ESTONIAN UNIVERSITY OF LIFE SCIENCES
Institute of Veterinary Medicine and Animal Sciences

Casper Kosonen

ANALYSIS OF ELEVATED TEMPERATURE READINGS IN DAIRY CATTLE USING TIME-SERIES ANALYSIS WITH SUBCUTANEOUS TEMPERATURE SENSOR DATA

KUUMASTRESSI ANALÜÜS PIIMAVEISTEL, KASUTADES NAHAALUSE TEMPERATUURIANDURI ANDMEID

Final Thesis
Curriculum in Veterinary Medicine

Supervisor: Tarmo Niine, DVM, PhD

Tartu 2024

www.emu.ee
Eesti Maaülikool
Estonian University of Life Sciences

Estonian University of Life Sciences
Kreutzwaldi 1, 51014, Tartu Estonia

Abstract of Final Thesis

Author: Casper Kosonen

Curriculum: Veterinary Medicine

Title: Analysis of elevated temperature readings in dairy cattle using time-series analysis with subcutaneous temperature sensor data

Pages: 58	Figures: 9	Tables: 4	Appendices: 5

Chair: Chair of Veterinary Biomedicine and Food Hygiene
Field of research and (CERC S) code: 3. Health, 3.2. Veterinary Medicine
B750 Veterinary medicine, surgery, physiology, pathology, clinical studies
Supervisor(s): Tarmo Niine, DVM, PhD
Place and year: Tartu 2024

Monitoring temperature is an important part of monitoring the overall health status of cattle. Increasing herd sizes cause problems to farmers, because among other problems, restraining and measuring individual cows’ temperature is becoming extremely labor-intensive and difficult. Technologies like the subcutaneous temperature sensors are being studied to ease monitoring this aspect of welfare in cattle. Our hypothesis was that there would be increased volatility in the subcutaneous sensor data during periods of elevated environmental temperatures. Secondary objective was to analyze circadian rhythm dynamics between two farms with different housing systems during the whole observation period. In current study, 6 bull calves (Farm 1) and 11 calves (Farm 2) with implanted subcutaneous temperature sensors were observed throughout the summer. The observation period for the Farm 1 bull calves was 48 days from July until August in 2020, and for the Farm 2 calves 78 days from June until August in 2022. The bull calves lived in an outside group pen while the calves were housed in an uninsulated indoor barn. Environmental data was collected on temperature and humidity, and temperature humidity index (THI) was calculated at each farm during the whole observation period. Generalized autoregressive conditional heteroskedasticity (GARCH) models with one lagged value (1h) were used to assess the subcutaneous (SC) temperature volatility. The autoregressive conditional heteroscedasticity (ARCH) test was performed to assess the performance of GARCH model. The results showed that the humidity in the indoor housing system was more erratic and frequently high, while in the outside pen, humidity showed lower values and a daily seasonality. GARCH model results showed that alpha was very low and beta very high which indicates that in the context of calves’ body temperature, the persistence of volatility was high, but recency was low. The ARCH test showed that the accuracy of GARCH model was poor for some calves from each farm. The circadian rhythm of the bull calves had temperature peaks during the day and lowest points during the night, while the calves had peaks during the evening and lowest readings in the morning.

Keywords: Calf, THI, circadian rhythm, GARCH model, volatility

www.emu.ee
Eesti Maaülikool
Estonian University of Life Sciences

Eesti Maaülikool Kreutzwaldi 1, 51014, Tartu		Lõputöö lühikokkuvõte	
Autor: Casper Kosonen		Õppekava: Veterinaarmeditsiin	
Pealkiri: Kuumasstressi analüüs piimaveistel, kasutades nahaaluse temperatuurianduri andmeid			
Lehekülgi: 58	Jooniseid: 9	Tabeleid: 4	Lisasid: 5

Õppetool: Veterinaarse biomeditsiini ja toiduhügieeni õppetool
ETIS-e teadusvaldkond ja CERC S-i kood: 3. Terviseuuringud, 3.2 veterinaarmeditsiin
B750 Veterinaarmeditsiin, kirurgia, füsioloogia, patoloogia, kliinilised uuringud
Juhendaja(d): Tarmo Niine, DVM, PhD
Kaitsmiskoht ja -aasta: Tartu 2024

Temperatuuri jälgimine on veiste üldise tervisliku seisundi jälgimise oluline osa. Piimaveise karjade looma arvu suurenemine valmistab loomakasvatajatele probleeme, sest muude kitsaskohtade hulgas muutub äärmiselt tööjõumahukaks ja keeruliseks ka üksikute lehmade kehatemperatuuri jälgimine ja mõõtmine. Veiste heaolu jälgimise hõlbustamiseks uuritakse lootustandvaid tehnoloogiaid nagu subkutaansed temperatuuriandurid. Uuringu hüpoteesiks oli, et kõrgema keskkonnatemperatuuriga perioodidel suureneb subkutaansete temperatuuriandurite registreeritud andmete volatiilsus. Teiseks eesmärgiks oli analüüsida ööpäevarütmi dünaamikat kahe erineva pidamissüsteemiga farmi vahel kogu vaatlusperioodi jooksul. Uuringu läbiviimiseks jälgiti kogu suve perioodi kuut pullvasikat (Farm 1) ja 11 lehmvasikat (Farm 2), kellele olid implanteeritud nahaalused temperatuuriandurid. Pullvasikate vaatlusperiood kestis 2020. aasta juulist augustini (48 päeva) ja 2022. aasta juunist augustini 78 päeva lehmvasikate puhul. Pullvasikad elasid õueaedikus, lehmvasikad aga soojustamata laudas. Kogu vaatlusperioodi jooksul koguti mõlemas farmis keskkonnaandmeid temperatuuri ja õhuniiskuse kohta ning arvutati temperatuuriniiskuse indeks (THI). Subkutaanse (SC) temperatuuri volatiilsuse hindamiseks kasutati üldistatud autoregressiivseid tingimusliku heteroskedastilisuse (GARCH) mudeleid ühe ajaperioodi viitega (1 tund). GARCH mudeli toimivuse hindamiseks viidi läbi autoregressiivne tingimusliku heteroskedastilisuse (ARCH) test. Tulemused näitasid, et siseruumides oli õhuniiskus ebastabiilsem ja sageli kõrge, samas kui õues oli õhuniiskus madalam ja esines märgatavat igapäevast, hooajalist muutlikkust. GARCH mudeli tulemused näitasid, et mudeli alfa oli väga madal ja beeta väga kõrge, mis viitab sellele, et vasikate kehatemperatuuri kontekstis oli volatiilsus püsiv, kuid hiljutisuse efekt madal. ARCH test näitas, et GARCH mudeli täpsus oli mõlema farmi mõne vasika puhul ebatäpne. Pullvasikate ööpäevarütmi temperatuuri tipud olid päeval ja madalaimad öösiti, lehmvasikatel aga õhtuti ja madalaimad hommikul.

Märksõnad: Vasikas, THI, ööpäevarütm, GARCH-mudel, volatiilsus

TABLE OF CONTENTS

LIST OF ABBREVATIONS

AR = autoregressive

ARMA = autoregressive moving average

BAT = brown adipose tissue

BMR = basal metabolic rate

CNS = central nervous system

CSV = comma-separated value

ETIC = equivalent temperature index for cattle

GARCH = generalized autoregressive conditional heteroskedasticity

GH = growth hormone

RR = respiratory rate

RT = rectal temperature

SD = standard deviation

THI = temperature-humidity index

TRP = transient receptor potentials

UCP1 = uncoupling protein 1

WCT = wind chill temperature

INTRODUCTION

Monitoring the body temperature of cattle is essential for the dairy industry. Most of the time body temperature is of no concern to most cattle owners, as the task of measuring it is labor-intensive, and in a healthy cow, it does not usually provide much valuable insight into the cow's health as a singular point of information. Adult cows have an estimated thermoneutral zone between -5 and 25 °C in which they are most productive and use minimal energy for thermoregulation (West, 2003). Thermoregulation is also affected by high productivity levels. A high milk yield is correlated with a high body temperature (Burgos Zimbelman et al., 2009).

Cows respond to cold and heat stress situations by altering their respiratory system, heart rate, circulation, feed consumption and behavior, to name a few. In a heat stress situation, a cow is over its thermoneutral zone and has to start expending energy to get rid of excess heat. Air temperature, relative humidity, air velocity, and solar radiation are the most important variables affecting the prevalence of heat stress. The temperature humidity index (THI) is often used in heat stress evaluation, as it combines the air temperature and relative humidity to a single value. Due to heat stress, the livestock industry in US is estimated to have annual losses of around \$1.69 to 2.36 billion, of which \$900 million is specific to the dairy industry (Polsky & von Keyserlingk, 2017).

Using standard methods, such as rectal temperature measurement, is labor-intensive and quite inefficient, especially when multiple cows are affected. However, rectal temperature measurement remains to be the industry standard for the time being, as other less invasive and labor-intensive temperature measurement systems, such as the tympanic membrane, vaginal, infrared, and microsensor measurements, have proven to be only slightly more efficient, quite a bit more inaccurate, not ready for the market, or too harmful for consecutive measurements. From a health perspective the rectal temperature is sufficient, but production would likely benefit from a more efficient temperature measurement system to better avoid or detect heat stress and its causes in individual production facilities (Bergen & Kennedy, 2000; Burfeind et al., 2010).

This study aimed to use subcutaneously implanted temperature sensors as a way to monitor the heat stress during high THI periods. Air temperature and humidity data from installed weather stations will be used with the implanted sensor data to evaluate the temperature reading volatility in heat stress time periods.

1. LITERATURE REVIEW

1.1 THERMOREGULATION

1.1.1 TYPES OF THERMOREGULATIONS

There are two types of thermoregulations in animals. Endothermic thermoregulation, which means that the animal can maintain a certain body temperature through energy expenditure and behavioral patterns. Ectothermic is used when talking about animals that are incapable of maintaining constant body temperature (Clarke & Pörtner, 2010).

All mammalian species are considered endotherms, as they are capable of maintaining a constant temperature, even if the outside ambient temperature changes drastically. Endothermic animals use energy to increase or lower their body temperature according to the changes in ambient temperature. This can be achieved by using brown adipose tissue reserves, increasing the metabolic rate, moving to a warmer space or near other animals, shivering, or activity. However, there are endothermic animals with different responses to changes in ambient temperature. There are hibernating mammals, often in the wild, that enter a state of prolonged torpor due to the increased cost of maintaining a high body temperature. These animals are capable of lowering their body temperature, metabolic rate, and other physiological functions to enter a state of low-energy expenditure (Geiser, 2004).

Ectothermic animals are vulnerable to the changes in the ambient temperature. Their bodies are incapable of maintaining a set body temperature by altering their bodily functions. These animals are prone seasonality in their activity levels. Ectothermic animals often have less energy expenditure than endothermic animals, because they do not maintain a set body temperature (Clarke & Pörtner, 2010).

1.1.2 IMPORTANCE OF TEMPERATURE TO TISSUE FUNCTION

The thermoregulatory system aims to maintain the temperature of the body within a narrow range to maintain homeostasis and allow for normal chemical and physiological processes to occur. The thermoregulatory system combines thermal receptors and thermoregulatory response mechanisms to effectively maintain the optimal body temperature in any given ambient temperature. The most important thermal sensors in the body are the skin and median pre-optic nucleus. There is also a concept of intramuscular thermal receptors, but these have yet to be physically confirmed (Gupta et al., 2013; Mrowka & Reuter, 2016).

Thermoregulatory response mechanisms include sweating, shivering, ventilating, basal metabolic rate (BMR) and brown adipose tissue. These combined systems allows the essential body functions, such as the kidneys and the nervous system, to function precisely and more effectively, regardless of the ambient temperature (Mrowka & Reuter, 2016).

If the core temperature exceeds the healthy range in either direction, the results can be fatal. In cows, the healthy range is considered to be 38 – 39.1 °C. If the body temperature falls below 38 °C, the metabolic processes are slowed, and thus the body does not function properly. In more extreme cases, a decrease in body temperature to 34 °C and below disrupts the animal's capability to thermoregulate, eventually leading to death. At the other end of the temperature spectrum, a temperature of 40.5 °C can lead to a disruption of the thermoregulatory system, and in extreme cases, a temperature of 43 °C and over can lead to fatal brain lesions (Cunningham & Klein, 2007).

1.1.3 HEAT PRODUCTION

Mammalian species can produce heat in the following three ways; as by-product of metabolic processes, shivering, or non-shivering thermogenesis. Heat generation in the body balances the heat inputs and outputs with the external temperatures of the environment. Heat is generated when ingested macronutrients are metabolized in the body. This heat is eventually dissipated from the body in passive heat exchange between the body and surrounding environment. The BMR acts as a foundation for metabolic thermoregulation. BMR is the rate of energy metabolism measured under minimal stress during fasting (Cunningham & Klein, 2007). Endotherms have a higher BMR than ectotherms because of the active thermoregulation and heat production requirements of endotherms. It is also notable that smaller animals have a higher BMR owing to their higher surface area-to-volume ratio (Polsky & von Keyserlingk, 2017).

The primary and most efficient way for an adult cow to increase its heat output is through non-shivering mechanisms or, more precisely, the brown adipose tissue (BAT). BAT generates heat via a special protein, uncoupling protein 1 (UCP1), on the inner membrane of the mitochondria. UCP1 facilitates proton movement between the intermembrane space and the mitochondrial matrix. This movement releases the chemiosmotic potential of protons directly as heat. This mechanism was not fully understood until chemiosmotic potential currents were measured (Fedorenko et al., 2012). Cows can perceive seasonal changes and reductions in photoperiod and temperature, which enables them to prepare for the upcoming colder periods by increasing their BAT reserves accordingly (Li et al., 2019). Newborn calves can use BAT reserves after birth. However, this BAT transforms into white fat in the next 2–3 weeks of life (Alexander G et al., 1975; Gemmell et al., 1972).

