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Use of an Ear Tag-Integrated Temperature Sensor to Depict Temperature Trends in Cows with Normo- and Hyperthermia

Diploma Thesis

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submitted by

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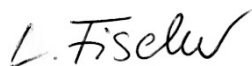
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Declaration of Independence

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Vienna, 06.03.2023

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ABSTRACT

Body temperature measurement in cattle is a useful tool for health status assessment and monitoring. To identify sick cows, manual rectal thermometry is still the gold standard. However, this method is labor intense and requires a high amount of time. Continuous and remote measurement of rectal temperature is not possible.

The aim of the presents study was to evaluate the use of an ear tag-integrated temperature sensor for detecting dairy cows suffering from fever. First, the reproducibility and accuracy of the ear tag-based thermometers were tested *ex vivo* utilizing a water bath. Subsequently it was investigated in fresh cows whether the transition from normo- to hyperthermia, determined by thermometry, can be traced via the ear tag-based temperature curves.

The measurements from the ear tag-based temperature sensor were very highly correlated with the reference temperature of the water bath ($r = 0.99$). The coefficient of variation between the ear tag-based temperature sensors was very low (0.82%). It failed to create a general graphical representation of transition from normo- to hyperthermia. The correlation between environmental and ear tag temperature ($r = 0.41 - 0.52$, $P = 0.01$) was significantly higher than that between rectal and SB temperature ($r = 0.11$, $P = 0.01$). The results of this study indicate that the ear tag-integrated temperature sensors were accurate and repeatable *ex vivo*. However, the environment seems to have a significantly greater influence, albeit rather low, on the ear tag temperature than the temperature of the animal. While the technology does not serve as an adequate alternative for rectal temperature measurement, it has the potential to passively monitor temperature. Further research is needed to differentiate animal-specific variations associated with disease from other influencing factors.

ZUSAMMENFASSUNG

Die Messung der Körpertemperatur bei Rindern ist ein nützliches Instrument zur Einschätzung und Überwachung des Gesundheitszustands. Um kranke Kühe zu identifizieren, ist die manuelle Rektalthermometrie immer noch der Goldstandard. Diese Methode ist jedoch arbeitsintensiv und erfordert einen hohen Zeitaufwand. Eine kontinuierliche und ferngesteuerte Messung der Rektaltemperatur ist nicht möglich.

Das Ziel der vorliegenden Studie war es, einen ohrmarkenbasierten Temperatursensor zur Erkennung von fiebernden Milchkühen zu evaluieren. Zunächst wurden die Reproduzierbarkeit und Genauigkeit des ohrmarkenbasierenden Thermometers *ex vivo* in einem Wasserbad getestet. Anschließend wurde an Kühen in der Früh-laktation untersucht, ob der thermometrisch ermittelte Übergang von Normo- zu Hyperthermie über die ohrmarkenbasierten Temperaturkurven dargestellt werden kann.

Die Korrelation zwischen der Messung des ohrmarkenbasierten Temperatursensors und der Referenztemperatur des Wasserbades war sehr hoch ($r = 0,99$). Der Variationskoeffizient innerhalb der ohrmarkenbasierten Temperatursensoren war sehr niedrig (0,82%). Es gelang nicht, den Übergang von Normo- zu Hyperthermie allgemein grafisch darzustellen. Die Korrelation zwischen Umgebungs- und Ohrmarkentemperatur ($r = 0,41 - 0,52$; $P = 0,01$) war signifikant höher als die zwischen rektaler und Ohrmarken-Temperatur ($r = 0,11$; $P = 0,01$). Die Ergebnisse dieser Studie zeigen, dass die in die ohrmarkenintegrierten Temperatursensoren *ex vivo* genau und wiederholbar waren. Allerdings scheint die Umgebung einen deutlich größeren Einfluss auf die Ohrmarkentemperatur zu haben als die Körpertemperatur des Tieres an sich. Die Technologie ist zwar keine adäquate Alternative für die rektale Temperaturmessung, hat aber das Potenzial, die Temperatur passiv zu überwachen. Weitere Forschung ist erforderlich, um tierspezifische Schwankungen, die mit Krankheiten zusammenhängen, von anderen Einflussfaktoren zu unterscheiden.

