18	28.44 ^{b} ±0.40	28.49 ^{b} ±0.17	38.52 ^{a} ±0.07	41.94 ^{a} ±0.72	75.35 ^{a} ±0.67
	Winter	-	38.43 ^{ab} ±0.04	-	28.11 ^{c} ±0.28	-	27.17 ^{c} ±0.29	-	27.53 ^{c} ±0.32	-	27.29 ^{c} ±0.30	-	35.30 ^{b} ±1.78	70.83 ^{c} ±2.44
Year	2012	38.46 ±0.03	38.31 ^{b} ±0.01	30.89 ^{b} ±0.12	29.16 ^{b} ±0.09	30.07 ^{b} ±0.14	28.51 ^{b} ±0.09	29.89 ^{b} ±0.17	28.82 ^{b} ±0.11	29.82 ^{b} ±0.14	28.50 ^{b} ±0.10	-	35.10 ^{b} ±0.46	75.98 ^{a} ±0.62
	2013	38.45 ±0.03	38.29 ^{b} ±0.01	30.43 ^{c} ±0.16	29.04 ^{b} ±0.08	29.36 ^{c} ±0.18	28.28 ^{c} ±0.08	29.42 ^{b} ±0.22	28.67 ^{b} ±0.09	29.27 ^{c} ±0.19	28.49 ^{b} ±0.08	38.38 ^{b} ±0.01	36.32 ^{a} ±0.44	74.88 ^{b} ±0.62
	2014	38.38 ±0.06	38.60 ^{a} ±0.05	31.75 ^{a} ±0.27	30.36 ^{a} ±0.35	30.75 ^{a} ±0.31	29.64 ^{a} ±0.36	30.55 ^{a} ±0.37	28.96 ^{a} ± 0.40	30.60 ^{a} ±0.32	29.79 ^{a} ±0.38	38.45 ^{a} ±0.06	34.44 ^{ab} ±1.8	67.54 ^{c} ±1.7

^{a,b} small letters denoting significant differences with $P < 0.05$

into a few distinct groups. Applying RRM is also useful for defining the onset of HS: as defined as THI threshold. RMM also depict HS sensitivity within the thermoneutral zone for cattle. Furthermore, observing the effect of the THI environment on functional animal traits by using RRM could provide precious information for animal responses to cold and heat stress.

Modifications of cooling and management systems are some of the most common strategies to cope with summer warming. These procedures succeed temporarily to reduce the detrimental effect of HS on lactating animals which are bred and adapted to moderate climates. Nevertheless, assuming that the global warming and climatic changes will severely modify the temperature, and assuming that selection in dairy cattle continues to increase milk yield, then classical selection strategies will not be sufficient to face the issue of thermal stress. During the next few decades, policymakers in collaboration with researchers in climate and farm animal sciences should establish new policies to cope with the predicted exorbitant high annual loss in the whole dairy industry sector (Key *et al.*, 2014). In addition to the development of new technologies, one solution could be the genetically improvement of dairy cattle adaptation to specific climate conditions.

Genome-wide association and heat tolerance in lactating cattle

Genome-wide association (GWAS)

Comparatively new methods for researchers to identify genes related to a trait are known as genome-wide association studies (**GWAS**). The base of these methods is an inspection of the whole genome for discovering common SNPs among different animals with specific traits (Bush and Moore, 2012). The associated SNPs reflect a sequence of the animal genome, contributing to gene expressions (Collier *et al.*, 2008). Although this thesis does not contain this technique, it strongly supports applying GWAS for the detection of HS in cattle in future studies.

GWAS application was used to identify specific SNPs and biomarkers associated with environmental sensitivity in cattle (Streit *et al.*, 2013), and were also studied in relation to feeding levels (Hayes *et al.*, 2009). Those authors concluded that the environmental sensitivity of production traits is a quantitative trait, controlled by many genes with small effects, and few genes with large effects. Howard *et al.* (2013) proved the existence of GxE interactions for the myostatin genotype by studying its relation to body temperature regulation in beef cattle during extreme temperature conditions. HS could be detected also by recognizing selenoprotein gene expressions, which play important roles in various physiological and pathological processes in tissues when animals are subjected to hot conditions (Cao *et al.*, 2015). The expression of ATPase beta family genes, responsible for establishing the electrochemical gradient across the plasma membrane during different environmental temperatures, has been examined in Holstein bulls (Deb *et al.*, 2015) and Jersey cows (Das *et al.*, 2015) crossbred with local Indian cattle. One method to improve the capacity of body temperature regulation and resistance to HS is applying gene transfer technique for an inclusion of the desired genes associated with better heat tolerance (e.g. Dikmen *et al.*, 2014). Using this methodology, Dikmen *et al.* (2014) introduced a specific single gene (*SLICK*; confers cattle with a short and sleek hair coat) from Senepol into Holstein cattle and evaluated its ability in thermoregulation by analyzing physiological traits during HS. Moreover, dairy cattle genotyped with superior thermoregulation ability also experience less depression in milk yield during the harsh environment (Dikmen *et al.*, 2012; Dikmen *et al.*, 2013; Dikmen *et al.*, 2014).