Shivering is a way to respond to an acute decrease in the environmental temperature. Shivering involves the generation of heat through muscle contraction. The heat generated by the muscles is then transferred to the internal part of the body, although this can take a while (Mrowka & Reuter, 2016). During shivering, the heat output of the body can reach up to four times the normal output in short bursts; however, more often, the product of shivering is two times the normal output for 2–4 h (Cunningham & Klein, 2007).

1.1.4 HEAT TRANSFER IN THE BODY

Animals use all four heat transfer methods: convection, conduction, radiation, and evaporation. Convection occurs when the body heats the surrounding air or water it is in contact with. This heated air or water then flows away, exposing the skin to cooler air/water (Cunningham & Klein, 2007). Convection can occur both naturally and forcefully. Natural convection is observed daily when the warmer air/water evaporates from the skin because its density is lower than that of colder air/water. Forced convection, such as fans, forcefully feeds colder new air towards the skin of the animals, thus providing the animal with a constant source of colder air. This leads to a decrease in the temperature of the air surrounding the animal as well as the skin surface temperature. Forced convection has a limited capability to cool animals in hot and humid environments because of the reduced moisture gradient between the skin surface and the ambient air (Gebremedhin et al., 2016).

Conduction is the transfer of heat between objects and animals in contact with each other. It is not a common effect to occur naturally because animals do not lay down on cool surfaces for the purpose of cooling down. Gebremedhin et al. (2016) suggested that conductive cooling systems, such as waterbeds or other heat exchangers under the bedding, could reduce humidity and improve farm hygiene.

Body tissues have poor heat conductivity, which means that the heat transfer between organs and other tissues is inefficient. The vast majority of the heat transfer in the body utilizes the circulatory system for heat transfer within and out of the body. As the blood perfuses through organs, such as the liver, it gathers heat with it, and then passes through the capillaries of the skin to relieve the heat on to the skin and out of the body. The cooled blood can then be utilized for cooling organs such as the brain (Cunningham & Klein, 2007). At temperatures over the cows thermoneutral zone around 15% of heat loss occurs via the respiratory tract. This has been connected to respiratory alkalosis, as the respiratory rate and depth increase, leading to the loss of CO2 (Blackshaw & Blackshawb, 1994). The rest of the heat is removed via evaporation, convection, and conduction as vessels in the peripheries undergo vasodilation to increase blood flow to the skin. The changes in blood flow to the skin might be expected to change the sweating rate of the cow owing to the close contact between the capillary beds and the sweat glands (Finch & Rockhampton, 1986).

Many mammals rely on heat dissipation via the extremities. Extremities are often less insulated than the core of an animal, allowing for greater heat dissipation via the circulatory system. This system also protects well-insulated animals from overheating during physical activity (Scholander, 1955). Peripheral temperature has been shown to decrease due to niacin, a nictonic acid, which causes vasodilation near the skin. Encapsulated niacin pills showed an increase in temperature loss when compared with the control group (Zimbelman et al., 2010).

1.1.5 ENVIRONMENTAL EFFECTS TO BODY TEMPERATURE

Cattle are affected by environmental factors such as air temperature, humidity, solar radiation, wind velocity, and precipitation every day. Heat stress occurs in a combination of these variables when the relative temperature of the environment rises over the thermoneutral zone of cows(Blackshaw & Blackshawb, 1994). Wind velocity and solar radiation data are often not publicly available from weather stations, which currently makes them rare occurrences in research. The most commonly used variables with regard to heat stress in cattle are air temperature and relative humidity, as they are often publicly available information from meteorological stations (Bohmanova et al., 2007).

Solar radiation and wind velocity are variables that can be minimized by using simple shade and cover structures. In one study, the rectal temperature of shaded and non-shaded cows showed that milk production was higher in shaded than in non-shaded cows. Additionally, neither group could get rid of excess heat during the night, but the non-shaded group lost more heat than the shaded group. This information suggests that non-shaded cows lose more heat when radiating heat to the sky, suggesting that shading entire loafed areas in hot climates is not optimal (Blackshaw & Blackshawb, 1994).

1.1.6 PERIPHERAL- AND CENTRAL THERMORECEPTORS

An animal's ability to thermoregulate relies on its ability to perceive changes in ambient and internal temperatures. Different animals have their own thermoneutral zones, which their bodies aim to maintain via neurons in the central nervous system (CNS) and peripheral thermoreceptors. These systems work together to make thermoregulation possible. One of the most well-known routes of thermal signaling is the spinothalamic–cortical pathway. Heat stimuli activate thermoreceptors, thermosensors, and cutaneous effectors that carry signals to the dorsal root ganglion of the spinal cord. From there, the signals go to the thalamus and eventually to the primary somatosensory cortex, where the signals are consciously perceived and realized (Lezama-García et al., 2022).

Sensory thermoreceptors are nerve endings with specialized ion channels that can cause changes in membrane permeability when reacting to external stimuli. Most of these receptors are made of transient receptor potentials (TRP), which perform various tasks such as participating in the transduction of mechanical and chemical sensory stimuli, maintaining the membrane resting potential, and controlling calcium and magnesium levels in neurons. They exist in nearly every tissue cell type and in all cell membranes, except for nuclear and mitochondrial membranes (Lezama-García et al., 2022).

1.1.7 HEAT- AND COLD STRESS

Heat stress is defined as a multifactorial problem in cows, that are unable to maintain homeostasis in their body due to changes in ambient temperature and humidity. It is difficult to assess heat stress universally as it is affected by individual heat tolerance. These individual differences include age, health status, breed, and prior exposure to heat. Therefore, heat stress analysis must be made individually while looking at the heat stress parameters. The most important parameters when examining heat stress are temperature-humidity index (THI), respiratory rate (RR), rectal temperature (RT), milk yield and body weight. THI combines air temperature and humidity to a single unit. In (Mader et al., 2006) the calculation of THI is described as:

$$THI = 0.8 * T + RH * (T - 14.4) + 46.4$$

T = Ambient temperature, RH = Relative humidity

With THI it is described that the value of 68 means a threshold for when a cow might start to experience heat stress. A value of 72 means a high risk for heat stress (Burgos Zimbelman et al., 2009; Fabris et al., 2017). RR of 80 per minute. RT 39.2 °C, decrease in milk yield of 10% and weight loss of 5% (Blackshaw & Blackshawb, 1994). Indicators for choosing heat stress thresholds and characteristics should be based on scientific literature and include breed, lactation stage, milk yield, husbandry system, climate region, bedding type, diet, and cooling management strategies (Galán et al., 2018).

Heat stress does not affect calves and heifers as often as fully grown adults because they generate less heat through metabolism and have a larger body surface area relative to body mass. Calves and heifers are still hardly immune to heat stress and its effects. Scientific evidence has shown that heat stress negatively influences physiology, feed conversion efficiency, dry matter intake, growth rate, and reproductivity (J. Wang et al., 2020).

Cold stress is a similar problem to heat stress, in which the cow is unable to maintain thermal homeostasis due to decreased air temperature. Analysis of cold stress uses the Wind Chill Temperature (WCT) index. The most commonly used calculation is that proposed by Tucker et al. (2007), which considers the velocity of ventilated air movement. Cold stress is not as common a problem as its counterpart because of the cow’s ability to create high metabolic heat and grow a thicker coat of fur. The effects of cold stress are similar to those of heat stress, and include decreased growth, fertility, and milk production. In cold stress, feed intake is markedly increased, unlike in heat stress, in which feed intake decreases. (Angrecka & Herbut, 2015).

1.1.8 FEVER VS HEAT STRESS

Fever is a common physiological response in calves and serves as an indicator of underlying infection, inflammation, or failure in thermoregulation. Understanding the causes, consequences, and management of fever in calves are crucial for effective veterinary care. During fever, the inflammatory system releases pyrogenic factors that stimulate febrile response. In addition to the inflammatory response that results from increased body temperature, temperature itself has been proven to kill certain pathogenic organisms (Eric D Reid, 2014).

Heat stress, similarly to fever, causes changes in the circulating hormone levels. For example, cortisol levels increase in both situations when cows experience stress. However, other hormonal changes, such as growth hormone (GH), differ significantly in heat stress and fever situations. In fever, GH levels rise to increase metabolic heat generation in response to a disease situation, whereas during heat stress, the cow's body tries to get rid of extra heat, such as metabolic heat generation, by decreasing the hypothalamic release of GH(Daniel et al., 2002; Mcguire M. A et al., 1991).

1.2 MEASURING TEMPERATURE IN CATTLE

1.2.1 REASONS OF MEASURING TEMPERATURE IN CATTLE

Cows suffering from heat stress are less productive than normally, and in high producing cows the effects of heat stress are even more pronounced than in less productive cows. Productivity concerns are especially high in dairy cattle due to a decrease in lactating ability during heat stress (Mayer et al., 1999).

Losses in productivity are greatly increased in high yielding dairy cattle, as it has been shown that each 10kg/day increase in milk yield will result in a 5°C decrease in heat stress threshold. This is attributed to the increased heat production of high-yielding cows. Heat stress also affects reproductivity in addition to productivity. It has been reported that during heat stress, up to 80% of estrus goes undetected owing to the decreased production of clear mucous discharge and decrease in mounting behavior (Cartwright et al., 2023).

In the United States alone, heat stress is estimated to cause annual losses of $1.69 to 2.36 billion for the whole livestock production industry, of which $900 million is specific to the dairy industry (Polsky & von Keyserlingk, 2017).

1.2.2 METHODS OF MEASURING TEMPERATURE

Measuring temperature in cows is often divided into two main categories: core temperature and peripheral temperature. Core temperature refers to the temperature of internal organs, such as the brain, intestines, and other vital organs. It is often the most accurate method for measuring temperature, as it is not affected by external variables. Measuring the core temperature is labor-intensive, which makes it difficult to use in situations where the temperature of multiple cows is of interest (Sato et al., 1996).

The peripheral temperature measures the temperature closer to the surface of the skin. It is less accurate with a larger variation between readings but is usually less labor-intensive (McManus et al., 2016). Peripheral temperature taken from the forehead of a cow using infrared technology correlated strongly with THI and moderately with rectal temperature (RT) (Salles et al., 2016).

The core temperature via rectal measurement will most likely remain the method of choice in cases of sickness in individual cows. Peripheral methods are more suited for following general trends in multiple cows affected by the same problem (Salles et al., 2016).

1.2.3 MEASURING CORE TEMPERATURE

Measurement of core temperature rectally is a good index of the general body temperature, even though core temperature might vary in different parts of the body during the day (Gupta et al., 2013). RT measurement is commonly used in studies, as it is a close representation of the cows’ core temperature. There is a chance of unreliable results due to possible user error, defective device or other reasons (Naylor et al., 2012).

The disadvantage of rectal temperature measurement is that it is labor-intensive. Measuring the rectal temperature requires personnel with knowledge of cattle behavior, around 30–60 seconds per reading, and having to be in contact with the animal during the measurement (Eric D Reid, 2014).

Tympanic membrane temperature has been used as an alternative to rectal temperature but has gained a somewhat controversial status. The tympanic membrane temperature is affected by facial cooling, which can produce faulty results (McCaffrey et al., 1975). Another study showed that tympanic membrane measurement was not affected by facial cooling if it was accurately placed in the ear canal (Sato et al., 1996). However, tympanic membrane temperature is widely accepted as the best predictor of thermoregulatory status because of the proximity of the tympanic membranes to the hypothalamus. Tympanic temperature data gathering can prove difficult because of possible dislodgement and ear canal infections (Bergen & Kennedy, 2000).

In a study where tympanic membrane temperature and vaginal temperature were measured via radiological telemetry, the vaginal temperature consistently produced core temperature results similar to the tympanic temperature (Bergen & Kennedy, 2000).

1.2.4 MEASURING PERIPHERAL TEMPERATURE

Peripheral temperature measurements provide us with skin or near-skin surface temperatures. It is often rapid, less invasive, and more labor-free, with different methods varying in these parameters. Thermography with an infrared device is fast, efficient, and noninvasive. These devices have been studied to produce reliable results and detect even small changes in temperature, with the most accurate results and the least variation coming from the eyes. Measuring the temperature with this method, however, is almost as labor-intensive as measuring the rectal temperature. Infrared devices require the user to be in close proximity to an animal and measure each cow individually (Salles et al., 2016).

Subcutaneous temperature sensors produce real-time results, and nearly no labor is required after initial surgery. Little is known about the correlation between peripheral implant data and rectal body temperature. In one study where cows were challenged with 0.1 ug/kg of LPS (*E. coli* 055:B5) into the bloodstream, the rectal temperatures quickly rose into fever, but peripheral sensor data negatively correlated as the temperature data in the peripheries showed a decrease in temperature (Eric D Reid, 2014).

1.2.5 ANALYSIS OF VOLATILITY

Generalized Autoregressive Conditional Heteroscedasticity (GARCH) modeling can be used to assess data for heteroscedasticity. Generalized means that the GARCH allows for more flexibility in modeling conditional variance by incorporating additional parameters and terms, such as an autoregressive moving average (ARMA) component, asymmetry in volatility responses, or long-term memory effects. The autoregressive (AR) part captures the dependence of the current conditional variance on past squared error terms (volatility shocks) and the conditional mean of the series. Conditional means that the variance of the current observation is conditioned on (depends on) past observations. Heteroscedasticity refers to the situation in which the variance of a variable is not constant over time. In other words, the variability of variables changes over time (Hautsch & Voigt, 2020; Hyndman & Athanasopoulos, 2018).

Conditional variance is used to analyze the volatility across a given time period. It refers to the estimated variability or dispersion of a variable given certain information or conditions, typically including past observations of the variable itself. It represents the uncertainty or volatility in the values of the variable at a particular time point, considering the available information up to that point. It reflects how much the variable's values are expected to fluctuate around their mean or expected value at that time, based on past observations and other relevant factors (Hautsch & Voigt, 2020; Hyndman & Athanasopoulos, 2018).

The GARCH model estimates the parameters that collectively define the dynamics of volatility in time-series data. These parameters include alpha (α), beta (β), omega (ω), and GARCH (p) parameters capturing autoregressive relationships; ARCH (q) parameters representing the impact of past volatility shocks; and mean equation parameters, if applicable. The values of alpha (α) and beta (β) determine volatility behavior (Hautsch & Voigt, 2020; Hyndman & Athanasopoulos, 2018).

1.3 ENVIRONMENTAL EFFECTS

1.3.1 TEMPERATURE HUMIDITY INDEX

Heat stress occurs when environmental factors such as air temperature, relative humidity, air movement, and solar radiation combine to create an effective environment with a temperature higher than the thermoneutral zone of the cow. The temperature–humidity index is a key parameter for the assessment of heat stress. It combines the temperature and relative humidity of the air, but does not consider solar radiation or air movement(Lim et al., 2021). The upper limit values for THI are commonly 69–72, depending on the calculation variables used. In body weight a marked loss of 0.2-0.41 kg/day has been reported for every unit over 69–72 THI. Higher yielding cows (>25 kg milk/day) experienced a reduction in milk yield of 16.1%, whereas lower yielding cows (<20 kg milk/day) experienced a decrease of 11.6% (Bouraoui et al., 2002; Ravagnolo et al., 2000).

There is also a marked difference in the ability of different breeds to resist heat stress. The Holstein breed tolerates cold quite well but is relatively susceptible to heat stress. Whereas the jersey cow which has a smaller body and produces less milk suffer no decrease in milk yield at a THI of 78 (Lim et al., 2021).

Climate change has increased concern for dairy cow productivity in the future. Increasing environmental temperature leads to larger losses in times of heat stress, especially with higher-yielding cows. Several studies have shown that during heat stress, production seems to correlate negatively with heat tolerance. As higher-yielding cows already suffer from higher metabolic heat production, their tolerance to heat stress diminishes. This has led researchers to discover variables that describe the overall level and slope of the response of milk production traits across rising THI values (Macciotta et al., 2017).

1.3.2 MICROCLIMATES IN DAIRY FARMS

The microclimate refers to the local environmental conditions surrounding dairy cattle, which can differ from other areas inside the building. The air surrounding the cow provides oxygen, but it also acts as a pathway for heat, gases, and particulate matter to enter the microclimate. Important parameters for assessing air quality and microclimates are temperature, air velocity, humidity, particulate matter, and gases, such as carbon dioxide, methane, ammonia, nitrous oxide, and oxygen (Teye et al., 2008).

Outdoor weather temperature directly affects the indoor temperature in uninsulated and semi-insulated dairy buildings because insulation is minimal and dairy cows themselves are the main source of heat generation. When moisture evaporates from the skin of cows, the surface temperature of the cow decreases due to evaporative heat loss. However, the amount of moisture and the temperature of the air surrounding the cow affect the rate of evaporation. A relative humidity (RH) over 90% at high indoor temperatures will induce heat stress in dairy cows owing to restricted evaporative heat losses. However, excessively low RH also has a negative impact on the microclimate, as it results in excessively dry bedding material in the dairy building and increases dust and the incidence of lung diseases in dairy cows (Seedorf et al., 1998).

1.3.3 CIRCADIAN RHYTHM

The circadian system is an inbuilt mechanism that tracks 24h days via circadian rhythms. These rhythms enable cattle to estimate, prepare, and act in response to environmental changes. This system is governed by homeostatic feedback loops and repair pathways. Exogenous and endogenous factors that function as inputs to the homeostatic system might disrupt natural circadian rhythms when applied at incorrect times; these events are called chrono disruptors. Common chrono disruptors include light, inappropriate times for feed intake, physical activity, and biological stress. These events can harm the temporal organization of physiology and lead to a decrease in welfare and productivity (Casey & Plaut, 2022).

2. AIMS OF THE STUDY

The aim of this study was to investigate temperature data using time-series analysis to describe calf body temperature volatility and its heteroscedastic nature during a relatively high-temperature period. The secondary objective was to describe the effects of circadian rhythm dynamics on calf body temperature during this period.

3. MATERIALS AND METHODS

3.1 STUDY POPULATION

This study was conducted with permission of animal testing license no. 161 (05.02.2020) issued by the Estonian Ministry of Rural Affairs. The study was conducted at a dairy cattle farm in Eastern Estonia (hereafter known as Farm 1) and another dairy cattle farm in northwest Estonia (hereafter known as Farm 2). After clinical examination and temperature measurement, calves between the ages of 3 and 12 days were implanted with a subcutaneous temperature sensor at both farms. If the calves showed clinical signs that would correlate with clinical disease or a temperature > 39 °C, they were excluded (data not shown).

Seventeen calves (11 from Farm 2 (all female) and 6 bull calves from Farm 1) between the ages of 20 and 45 days at the beginning of the observation period were used in this study. The full length of the observation period for the calves from Saaremaa was 78 days, starting from 15th of June 2022 till 31st to August 2022. The study period for bulls was 48 days, starting from 15th of July 2020 till 31st of August 2020. These observation periods were June, July, and August, which usually have the highest mean temperatures in Estonia. There were no data gaps in the implant readings during these periods. As seen in (Fig. 1), the calves in Farm 1 were outside in a group pen, and calves from Farm 2 were inside in a group pen for the duration of the study. Bull calves from Farm 1 were given a number from 1–6 and calves from Farm 2 were given a number from 1–11.

Figure 1. Calves' husbandry systems. (A) Bull calves from Farm 1, living in an outside pen with shelter. (B) Calves from Farm 2, in an indoor system. (Author of photo A: Margit Sarits, EMÜ and photo B: Tarmo Niine, EMÜ)

3.2 IMPLANTATION OF THE TEMPERATURE SENSORS

To measure peripheral temperature, six sensors were surgically implanted in Farm 1 bull calves: one in the neck, one in the prescapular groove, and one behind the elbow on both the left and right flanks (Fig. 3). On Farm 2, only one sensor was implanted in the prescapular groove. To make the data more comparable between farms 1 and 2, only one sensor from farm 1 was used. The temperature sensor implant was approximately 50 mm long and 5 mm in diameter (Fig. 2).

Figure 2. Example of the temperature sensor implant that was used for all calves in this study (Author: Tarmo Niine, EMÜ)

Sedation was done intramuscularly via alpha2-agonist xylazine (Xylapan inj 20 mg/ml – Vetoquinol Biowet Sp.z o.o) with doses varying from 1.0 ml to 1.5 ml between individuals according to their size and alertness. After sedation, the implantation area was prepared for the surgery. An area of approximately 10 cm × 10 cm was clipped and washed. The clipped area was scrubbed three times with swabs soaked in chlorhexidine soap (Hibiscrub chlorhexidine gluconate 40 mg/ml; Mölnlycke Health Care Group), and the area was cleaned with ethyl alcohol until the swabs were visually clean. With the surgical area cleaned, the local anesthetic amino ester group procaine (Procamidor 20 mg/ml; Richter Pharma AG) was subcutaneously administered at a dose of 1.0 ml. Procaine was injected in the form of a line to the area that would later become the incision line. An incision was made to reach the subcutaneous tissues, and a modified trocar was used to push the implant deeper into the subcutaneous tissues (Fig. 3). With the sensor in place, the incisions were closed with a single cross suture using Vicryl 2-0 FS-1 (Johnson and Johnson International). An antibiotic spray (Pederipra Spray, Laboratorios HIPRS S.A.) was sprayed on the closed incisions, as shown in (Fig. 4). A postsurgical analgesic (Metacam 20 mg/ml; Boehringer Ingelheim Vetmedica GmbH) was administered subcutaneously on the completion of surgery.

Figure 3. Trocar was used to push the temperature sensor implant deeper into the subcutaneous tissue. Blue arrow shows the implant in the prescapular area which was used in this study. Red arrows point to areas from which implant sensor data was used in other studies. (Author: Tarmo Niine, EMÜ)

Disbudding of calves that had horn buds via the hot iron method was performed before implantation. Three milliliters of procaine was injected halfway between the base of the ear and corner of the eye, approximately 1 cm deep on both sides, to block the cornual branch of the lacrimal nerve. A hot disbudding iron was then used to cauterize and remove the horns. Antibiotic spray was used on the cauterized areas to prevent infection.

Figure 4. One of the bull calves a day after surgical implantation. Antibiotic spray was used in all surgical sites and on cauterized areas. Blue arrow shows the prescapular area from which the temperature data was used for this study. Red arrow shows the wound from disbudding procedure. (Author: Margit Sarits, EMÜ)

3.3 SAMPLING

The temperature sensors measured and sent the calf temperature to a receiver situated in the barn. On Farm 1, readings were taken every 10 min, whereas on Farm 2, readings were taken every 20 min. The receiver is connected to the Internet and the data can be viewed using an Internet-connected device. Weather temperature and humidity were recorded hourly by the receiver.

3.4 TEMPERATURE HUMIDITY INDEX

We used the following calculation developed by Mader et al. (2006):

$$\mathrm{THI} = 0.8 * T + \mathrm{RH} * (T - 14.4) + 46.4$$

T = Ambient temperature, RH = Relative humidity

This calculation method was chosen because our data could be used in it. The reference points we used were, 68 a threshold for when a cow might start to experience heat stress, and a value of 72 and over, a high risk for heat stress. These reference values were chosen because they are specific to the Holstein breed, as described in (Fabris et al., 2019).

3.5 STATISTICAL ANALYSIS

Data handling, analysis, and figures were performed using RStudio version 2023.06.2+561 "Mountain Hydrangea" (RStudio Team, 2020), R version 4.3.2 (R core team, 2022). Temperature and humidity readings were saved as comma-separated value (CSV) files.

Descriptive statistics, such as minimum, maximum, average, and standard deviation, were used to create a table based on the weather temperature, humidity, and THI values. Environmental temperature and humidity data were also analyzed using time-series plots to visualize seasonality and trends. Weather temperature and humidity (expressed as a proportion) were used to calculate THI values for the entire study period. To analyze the individual calf heat stress dynamics, environmental temperature and humidity data were analyzed to determine the possible heat stress periods.

The volatility of subcutaneous temperature was evaluated in all calves during the entire observation period, with a focus on THI ≥ 72 (Burgos Zimbelman et al., 2009; Fabris et al., 2017). Volatility was tested using generalized autoregressive conditional heteroscedasticity (GARCH) models (Hautsch & Voigt, 2020), autoregressive moving average (ARMA), and GARCH, with both set to (1,1). In ARMA the first 1 in (1,1) indicates that there is one lagged term in the autoregressive (AR) part of the model, and the second 1 indicates a lagged term in the Moving Average (MA) part. The GARCH section of the model was set to (1,1), with the first 1, the Autoregressive Conditional Heteroskedasticity (ARCH) term representing the extent to which past squared residuals (past shocks to the variance) affect the current variance. The GARCH term, the second in Garch (1,1), measures the impact of past conditional variances on the current variance, indicating persistence in volatility. Other specifications for the model parameters were not tested in order to keep the model as simple as possible. The autoregressive conditional heteroskedasticity (ARCH) test was used to test GARCH models, in which a p-value ≤ 0.05, was considered significant. A significant result ($p \leq 0.05$) from the ARCH test indicates that the assumption of constant variance (homoscedasticity) in the residuals of a time-series model is violated. If the ARCH test p-value was greater than 0.05, then there was insufficient evidence to reject the null hypothesis of no ARCH in the model's errors. In this case, it could mean that there might be false data, lack of data, or some unforeseen complications in the data provided to the GARCH model, leading to “incomplete” results.

GARCH model parameters ‘Alpha’ and ‘Beta’ were used in the current study. ‘Alpha’ is the Recency Effect / Reaction to Recent Shock, that in the context of calves’ body temperature, refers to the immediate effect of a recent event or shock on the body temperature. For example, if a calf is exposed to a sudden change in ambient temperature, the ‘Alpha’ would represent how much the calf’s body temperature is expected to change in response to this new environment. A high ‘Alpha’ value (close to 1) suggests that recent shocks have a significant impact on current volatility. ‘Beta’ is the Persistence / Decay of Conditional Variance, which represents the persistence of volatility over time. A high ‘Beta’ value (close to 1) implies that past volatility persists into the future. In other words, if volatility was high yesterday, it is likely to remain high today (Bollerslev, 1987; Maheu & Shamsi Zamenjani, 2021).

Conditional variance was used to visualize the volatility during the entire observation period. Calf body temperature readings and THI were evaluated simultaneously to describe patterns and similarities in the data.

With circadian rhythm, it was expected that the temperature would fluctuate throughout the day, lowest in the morning, increase towards the end of the day, and decrease again during the night (Mrowka & Reuter, 2016). To describe the circadian rhythm, we separated the body temperature values into the following times of the day: “morning” – 06:00-11:59, “day” – 12:00-17:59, “evening” – 18:00-23:59, and “night” – 00:00-05:59. The average, maximum, minimum, and SD values were calculated for each period.

4. RESULTS

The Farm 1 weather temperature, humidity %, and THI from the weather stations are summarized in Table 1. A similar table with descriptive statistics for Farm 2 is presented in Table 2.