Although a substantial number of genes was recently identified, still the best suited set of genes for thermal stress are genes expressing heat shock proteins (**HSP**s) (e.g. Das *et al.*, 2015; Sailo *et al.*, 2015a). HSPs are defined as an evolutionary conserved family of proteins which are induced by living cells in response to biological stresses, and play a crucial role in cellular thermotolerance and HS response (Sajjanar *et al.*, 2015). Several studies were conducted to investigate the relationship between the expression of HSPs and physiological traits of cattle (e.g. Kumar *et al.*, 2015a) and buffalo heifers (Kumar *et al.*, 2015b), as well as physiological traits of bulls semen characteristics (Rajoriya *et al.*, 2014).

Heat tolerance in lactating cattle

The heat tolerance coefficient (**HTC**) is a formula to assess the ability of thermoregulation, and reflect the adaptability of animals exposed to acute heat stress. Previously, the HTC formula for cattle was developed by Rhoad (1944) and is based on the average RT and the physiological bovine body temperature (38.3 °C) with the correlation factor (10) to convert deviations in body temperature to a unit basis. This equation is also called “Iberia heat tolerance test” and is defined as follows:

$$\mathrm{HTC} = 100 - 10\,(\mathrm{RT} - 38.3)$$

Higher HTC values indicate lower adaptability to heat stress. Charoensook *et al.* (2012) measured individual Iberia HTC as physiological responses of HS in Thai indigenous cattle. They identified nine SNPs related to a specific gene and suggested using those SNP as genetic biomarkers to select appropriate breeds for hot climates. Sajjanar *et al.* (2015) found that cows with a favorable genotype had better physiological responses during summer stress (HTC and RR), lower RT, and are better in milk production. This formula was also used by Abdoun *et al.* (2013) to investigate the variation in thermophysiological responses, and indicate that coat color does not influence heat tolerance of different camel breeds.

Benezra (1954) developed another thermal index for measuring the heat tolerance coefficient based on both respiration rate (RR) and rectal temperature (RT):

$$\mathrm{HTC} = \frac{\mathrm{RR}}{23} + \frac{\mathrm{RT}}{38.33}$$

23 breaths/min and 38.33 (°C) are normal for RR and RT, respectively, for cattle kept under ideal conditions. The lower value determined by this equation represent the high degree of adaptability. This formula was applied in genetic polymorphism studies concerning ATPase (Das *et al.*, 2015) and HSPs (Sailo *et al.*, 2015a) heat stress biomarkers, discussed in the context of associations with thermotolerance in Jersey crossbred and in Sahiwal (Sailo *et al.*, 2015a; Kumar *et al.*, 2015c) cows. These studies were conducted during the humid subtropical hot summer climate of India. Those authors found a novel SNP association with RR, RT, and HTC, which are ubsequently associated with production traits.

From the above studies, the importance of applying GWAS technology for improving heat tolerance in cattle is obvious. Exploring new biomarkers and SNPs will enhance the adaptation of HS at the cellular level in cattle, in addition to selection for high milk production with low feeding input under the expected climate change scenarios (Hayes *et al.*, 2009).

Future aspects studies

1. Intensified attention is required to study influences of HS (e.i. THI) on semen traits of AI bulls. These traits are represented by the physiological and morphological structure of semen and testicular traits, and show alterations of genetic parameters by THI in the temperate climate of Europe. Further, determining the physiological responses of bulls (e.g. body temperature and respiration) during hot summers could be effective to

realize the goal of improving the adaptation of these sires, also to the harshest environments.

2. Deep investigations of physiological, behavioral, and biochemical responses of cattle (as thermal stress indicators) for different age structures, sexes, production levels within production systems, and breeds are imperative to explore the HS phenomenon in detail.
3. Defining new physiological traits not included in this work (e.g. sweating and panting) and visualizing surface body temperature by infrared thermography from new body areas (like on eye, muzzle, ear, leg, and udder) as further reliable indicator of HS
4. We support investigations of physiological responses of cattle to hot summers in collaborative projects across European climates, in order to include a large number of data for different breeds and housing systems. In addition to the identification of thresholds and the optimal environment, these longitudinal studies should also assess the effects of climatic changes and HS on production, reproduction, and health disorders (e.g. mastitis, acidosis, ketosis, and laminitis) on both scales genetic and phenotypic.
5. Genetically, the best choice of improving the adaptability of cattle is an estimation of genetic components of heat resistance (using THI specific breeding values) and selecting animals within a breed for highest heat tolerance. This selection should be applied with regard to physiological and traditional animal traits.
6. Focusing on the local adapted cattle (e.g. DSN cows) kept in pasture based systems, responses to stress should be included in overall breeding goals.
7. Application of GWAS technique, and defining new SNPs and biomarkers associated with HS in cattle might be helpful for a better understanding of cellular thermoregulation and genes attributed to heat tolerance.
8. Ongoing studies concerning the effect of indoor and outdoor climatic conditions via the recording of meteorological data in close distance to the animal are suggested (instead of using data from weather stations).
9. Applying RRM is effective to identify THI thresholds and comfort zones, as well as to explore genotype by environment interaction.
10. Some other traits like hair coat color and thickness, dehorning, and body structure could be beneficial with regard to the evaluation of thermal stress.

11. The effects of HS can be reduced in dairy cattle viaby the application of appropriate countermeasures such as:
    a. Implication of cooling systems (e.g. fans, heads showers, sprinkling).
    b. Adjustment of feeding programs (e.g. decreasing fibres and increasing protein, increase ration numbers, provide cold water, and studying feeding behaviours)
    c. Modification of management strategies (e.g. decreasing overcrowding in barns and parlour, providing shade in pastures and change to night pasture plan).

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