Overall, on Farm 1, the average weather temperature during the observation period was approximately 20.56 °C, with fluctuations ranging from 13.19 °C to 31.12 °C. These temperatures were associated with a standard deviation of approximately 2.11 °C. The humidity was approximately 53.17 % with a standard deviation of 0.95. The average THI was approximately 66.09, with values fluctuating between 80.23 and 56.28. The standard deviation of the THI was 2.78. For more detailed information, please refer to Table 1.

In Farm 2, Throughout the observation period, the average weather temperature fluctuated around 20.29 °C, with maximum and minimum temperatures ranging from 11.70 °C to 30.96 °C, with standard deviation of approximately 2.62 °C. The humidity was 76.37 % with standard deviation 9.05 %. THI averaged approximately 66.97, with values ranging from 79.41 as the maximum to 53.53 as the minimum. The standard deviation of the THI was 3.75. For more detailed information, please refer to Table 2.

Table 1. Farm 1 weather data from 15.07.2020 – 31.08.2020. Average (avg), maximum (max), minimum (min), and standard deviation (sd) of weather temperature, humidity % and temperature humidity index (THI) presented from each week from the study period. Overall is the average of all the weekly results.

Week	*Weather Temp*				*Humidity %*				*THI*			
	avg	max	min	sd	avg	max	min	sd	avg	max	min	sd
1	22.78	28.01	16.14	2.39	49.98	53.00	45.80	1.48	68.78	75.33	60.24	3.01
2	19.68	25.35	14.40	2.76	51.19	53.60	49.70	0.85	64.84	72.21	57.92	3.60
3	19.73	22.80	16.79	1.46	53.84	56.23	52.00	0.89	65.05	69.18	61.11	1.94
4	22.38	31.12	17.92	2.43	54.49	56.00	51.67	0.79	68.65	80.23	62.64	3.25
5	20.76	26.84	16.78	2.43	53.74	56.00	52.00	0.73	66.42	74.59	61.10	3.25
6	21.17	25.30	17.10	1.92	53.16	55.00	51.00	1.18	66.93	72.25	61.55	2.51
7	17.39	19.76	13.19	1.41	55.77	57.00	54.83	0.70	61.98	65.16	56.28	1.91
Overall	20.56	25.60	16.05	2.11	53.17	55.26	51.00	0.95	66.09	72.71	60.12	2.78

Table 2. Farm 2 weather data from 15.06.2022 – 31.08.2022. Average (avg), maximum (max), minimum (min), and standard deviation (sd) of weather temperature, humidity % and temperature humidity index (THI) presented from each week from the study period. Overall is the average of all the weekly results.

Week	*Weather °C*				*Humidity %*				*THI*			
	avg	max	min	sd	avg	max	min	sd	avg	max	min	sd
1	18.54	21.56	14.88	1.56	75.98	91.12	51.41	9.78	64.31	68.09	58.70	2.24
2	22.63	30.96	12.58	4.76	67.70	90.03	43.37	13.14	69.64	79.41	54.95	6.33
3	23.41	29.49	15.71	2.95	72.80	90.31	44.08	11.75	71.59	79.17	60.02	4.37
4	18.78	23.70	14.53	2.04	80.60	93.79	53.23	9.42	64.83	71.24	58.12	2.99
5	18.25	23.31	12.31	2.64	80.16	95.79	53.27	10.48	63.97	71.40	54.38	4.13
6	20.97	28.61	13.23	3.03	77.19	91.97	52.66	8.92	68.06	77.54	56.05	4.20
7	18.35	23.99	11.70	2.78	75.44	91.23	50.03	8.25	63.93	71.78	53.53	4.12
8	20.38	27.01	13.13	3.11	74.29	87.55	52.82	7.87	66.97	75.57	55.88	4.39
9	22.77	27.90	13.55	3.14	75.66	89.44	53.05	8.32	70.77	77.81	56.57	4.46
10	23.58	28.36	18.65	2.01	77.94	89.96	54.65	8.24	72.28	77.19	64.92	2.56
11	20.61	27.53	16.27	2.61	82.71	95.36	64.82	6.27	67.97	77.18	61.03	3.99
12	15.25	16.59	14.05	0.76	76.01	84.52	65.68	6.17	59.26	61.47	57.36	1.23
Overall	20.29	25.75	14.22	2.62	76.37	90.92	53.26	9.05	66.97	73.99	57.63	3.75

4.1 Analysis of environmental temperature and humidity data

In the Farm 1, bull calves' dataset (Fig. 5) we can see environmental temperature peaks in early July as well as early August, with peaks reaching up to 28 °C. Humidity seemed to decrease when the temperature rose, with a few exceptions around 6th–8th and 15th–17th of August when there was no obvious decrease in humidity during increased temperatures. The mean temperature was higher at the beginning of the observation period and seemed to decrease towards the end. Humidity was lower at the beginning and higher towards the end. THI values over 72 were only observed during or after high temperature and humidity periods in our data. Most of the time, it seems that THI is below the threshold of 68. Between all the peaks where THI $\geq$ 72, the value dropped below 72 before the next peak.

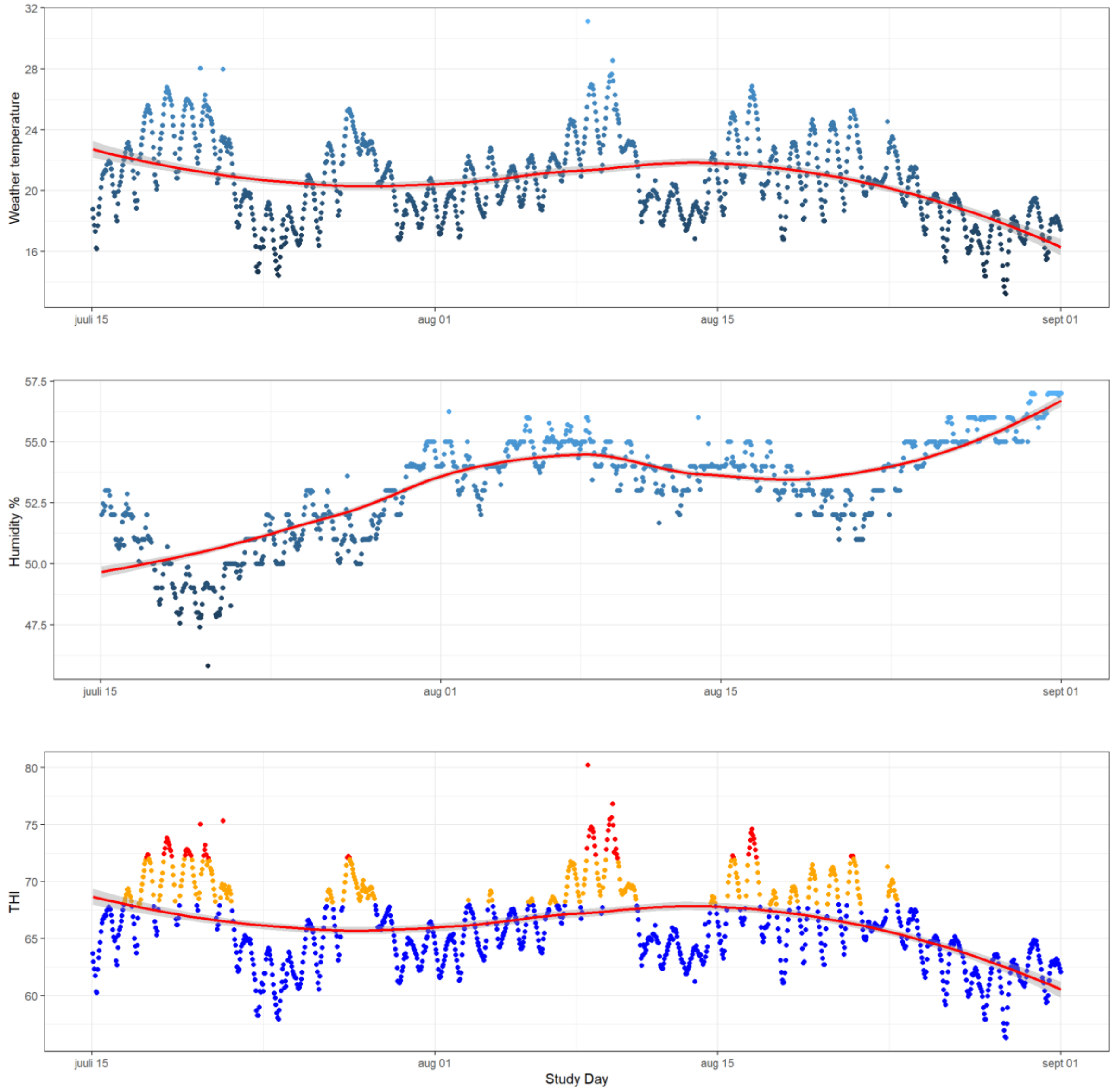


Figure 5. Farm 1 Weather temperature, Humidity % and temperature humidity index (THI) shown in separate graphs. Dots represent a value from every hour during the study period (48 days). Mean (red lines) and 95% confidence interval (grey lines). In THI graph, Blue = THI<68 no risk of heat stress, Orange = THI 68–72 calf can experience heat stress and Red = THI≥72 high chance of heat stress.

Farm 2 weather data (Fig. 6) show a temperature peak of approximately 32 °C at the end of June, but subsequent peaks at 28 °C in July and August. There was no obvious increase or decrease in temperature between the beginning and end of the observation period. Humidity is erratic across the observation period but seems to increase slightly towards the end. Humidity on Farm 2 was consistently very high at 50–90 (mostly 70–90). There seems to be some periods when THI stays over 72 between peaks of THI ≥ 72.

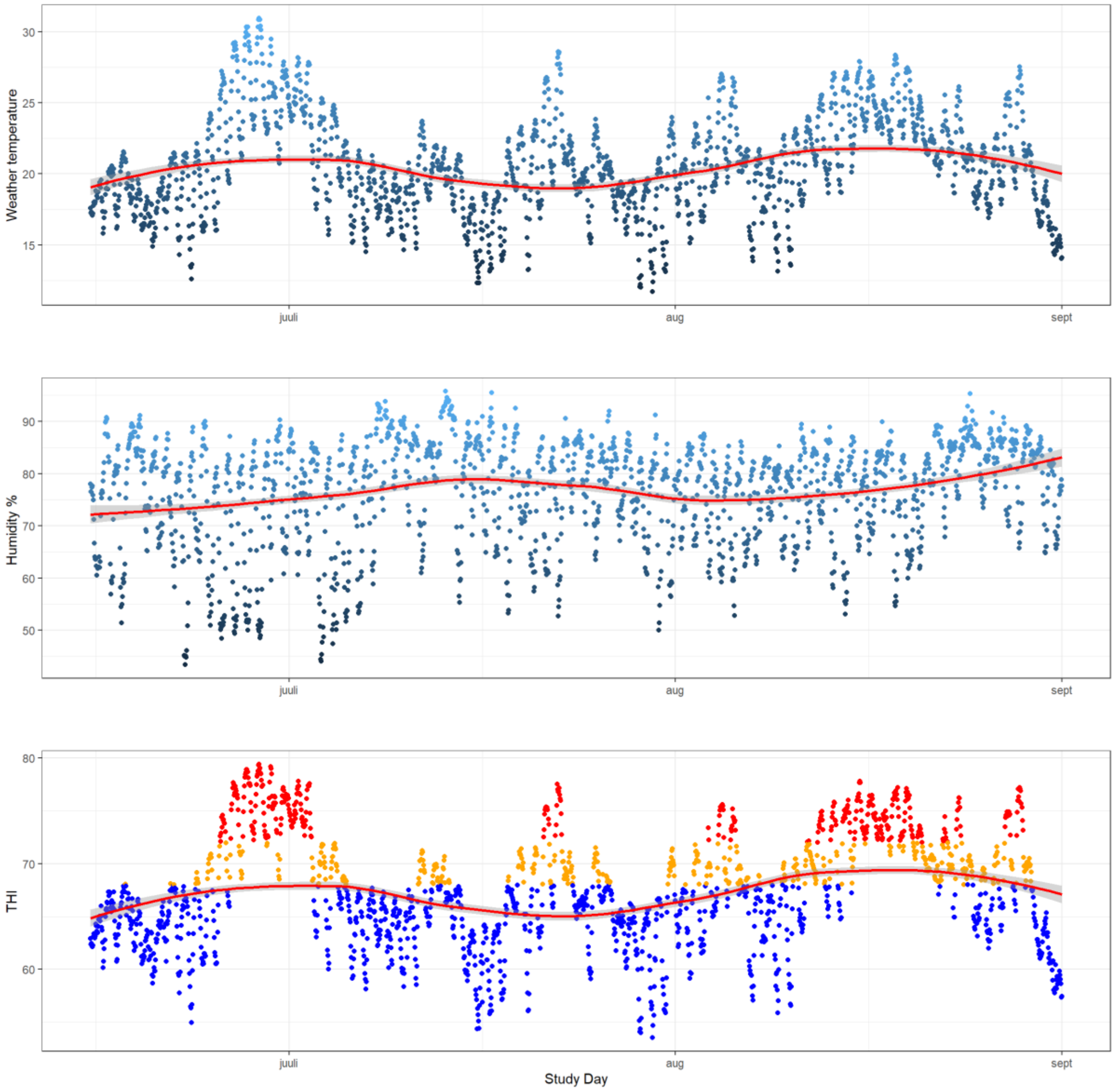


Figure 6. Farm 2 Weather temperature, Humidity % and temperature humidity index (THI) value from every hour during the study period (78 days). Mean (red lines) and 95% confidence interval (grey lines). In THI graph, Blue = THI<68 no risk of heat stress, Orange = THI 68–72 calf can experience heat stress and Red = THI ≥ 72 high chance of heat stress.

4.2 Body temperature volatility

Volatility was tested for the whole observation period with GARCH model analysis with a focus on 'Alpha' and 'Beta' values. The 'Alpha' values were low across all individual calves, reaching a maximum value of 0.15 on Farm 1 (Table 3), and 0.06 in Farm 2 (Table 4). The sum of 'Alpha' and 'Beta' in Farm 1 was the highest at 0.85, and close to 1 in all calves from Farm 2, except for calf 5. 'Beta' values, reached up to 0.98 in Farm 2, and 0.77 in Farm 1.

Except for bull calf 6 at Farm 1 (Table 3) and calf 5 at Farm 2 (Table 4), all calves had a sum of 0.5–1. On Farm 1, the ARCH test p-value was significant in bull calves 3, 4, and 6. On Farm 2, calves 3, 6, 7, 8, 9, and 10 had a significant ARCH test p-value.

Table 3. Farm 1. Generalized Autoregressive Conditional Heteroskedasticity (GARCH) model analysis of volatility during the observation period (48 days). All the hourly values were used. Alpha describes the effect of the baseline volatility on current volatility and Beta describes the effect of past observations on current volatility. If $\alpha + \beta$ is close to 1, it indicates a high level of persistence in volatility, meaning that shocks to the system will have long-lasting effects. ArchTest p-value ≤ 0.05 describes the presence of heteroskedasticity in the model residuals.

Bull calf	*Alpha*	*Alpha p-value*	*Beta*	*Beta p-value*	*ArchTest p-value*	*Alpha + Beta*
1	0.08	<0.001	0.77	<0.001	0.39	0.85
2	0.12	<0.001	0.60	<0.001	0.60	0.71
3	0.14	<0.001	0.50	<0.001	<0.001	0.64
4	0.11	<0.001	0.67	<0.001	<0.001	0.78
5	0.09	0.02	0.49	0.05	0.26	0.57
6	0.15	<0.001	0.30	0.06	0.05	0.45
Average	0.11	<0.01	0.55	0.02	0.22	0.67

Table 4. Farm 2. Generalized Autoregressive Conditional Heteroskedasticity (GARCH) model analysis of volatility during the observation period (78 days). All the hourly values were used. Alpha describes the effect of the baseline volatility on current volatility and Beta describes the effect of past observations on current volatility. If $\alpha + \beta$ is close to 1, it indicates a high level of persistence in volatility, meaning that shocks to the system will have long-lasting effects. ArchTest p-value ≤ 0.05 describes the presence of heteroskedasticity in the model residuals.

Calf	*Alpha*	*Alpha p-value*	*Beta*	*Beta p-value*	*ArchTest p-value*	*Alpha + Beta*
1	0.03	<0.001	0.96	<0.001	0.14	0.99
2	0.02	<0.001	0.97	<0.001	0.43	0.99
3	0.05	<0.001	0.95	<0.001	<0.001	1.00
4	0.03	<0.001	0.96	<0.001	0.11	0.99
5	0.06	0.03	0.35	0.10	0.24	0.41
6	0.03	<0.001	0.97	<0.001	<0.001	1.00
7	0.02	<0.001	0.97	<0.001	0.05	0.99
8	0.03	<0.001	0.96	<0.001	<0.001	0.99
9	0.02	<0.001	0.97	<0.001	<0.001	0.99
10	0.02	<0.001	0.98	<0.001	<0.001	1.00
11	0.03	<0.001	0.97	<0.001	0.06	1.00
Average	0.03	<0.01	0.91	0.01	0.09	0.94

In (Fig. 7) the bull calves from Farm 1 show erratic variability in the volatility of subcutaneous (SC) temperature readings. The greatest variability in volatility was seen in and around the middle to late August when the THI was between 60 and 75. There is considerable variation among individuals. From Appendix 2, it can be seen that most bull calves had an increase in mean body temperature from middle to late August.

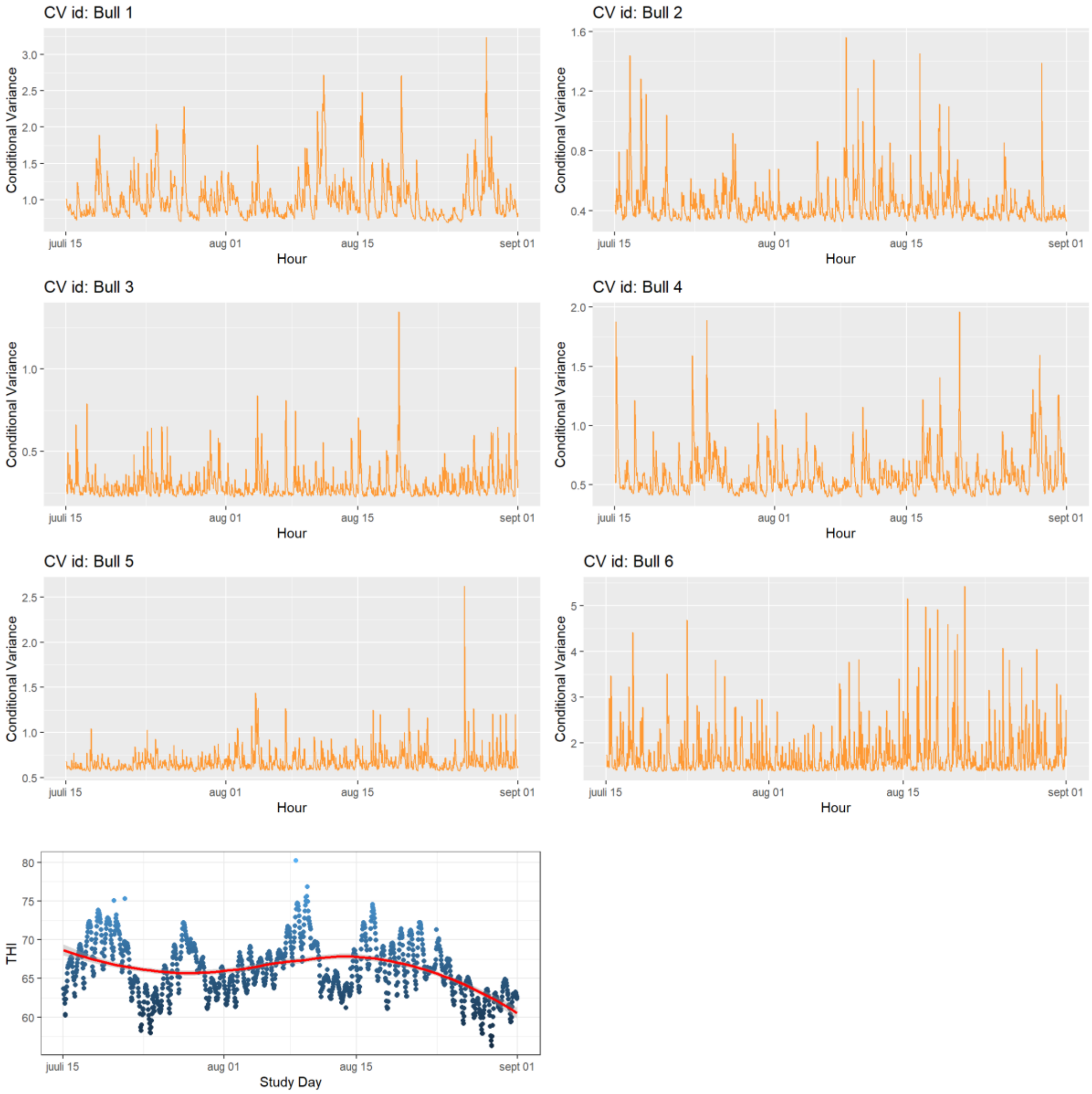


Figure 7. Farm 1 variation of individual subcutaneous (SC) temperatures with 1 lagged value (1 hour) visualized with conditional variance (CV) and temperature humidity index (THI) from the observation period (48 days).

Volatility is presented as conditional variance for all Farm 2 calves in Figure 8. The conditional variance seemed to be the lowest during and slightly after the periods when THI ≥ 72. These events can be observed between June and July and near the end of September. Calf body temperatures also seemed to increase among all individuals during these periods, as shown in Appendix 1. The volatility of calf body temperature was lower than average during these periods. There was also a similar event near the end of August, but with more variation among individuals as to when the period began and ended.

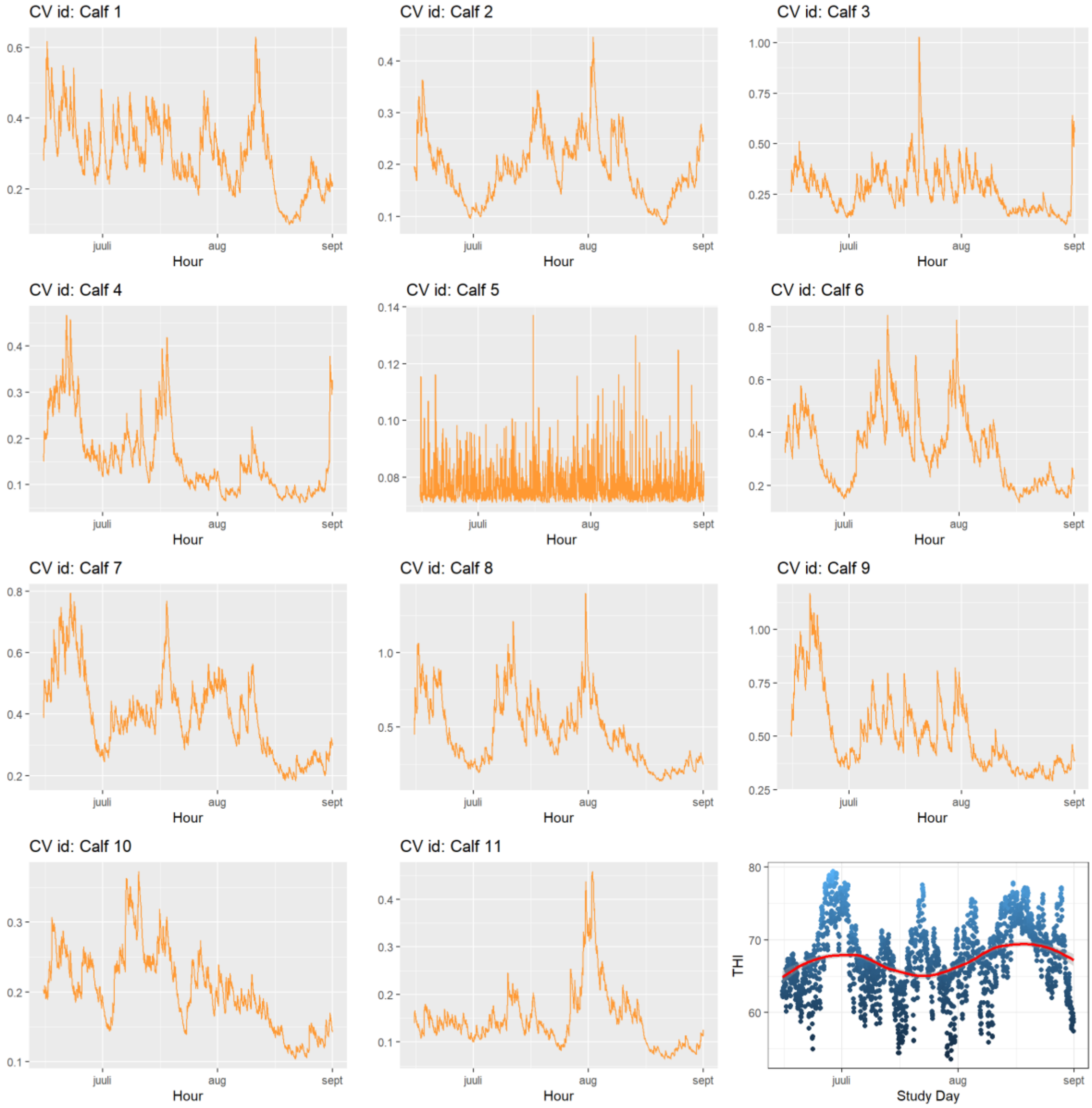


Figure 8. Farm 2 variation of individual subcutaneous (SC) temperatures with 1 lagged value (1 hour) visualized with conditional variance (CV) and temperature humidity index (THI) from the observation period (78 days).

4.3 Circadian rhythm dynamics

Calves from Farm 2 overall average temperatures ranged from approximately 36.43 °C to 38.38 °C (Appendix 4), with slight fluctuations observed between morning, day, evening, and night. Evening temperatures tended to peak slightly higher than those in the morning, with temperatures during the day falling between. The average temperature was lowest in the morning, higher during the day, and highest towards the end of the day in the evening. On Farm 1, the bull calves generally seemed to show higher average temperatures during the day than at other times of the day-night cycle (Appendix 3). Body temperature was lowest during the night, increased in the morning, peaked during the day, and lowered in the evening towards the night again.

At Farm 1, the data show body temperature readings of up to 42.15 °C during the day and 29.04 °C during night. Farm 2 shows comparable values of 40.67 °C in the evening and 32.30 °C in the morning.

5. DISCUSSION

The data from Farm 1 suggest that the THI drops below 72 between peaks, and a reasonable seasonality in the humidity readings can be seen. Humidity in Farm 1 seemed seasonal, as there was variation between the days and during the observation period, and the mean value increased towards the end of the study period. The humidity data from Farm 2 do not seem seasonal in the day cycle; instead, the humidity seems constantly high, and the readings during the observation period seem more random. It was theorized that since Farm 2 calves lived indoors in an uninsulated building with hundreds of other calves, heifers, and cows, the humidity is greatly increased by excrement, gases, and breathing, but since Farm 1 bull calves lived outside, where various weather conditions apply, there were no similarities in the humidity data. The animals all have a part in creating a microclimate, the effect of which is emphasized in uninsulated dairy buildings that rely on natural ventilation (Teye, 2008). Similar humidity and microclimatic effects were seen as in the current study at Farm 2. It seems that due to the consistently high humidity, Farm 2 experienced more consecutive days where the THI ≥ 72 compared to bull calves from Farm 1. The housing system used in Farm 2 seems to increase the possibility of heat stress experienced in cows because it allows for the same temperature as outside but likely provides less ventilation, which is a key factor in decreasing humidity. On the other hand, this type of housing system provides protection from wind and rain during colder periods, an important feature, especially in Estonia and Nordic countries. The primary limitation of the current study was the lack of air velocity data. Ventilation can greatly reduce humidity and, as such, diminish the occurrence and effect of heat stress symptoms (Zhou et al., 2022).

The data from the GARCH models suggest that calf body temperatures were primarily affected by past volatility instead of recent shocks. It can be deduced that the 'Alpha' values were low mostly, even if the sum of 'Alpha' and 'Beta' was close to 1, and 'Beta' values were often high. Comparing the conditional variance in Farm 1 bull calves and Farm 2 calves, it can be seen that the calves from Farm 1 had much more volatility in body temperature throughout the observation period. The bull calves experienced more direct weather conditions, such as rain, wind, and direct sunlight. They also had the possibility to alternate between shade/cover and these direct weather conditions. In addition, the humidity was likely affected by wind and other weather conditions at Farm 1. It is possible that these weather conditions adversely affected the ‘Alpha’ and ‘Beta’ readings at Farm 1. In Farm 2 calves, the effect of past observations seemed to be pronounced, suggesting that the effect of a humid microclimate and no direct weather conditions emphasize the effect of past volatility on current volatility. In 15 out of 17 calves the sum of ‘Alpha’ and ‘Beta’ was > 0.5, with an emphasis on the ’Beta’ values. This suggests a moderate to high level of persistence in volatility, meaning there are relatively low impact recent shocks to the system, but a larger impact comes from past volatility that likely persists into the near future. For example, if a calf is experiencing heat stress, the body temperature fluctuates slightly, but the effect of these fluctuations to the next hour is small, instead the current volatility is affected more by past observations of low volatility during this period. This indicates that the observed temperature dynamics are primarily driven by the persistence of past volatility patterns rather than immediate responses to recent events.

Some calves from both farms showed a significant p-value in the ARCH test. This means that the SC temperature volatility of these calves was poorly predicted by the model, as there were large differences between the predicted volatility and current volatility. It is possible that these individuals suffered from heat stress, sickness, or other outside interventions, which the model did not account for. In the current study, some calves were sick (data not shown) during the observation period. The rest of the calves showed a non-significant p-value, suggesting that there were smaller differences between the models’ predicted volatility and current volatility. It seems that the constructed GARCH models with set parameters did not fit accurately given the data.

Volatility was visualized using conditional variance from the entire observation period. Based on visual observation, it seems that the effect of high THI on body temperature is not immediate and instead comes with a small delay. Contrary to our presumed hypothesis, during the high THI periods, volatility seemed to decrease after the initial increase in the SC temperature. This could be because, if the environmental temperature is high, the temperature of the calf also remains high, because of its limited ability to lose heat. Conditional variance profiles seemed to differ between the farms. This might be caused by the differences in the microclimates and environmental effects, in their respective housing systems.

Most of the calves experienced increased persistence of volatility from past shocks and decreased volatility during high THI periods. This could be due to the fact that calves lack innate active cooling mechanisms and thus, rely on the passive heat exchange between the environment and themselves. The efficiency of passive heat exchange is decreased during periods of high THI (J. Wang et al., 2020), which adds to the difficulty of losing heat and the length of persistence in volatility.

It was expected that the temperature measurements would fluctuate during the day due to circadian rhythm dynamics and environmental effects, as observed in (Lee et al., 2016). Indeed, with Farm 2 calves, the results showed that there was fluctuation in the sensor measurements, similar to the temperature changes due to circadian rhythm, where the average temperature would be at its lowest in the morning and highest around midnight. In a study by Lee et al. (2016), in which subcutaneous temperature sensors were used, a similar display of daily temperature fluctuations was observed. The lowest reading was in the early morning, increased throughout the day, and the highest point was around midnight.

Farm 1 bull calves, however, showed a deviation from this rhythm, having the highest temperatures during the day and the lowest temperatures during the night. The subcutaneous location of the temperature sensors makes them more vulnerable to environmental temperature fluctuations (Torrao et al., 2011). In bull calves, this could explain some of the abnormally high temperature measurements that the sensors provided during the observation period, as the observation period was in the summer, during which direct sunlight could affect the sensor readings. Some implants were also placed under black fur patches (Fig. 9), which could intensify the effect of direct sunlight.

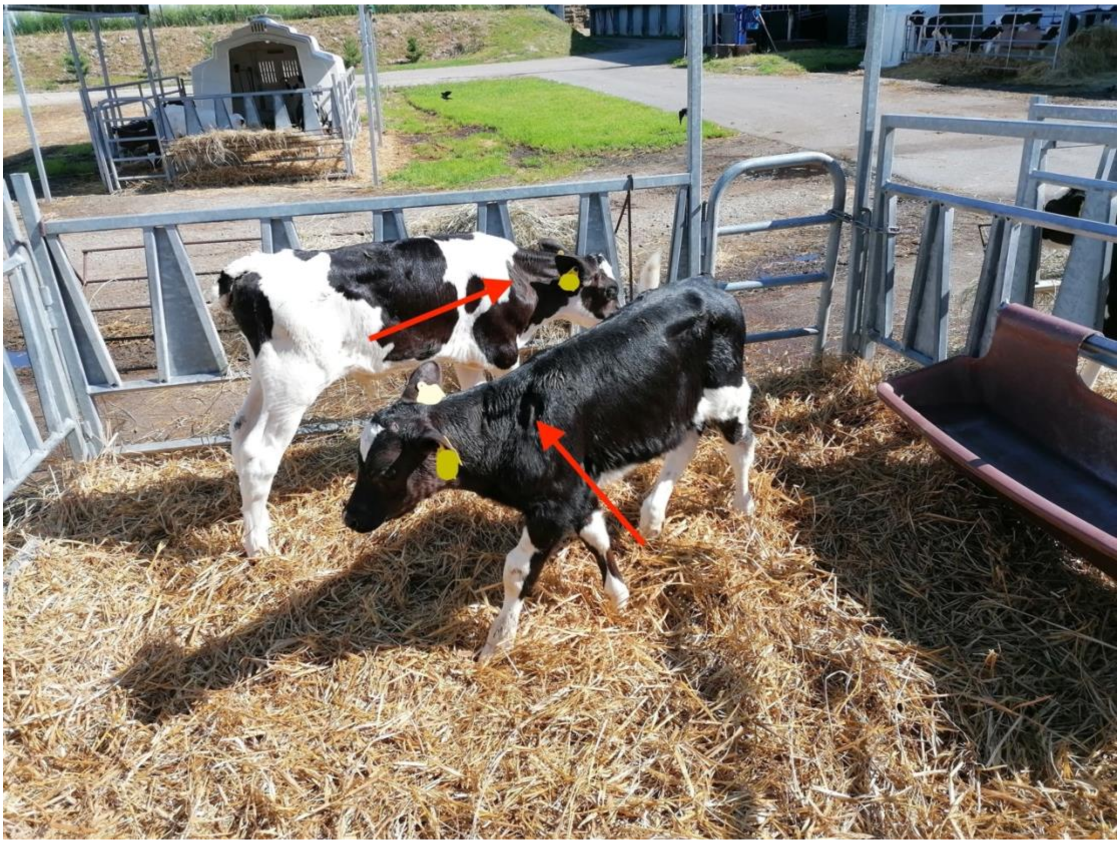


Figure 9. Bull calves 6 (in front) and 2 (in the back) under direct sunlight. There was variation of pigmentation in the areas (Red arrows) where temperature sensors were implanted.

There could have been opposite results if the observation period was during winter. In the future, it would be important to see how temperature readings react in animals exposed to direct weather conditions during other seasons. Lee et al. (2016) found that temperature readings did not change significantly compared to winter and summer conditions, although their research was conducted in a more controlled shed environment; thus, it could have some influence on the results as environmental elements, such as wind or direct sunlight, are mostly blocked out. In the current study, calf temperature measurements were limited to the peripheral temperature data. The core temperature via the rectal or tympanic method could provide more stable measurements from calves in situations where environmental effects cannot be avoided (Gupta et al., 2013; Sato et al., 1996). Environmental factors (i.e., humidity, wind speed, outside temperature, direct sunlight, etc.) affect the volatility of SC temperature readings, but to what extent remains unknown.

The surgical implantation of subcutaneous temperature sensors varies according to the type of sensor, location, and surgical plan used; however, all these procedures require invasion into the subcutaneous tissues. If done properly, the surgical process of the implantation takes quite a long time, as it requires sedation of the animal or a qualified person, for example a veterinarian, to perform the surgery. A simpler and quicker solution for implantation could make it more enticing among dairy farmers, even more so if the implantation could be done by the farmers themselves. Simplifying the implantation procedures and reducing the associated costs could make temperature monitoring more accessible. The prescapular location used in this thesis, might be too affected by environmental aspects. To get accurate core temperature readings, a temperature sensor could be implanted into the retroperitoneum, between the internal oblique muscle and abdominal wall of the animal (Torrao et al., 2011). The problem with this approach is that it would require deeper anesthesia and preferably a more sterile operating area. The surgery itself is more complicated than a subcutaneous implantation, since it would require accurate identification of abdominal tissue layers and a risk or perforating abdominal wall is present.

The use of THI to assess thermal comfort levels and heat stress in calves has limitations, as it does not consider any environmental effects other than air temperature and relative humidity. More advanced thermal indices, such as the Equivalent Temperature Index for Cattle (ETIC), have been developed that consider air velocity and solar radiation in addition to humidity and air temperature (X. Wang et al., 2018). In future studies, substituting THI with ETIC in outside conditions could make assessing thermal comfort levels and heat stress more accurate.

The application of GARCH models and conditional variance with a sufficient amount of data can offer a nuanced understanding of temperature volatility and calf sensitivity to environmental stressors.

CONCLUSION

This study highlights the importance of environmental factors and microclimates in influencing temperature volatility during periods of high Temperature-Humidity Index (THI). The results show that the Subcutaneous (SC) temperature volatility was lower in high THI periods, and that the persistence of volatility had a greater effect on current volatility, than recent shocks. In addition, the circadian rhythm of calves living outside deviated from prior studies by having peak temperatures during the day and lowest during the night. SC temperature sensors are prone to greater measurement variability due to direct environmental conditions. Outside and indoor housing systems would likely benefit from separate thermal indices to account for the differences in their respective environmental conditions. Future research should incorporate air velocity, solar radiation, and relevant health status information with the SC temperature sensor data to assess the severity of heat stress in cows.

ACKNOWLEDGEMENTS

This research was conducted in partnership with Piimaklaster MTÜ and co-funded by Estonian Rural Development Plan project no. 616118790023.

I would like to thank my supervisor Tarmo Niine for his superb guidance and relentless attitude for helping me since we started this thesis and until the very end. It is amazing how you had the patience to deal with me and my struggle with Rstudio. I am also thankful for your feedback and comments on this thesis, it is all the better for it.

Thank you for the whole 6th year class for all the memories and experiences we had during our studies.

Thank you to my family for all their support during these six years.

REFERENCES

Alexander G, Bennett J.W, & Gemmell R.T. (1975). Brown adipose tissue in the new-born calf (Bos taurus). *The Journal of Physiology*, *244*(1), 223–234.

Angrecka, S., & Herbut, P. (2015). Conditions for cold stress development in dairy cattle kept in free stall barn during severe frosts. *Czech Journal of Animal Science*, *60*(2), 81–87. https://doi.org/10.17221/7978-CJAS

Bergen, R. D., & Kennedy, A. D. (2000). Relationship between vaginal and tympanic membrane temperature in beef heifers. *Canadian Journal of Animal Science*, *80*(3), 515–518. www.nrcresearchpress.com

Blackshaw, J. K., & Blackshawb, A. W. (1994). Heat stress in cattle and the effect of shade on production and behaviour: a review. *Australian Journal of E.Uperimenta1 Agriculture*, *34*, 285–295.

Bohmanova, J., Misztal, I., & Cole, J. B. (2007). Temperature-humidity indices as indicators of milk production losses due to heat stress. *Journal of Dairy Science*, *90*(4), 1947–1956. https://doi.org/10.3168/jds.2006-513

Bollerslev, T. (1987). A Conditionally Heteroskedastic Time Series Model for Speculative Prices and Rates of Return. *The Review of Economics and Statistics*, 542–547.

Bouraoui, R., Lahmar, M., Majdoub, A., Djemali, M., & Belyea, R. (2002). The relationship of temperature-humidity index with milk production of dairy cows in a Mediterranean climate. *Animal Research*, *51*(6), 479–491. https://doi.org/10.1051/animres:2002036

Burfeind, O., von Keyserlingk, M. A. G., Weary, D. M., Veira, D. M., & Heuwieser, W. (2010). Short communication: Repeatability of measures of rectal temperature in dairy cows. *Journal of Dairy Science*, *93*(2), 624–627. https://doi.org/10.3168/jds.2009-2689

Burgos Zimbelman, R., Rhoads, R. P., Lynn Rhoads, M., & Duff, G. (2009). A Re-Evaluation of the Impact of Temperature Humidity Index (THI) and Black Globe Humidity Index (BGHI) on Milk Production in High Producing Dairy Cows. *Proceedings of the Southwest Nutrition Conference*, 158–169. https://www.researchgate.net/publication/251735409

Cartwright, S. L., Schmied, J., Karrow, N., & Mallard, B. A. (2023). Impact of heat stress on dairy cattle and selection strategies for thermotolerance: a review. *Frontiers in Veterinary Science*, *10*. https://doi.org/10.3389/fvets.2023.1198697

Casey, T. M., & Plaut, K. (2022). Circadian clocks and their integration with metabolic and reproductive systems: our current understanding and its application to the management of dairy cows. *Journal of Animal Science*, *100*(10). https://doi.org/10.1093/jas/skac233

Clarke, A., & Pörtner, H. O. (2010). Temperature, metabolic power and the evolution of endothermy. *Biological Reviews*, *85*(4), 703–727. https://doi.org/10.1111/j.1469-185X.2010.00122.x

Cunningham, J. G., & Klein, B. G. (2007). *Textbook of veterinary physiology: Vol. 4th edition* (Issue Fourth Edition).

Daniel, J. A., Whitlock, B. K., Wagner, C. G., & Sartin, J. L. (2002). Regulation of the growth hormone and luteinizing hormone response to endotoxin in sheep. *Domestic Animal Endocrinology*, *23*, 361–370.

Eric D Reid. (2014). *The use of implantable microchips for body temperature collection in cattle*.

Fabris, T. F., Laporta, J., Corra, F. N., Torres, Y. M., Kirk, D. J., McLean, D. J., Chapman, J. D., & Dahl, G. E. (2017). Effect of nutritional immunomodulation and heat stress during the dry period on subsequent performance of cows. *Journal of Dairy Science*, *100*(8), 6733–6742. https://doi.org/10.3168/jds.2016-12313

Fabris, T. F., Laporta, J., Skibiel, A. L., Corra, F. N., Senn, B. D., Wohlgemuth, S. E., & Dahl, G. E. (2019). Effect of heat stress during early, late, and entire dry period on dairy cattle. *Journal of Dairy Science*, *102*(6), 5647–5656. https://doi.org/10.3168/jds.2018-15721

Fedorenko, A., Lishko, P. V., & Kirichok, Y. (2012). Mechanism of fatty-acid-dependent UCP1 uncoupling in brown fat mitochondria. *Cell*, *151*(2), 400–413. https://doi.org/10.1016/j.cell.2012.09.010

Finch, V. A., & Rockhampton, N. (1986). Body temperature in beef cattle: its control and relevance to production in the tropics. *Journal of Animal Science*, *62*(2), 531–542. https://academic.oup.com/jas/article-abstract/62/2/531/4658509

Galán, E., Llonch, P., Villagrá, A., Levit, H., Pinto, S., & Del Prado, A. (2018). A systematic review of non-productivityrelated animal-based indicators of heat stress resilience in dairy cattle. *PLoS ONE*, *13*(11). https://doi.org/10.1371/journal.pone.0206520

Gebremedhin, K. G., Wu, B., & Perano, K. (2016). Modeling conductive cooling for thermally stressed dairy cows. *Journal of Thermal Biology*, *56*, 91–99. https://doi.org/10.1016/j.jtherbio.2016.01.004

Geiser, F. (2004). Metabolic rate and body temperature reduction during hibernation and daily torpor. *Annual Review of Physiology*, *66*, 239–274. https://doi.org/10.1146/annurev.physiol.66.032102.115105

Gemmell, R. T., Bell, A. W., & Alexander, G. (1972). Morphology of Adipose Cells in Lambs at Birth and during Subsequent Transition of Brown to White Adipose Tissue in Cold and in Warm Conditions. *American Journal of Anatomy*, *133*(2), 143–163.

Gupta, M., Kumar, S., Dangi, S., & Jangir, B. (2013). Physiological, Biochemical and Molecular Responses to Thermal Stress in Goats. *International Journal of Livestock Research*, *3*(2), 27. https://doi.org/10.5455/ijlr.20130502081121

Hautsch, N., & Voigt, S. (2020). *fGarch: Rmetrics - Autoregressive Conditional Heteroskedastic Modelling.* (3042.86). https://cran.r-project.org/web/packages/fGarch/index.html

Hyndman, R. J., & Athanasopoulos, G. (2018). *Forecasting: Principles and Practice: Vol. Otexts* (3rd edition).

Lee, Y., Bok, J. D., Lee, H. J., Lee, H. G., Kim, D., Lee, I., Kang, S. K., & Choi, Y. J. (2016). Body temperature monitoring using subcutaneously implanted thermo-loggers from holstein steers. *Asian-Australasian Journal of Animal Sciences*, *29*(2), 299–306. https://doi.org/10.5713/ajas.15.0353

Lezama-García, K., Mota-Rojas, D., Pereira, A. M. F., Martínez-Burnes, J., Ghezzi, M., Domínguez, A., Gómez, J., Geraldo, A. de M., Lendez, P., Hernández-ávalos, I., Falcón, I., Olmos-Hernández, A., & Wang, D. (2022). Transient receptor potential (Trp) and thermoregulation in animals: Structural biology and neurophysiological aspects. *Animals*, *12*(1). https://doi.org/10.3390/ani12010106

Li, L., Li, B., Li, M., & Speakman, J. R. (2019). Switching on the furnace: Regulation of heat production in brown adipose tissue. *Molecular Aspects of Medicine*, *68*, 60–73. https://doi.org/10.1016/j.mam.2019.07.005

Lim, D. H., Kim, T. Il, Park, S. M., Ki, K. S., & Kim, Y. (2021). Evaluation of heat stress responses in Holstein and Jersey cows by analyzing physiological characteristics and milk production in Korea. *Journal of Animal Science and Technology*, *63*(4), 872–883. https://doi.org/10.5187/jast.2021.e62

Macciotta, N. P. P., Biffani, S., Bernabucci, U., Lacetera, N., Vitali, A., Ajmone-Marsan, P., & Nardone, A. (2017). Derivation and genome-wide association study of a principal component-based measure of heat tolerance in dairy cattle. *Journal of Dairy Science*, *100*(6), 4683–4697. https://doi.org/10.3168/jds.2016-12249

Mader, T. L., Davis, M. S., Brown-Brandl, T., Mader, T. L., & Brown-Brandl, T. (2006). Environmental Factors Influencing Heat Stress in Feedlot Cattle. *Journal of Animal Science*, *84*(3), 712–719. https://digitalcommons.unl.edu/animalscifacpub/608

Maheu, J. M., & Shamsi Zamenjani, A. (2021). Nonparametric dynamic conditional beta. *Journal of Financial Econometrics*, *19*(4), 583–613. https://doi.org/10.1093/jjfinec/nbz024

Mayer, D. G., Davison, T. M., Mcgowan, M. R., Young, B. A., Matschoss, A. L., Hall, A. B., Goodwin, P. J., Jonsson, N. N., & Gaughan, J. B. (1999). Extent and economic effect of heat loads on dairy cattle production in Australia THI Temperature-humidity index. *Australian Veterinary Journal*, *77*(12).

McCaffrey, T. V, McCook, R. D., & Wurster, R. D. (1975). Effect of head skin temperature on tympanic and oral temperature in man. *Journal of Applied Physiology*, *39*(1), 114–118. www.physiology.org/journal/jappl

Mcguire M. A, Beede D. K, Collier R. J, Buonomo F. C, DeLorenzo M. A, Wilcox C. J, Huntington G. B, & Reynolds C. K. (1991). Effects of acute thermal stress and amount of feed intake on concentrations of somatotropin, insulin-like growth factor (IGF)-I and IGF-II, and thyroid hormones in plasma of lactating holstein cows. *Journal of Animal Science*, *69*(5), 2050–2056. https://academic.oup.com/jas/article-abstract/69/5/2050/4705371

McManus, C., Tanure, C. B., Peripolli, V., Seixas, L., Fischer, V., Gabbi, A. M., Menegassi, S. R. O., Stumpf, M. T., Kolling, G. J., Dias, E., & Costa, J. B. G. (2016). Infrared thermography in animal production: An overview. In *Computers and Electronics in Agriculture* (Vol. 123, pp. 10–16). Elsevier B.V. https://doi.org/10.1016/j.compag.2016.01.027

Mrowka, R., & Reuter, S. (2016). Thermoregulation. *Acta Physiologica*, *217*(1), 3–5. https://doi.org/10.1111/apha.12664

Naylor, J. M., Streeter, R. M., & Torgerson, P. (2012). Factors affecting rectal temperature measurement using commonly available digital thermometers. *Research in Veterinary Science*, *92*(1), 121–123. https://doi.org/10.1016/j.rvsc.2010.10.027

Polsky, L., & von Keyserlingk, M. A. G. (2017). Invited review: Effects of heat stress on dairy cattle welfare. *Journal of Dairy Science*, *100*(11), 8645–8657. https://doi.org/10.3168/jds.2017-12651

R core team. (2022). *R: A language and environment for statistical computing.* R Foundation for Statistical Computing, Vienna, Austria. URL https://www.R-project.org/.

Ravagnolo, O., Misztal, I., & Hoogenboom, G. (2000). Genetic component of heat stress in dairy cattle, development of heat index function. *Journal of Dairy Science*, *83*(9), 2120–2125. https://doi.org/10.3168/jds.S0022-0302(00)75094-6

RStudio Team. (2020). *RStudio: Integrated Development for R.* (4.3.2). RStudio, PBC. http://www.rstudio.com/

Salles, M. S. V., da Silva, S. C., Salles, F. A., Roma, L. C., El Faro, L., Bustos Mac Lean, P. A., Lins de Oliveira, C. E., & Martello, L. S. (2016). Mapping the body surface temperature of cattle by infrared thermography. *Journal of Thermal Biology*, *62*, 63–69. https://doi.org/10.1016/j.jtherbio.2016.10.003

Sato, K. T., Kane, N. L., Soos, G., Gisolfi, C., Kondo, N., Sat, K., Gisolfi, C. V, & Sato Reexamination, K. (1996). Reexamination of tympanic membrane temperature as a core temperature. *J. Appl. Physiol*, *80*(4), 1233–1239. www.physiology.org/journal/jappl

Scholander, P. F. (1955). Evolution of climatic adaptation in homeotherms. *Evolution*, *9*(1), 15–26. https://doi.org/10.1111/j.1558-5646.1955.tb01510.x

Seedorf, J., Hartung, J., Schröder, M., Linkert, K. H., Pedersen, S., Takai, H., Johnsen, J. O., Metz, J. H. M., Groot Koerkamp, P. W. G., Uenk, G. H., Phillips, V. R., Holden, M. R., Sneath, R. W., Short, J. L., White, R. P., & Wathes, C. M. (1998). Temperature and Moisture Conditions in Livestock Buildings in Northern Europe. *Journal of Agricultural Engineering Research*, *70*(1), 49–57.

Teye, F. Kwame., & Helsinki University Printing House). (2008). *Microclimate and gas emissions in dairy buildings : instrumentation, theory and measurements*.

Torrao, N. A., Hetem, R. S., Meyer, L. C. R., & Fick, L. G. (2011). Assessment of the use of temperature-sensitive microchips to determine core body temperature in goats. *Veterinary Record*, *168*(12), 328. https://doi.org/10.1136/vr.c6200

Tucker, C. B., Rogers, A. R., Verkerk, G. A., Kendall, P. E., Webster, J. R., & Matthews, L. R. (2007). Effects of shelter and body condition on the behaviour and physiology of dairy cattle in winter. *Applied Animal Behaviour Science*, *105*(1–3), 1–13. https://doi.org/10.1016/j.applanim.2006.06.009

Wang, J., Li, J., Wang, F., Xiao, J., Wang, Y., Yang, H., Li, S., & Cao, Z. (2020). Heat stress on calves and heifers: A review. *Journal of Animal Science and Biotechnology*, *11*(1). https://doi.org/10.1186/s40104-020-00485-8

Wang, X., Gao, H., Gebremedhin, K. G., Bjerg, B. S., Van Os, J., Tucker, C. B., & Zhang, G. (2018). A predictive model of equivalent temperature index for dairy cattle (ETIC). *Journal of Thermal Biology*, *76*, 165–170. https://doi.org/10.1016/j.jtherbio.2018.07.013

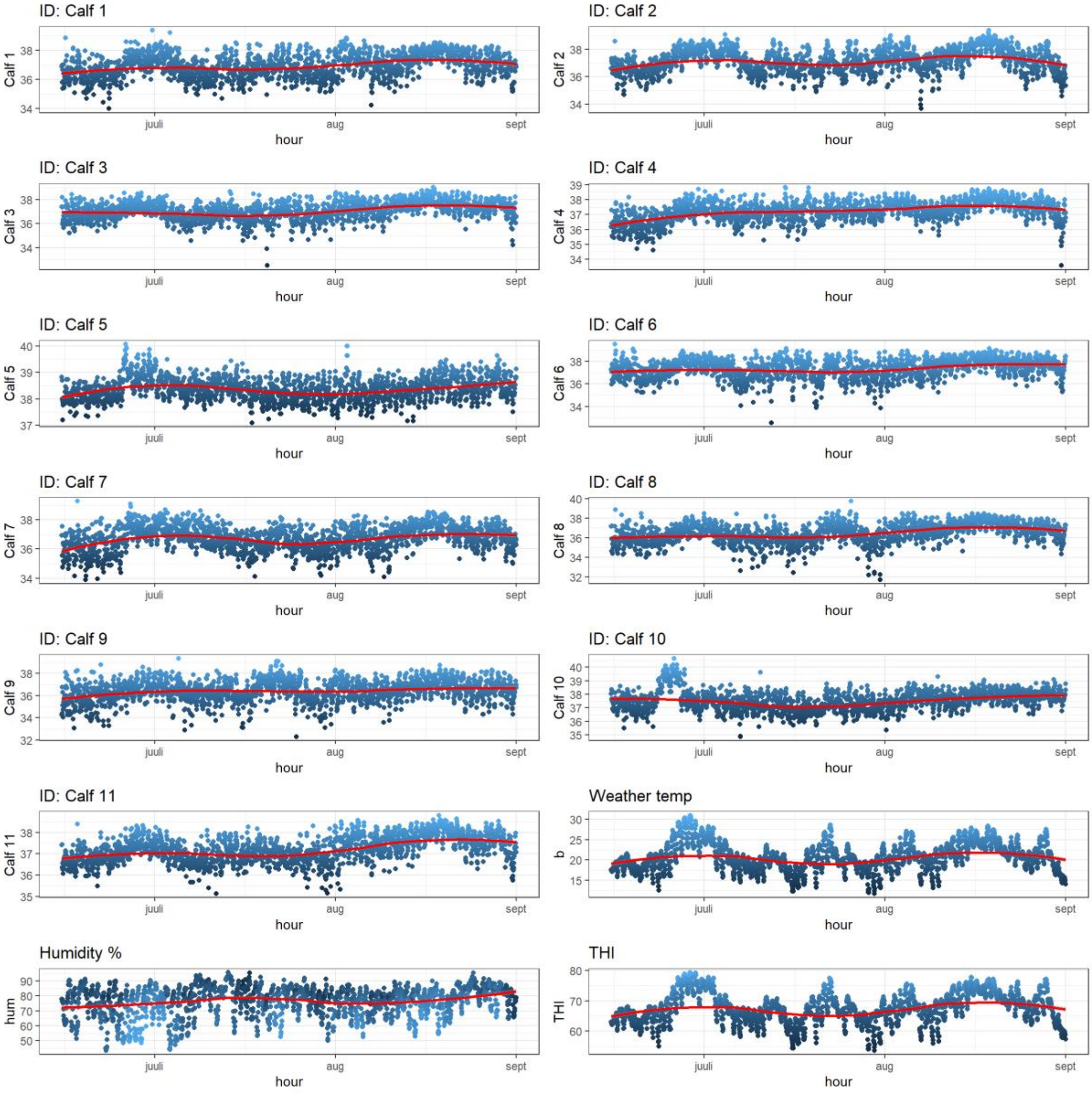
West, J. W. (2003). Effects of heat-stress on production in dairy cattle. *Journal of Dairy Science*, *86*(6), 2131–2144. https://doi.org/10.3168/jds.S0022-0302(03)73803-X

Zhou, M., Aarnink, A. J. A., Huynh, T. T. T., van Dixhoorn, I. D. E., & Groot Koerkamp, P. W. G. (2022). Effects of increasing air temperature on physiological and productive responses of dairy cows at different relative humidity and air velocity levels. *Journal of Dairy Science*, *105*(2), 1701–1716. https://doi.org/10.3168/jds.2021-21164

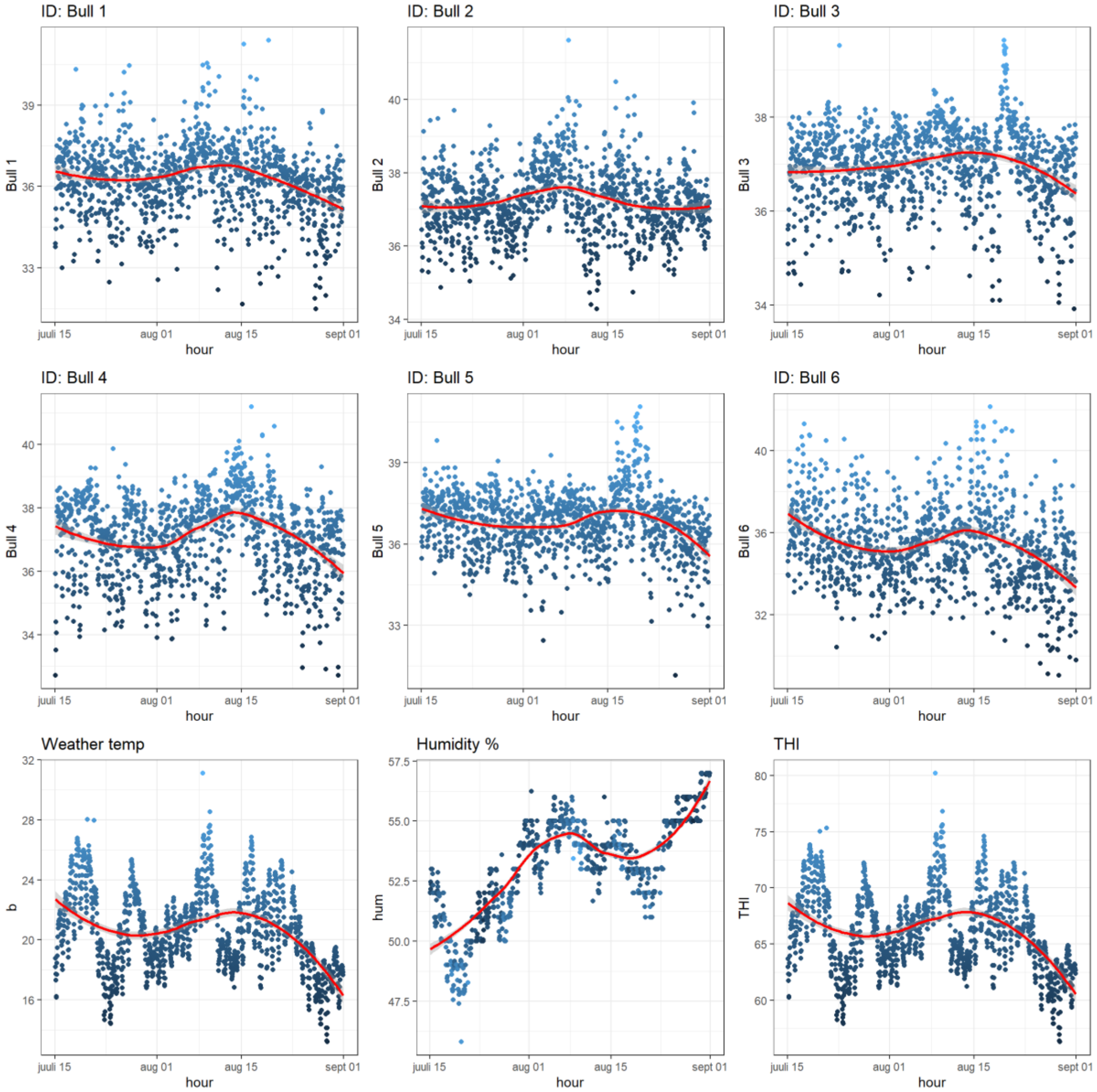
Zimbelman, R. B., Baumgard, L. H., & Collier, R. J. (2010). Effects of encapsulated niacin on evaporative heat loss and body temperature in moderately heat-stressed lactating Holstein cows. *Journal of Dairy Science*, *93*(6), 2387–2394. https://doi.org/10.3168/jds.2009-2557

APPENDICES

Appendix 1. Farm 2 individual calf body temperatures from the whole observation period with weather temperature, humidity % and emperature humidity index (THI).



Appendix 2. Farm 1 individual bull calf body temperatures from the whole observation period with weather temperature, humidity % and temperature humidity index (THI).



Appendix 3. Farm 1 individual calf mean body temperatures from the whole observation period. Divided into "morning" – 06:00-11:59, "day" – 12:00-17:59, "evening" – 18:00-23:59, and "night" – 00:00-05:59.

Bull	*Time_of_Day*	*Average*	*Maximum*	*Minimum*	*SD*	*No. Of Observations*
Bull 1	Overall	36.27	41.39	31.47	1.32	1151
	morning	36.42	41.24	31.47	1.41	288
	day	36.88	41.39	33.51	1.28	288
	evening	36.44	38.96	33.42	0.81	288
	night	35.36	38.31	31.66	1.22	287
Bull 2	Overall	37.20	41.61	34.28	0.91	1137
	morning	37.26	40.48	34.41	1.08	282
	day	37.53	41.61	34.98	0.85	283
	evening	37.30	39.94	35.13	0.67	285
	night	36.72	38.93	34.28	0.79	287
Bull 3	Overall	36.97	39.63	33.92	0.84	1152
	morning	36.88	39.47	34.10	0.87	288
	day	37.24	39.52	33.92	0.66	288
	evening	37.31	39.08	34.92	0.56	288
	night	36.46	39.63	34.05	0.95	288
Bull 4	Overall	37.10	41.20	32.71	1.26	1148
	morning	36.87	39.35	33.86	1.25	288
	day	37.56	41.20	33.88	1.10	288
	evening	37.52	39.70	34.58	0.89	286
	night	36.44	39.86	32.71	1.38	286
Bull 5	Overall	36.81	41.07	31.14	1.15	1152
	morning	36.54	41.07	31.14	1.26	288
	day	37.31	40.71	32.96	1.03	288
	evening	37.08	40.81	33.26	1.01	288
	night	36.30	39.14	33.54	0.98	288
Bull 6	Overall	35.41	42.15	29.04	2.01	1150
	morning	35.71	41.40	29.12	2.14	287
	day	36.83	42.15	32.30	1.74	288

Bull 6	evening	35.20	41.32	30.25	1.43	287
	night	33.89	37.20	29.04	1.41	288

Appendix 4. Farm 2 individual calf mean body temperatures from the whole observation period. Divided into "morning" – 06:00-11:59, "day" – 12:00-17:59, "evening" – 18:00-23:59, and "night" – 00:00-05:59.

Calf	*Time_of_Day*	*Average*	*Maximum*	*Minimum*	*SD*	*No. Of Observations*
Calf 1	Overall	36.96	39.37	34.00	0.83	1871
	morning	36.46	38.17	34.70	0.66	468
	day	36.96	39.37	34.70	0.73	468
	evening	37.39	38.83	35.13	0.71	468
	night	37.04	38.57	34.00	0.90	467
Calf 2	Overall	37.18	39.40	33.67	0.86	1872
	morning	36.76	38.53	34.60	0.70	468
	day	37.30	39.27	35.43	0.75	468
	evening	37.55	39.40	35.23	0.81	468
	night	37.10	39.00	33.67	0.94	468
Calf 3	Overall	37.08	39.03	32.50	0.74	1872
	morning	36.72	38.13	32.50	0.66	468
	day	37.07	39.03	35.30	0.65	468
	evening	37.47	38.93	34.90	0.67	468
	night	37.08	38.67	33.90	0.77	468
Calf 4	Overall	37.25	38.83	33.57	0.68	1872
	morning	36.86	38.07	33.57	0.58	468
	day	37.16	38.53	35.13	0.61	468
	evening	37.65	38.83	35.60	0.57	468
	night	37.33	38.70	34.90	0.70	468
Calf 5	Overall	38.38	40.07	37.10	0.45	1872
	morning	38.06	39.33	37.17	0.35	468
	day	38.24	39.63	37.10	0.38	468
	evening	38.63	40.07	37.73	0.36	468
	night	38.58	40.00	37.20	0.43	468
Calf 6	Overall	37.35	39.50	32.57	0.86	1872

Calf 6	morning	36.84	38.93	33.87	0.78	468
	day	37.30	39.50	32.57	0.71	468
	evening	37.80	39.13	34.33	0.72	468
	night	37.48	39.13	33.90	0.92	468
Calf 7	Overall	36.74	39.27	33.90	0.86	1872
	morning	36.37	38.27	34.10	0.74	468
	day	36.80	38.47	34.20	0.76	468
	evening	37.13	39.27	34.63	0.77	468
	night	36.67	38.47	33.90	0.95	468
Calf 8	Overall	36.48	39.73	31.70	1.01	1872
	morning	36.02	38.07	32.43	0.86	468
	day	36.45	38.43	33.97	0.83	468
	evening	36.92	38.87	33.97	0.89	468
	night	36.54	39.73	31.70	1.21	468
Calf 9	Overall	36.43	39.37	32.30	0.97	1872
	morning	35.84	37.90	32.30	0.77	468
	day	36.45	38.77	34.03	0.73	468
	evening	37.00	39.07	33.17	0.80	468
	night	36.46	39.37	32.90	1.14	468
Calf 10	Overall	37.53	40.67	34.87	0.74	1872
	morning	37.19	40.20	35.37	0.71	468
	day	37.48	40.20	35.93	0.71	468
	evening	37.77	40.67	35.90	0.69	468
	night	37.68	39.80	34.87	0.72	468
Calf 11	Overall	37.21	38.80	35.13	0.61	1872
	morning	36.91	38.17	35.30	0.51	468
	day	37.13	38.80	35.13	0.58	468
	evening	37.48	38.63	35.33	0.58	468
	night	37.32	38.53	35.43	0.61	468

Appendix 5. Non-exclusive licence for depositing the final thesis and opening it for the public and the supervisor's confirmation for allowing the thesis for the defence

Hereby I, **Casper Erno Nikolai Kosonen**

27.03.1995

1. grant Eesti Maaülikool, the Estonian University of Life Sciences, a free-of-charge non-exclusive licence to store the final thesis titled **Analysis of elevated temperature readings in dairy cattle using time-series analysis with subcutaneous temperature sensor data**, supervised by **Tarmo Niine** for

   1.1. preservation;
   1.2. depositing a digital copy of the thesis in the archive of DSpace and
   1.3. opening it for the public on the Web
   until the validity of the term of protection of copyright.

2. I am aware that the author retains the same rights as listed in point 1;

3. I confirm that by being issued the CC licence no rights deriving from the Personal Data Protection Act and the Intellectual Property Rights Act have been infringed.

Author of the final thesis ____________________
signature

In Tartu, **16.05.2024**

The core supervisor's approval for the final thesis to be allowed for defence

This is to confirm that the final thesis is allowed for defence.

………………………………………………… ………………………………..
Supervisor's name and signature Date

………………………………………………… ………………………………..
Supervisor's name and signature Date