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1. INTRODUCTION

Body temperature measurement in cattle is a useful tool primarily for health status assessment and monitoring, but it is also helpful in determining estrus and predicting parturition. Assessing the health status (sick, healthy, and intermediate stages) regarding body core temperature means inferring the presence of elevated temperature due to fever and disease, respectively, which is a sign of a certain immune responses (McGuirk, 2008) but can also be triggered by heat stress. Generally, the average daily body temperatures for cattle fall within a range of 38.0–39.4°C (Hewitt, 1921; Lefcourt et al., 1999; L. C. Hicks et al., 2001). Further, thresholds for fever of $\geq 39.4^{\circ}\text{C}$ and $\geq 39.7^{\circ}\text{C}$, respectively, have been recommended to differentiate between healthy and diseased cows (Smith and Risco, 2005; Benzaquen et al., 2007; Wagner et al., 2008). Health monitoring is the continued evaluation of the health status and plays an important role especially in risk periods, when dairy cattle are predisposed to certain diseases. During the transition period, 3 weeks before to 3 weeks after parturition, there is a high incidence of metabolic diseases due to enormous increases in performance. While the animals are exposed to extreme environmental temperatures, the immune system is also vulnerable. Lameness and udder disease are critical during the dry period. While the animals are not under tremendous performance, they often fly under the radar in terms of health monitoring. Calf diseases occur mainly at the time of immunological gap or are caused by stressful situations such as transport or rehousing. The aforementioned health monitoring can be applied in the form of general health checks, noting abnormalities, during estrus monitoring or milking, conscious or permanent lameness scoring, and via remote sensor systems that measure the various health parameters of the animals in real time. In the context of body temperature measurement, patterns or peaks of body temperature can be captured through continuous diurnal, hourly, or even closer surveillance. For measuring body core temperature in cattle and to identify sick cows, manual rectal thermometry is still the gold standard (Burfeind et al., 2010). However, the weak points of this method are the high labor intensity and time consumption. In addition, it is influenced by the presence or absence of feces and by the depth of penetration of the thermometer. In the worst case, it results in rectal injury. Furthermore, restraining animals to manually collect temperature data can cause stress, which in turn alters the temperature. A reliable method of collecting temperature without human intervention should allow the more accurate measurement of temperature in dairy cows (Prendiville et al., 2002; Liang et al., 2013). The field of

precision dairy farming follows in these footsteps. It involves the use of technology to measure automatically and remotely physiological, behavioral, and production indicators in individual animals. The main objectives are to maximize animal performance, detect diseases early in individual cows, detect health and production problems early at herd level, and minimize the use of medications through preventive health measures (Precision Dairy Farming, 2021). Next to milk yield recording systems, milk component monitors, activity monitors, lying and rumination behavior monitors, milk conductivity indicators, and heat detection monitors, there are already many approaches to measure body temperature (Sellier et al., 2014). The following is an overview of the current methods that are either in use or still in research.

Burfeind et al. (2010) were the first to confirm that rectal temperature can be measured repeatedly in dairy cows. Very high correlation coefficients ($r = 0.98$) and small differences among rectal measurements showed high repeatability of measurements within and among the investigator(s). Nevertheless, the measurement is influenced by the procedure itself (up to 0.5°C), the type of thermometer (up to 0.3°C), and the depth of penetration of the thermometer into the rectum (up to 0.4°C difference between penetration depths of 11.5 and 6.0 cm). Automated direct rectal temperature measurement plays a role mainly in the research field. An overview of the devices used are provided in Table 1.

Gaughan et al. (2008) used a thermistor sensor placed in the tip of a rectal probe and connected to a data logger. These probes were attached to the steer with an elastic cord attached to a heart harness. Rectal temperature was recorded every 5.4 minutes. A digital display for each animal in an outside room provided continuous monitoring. Reuter et al. (2010) developed a device for the automatic monitoring of rectal temperature, consisting of a custom-made aluminum tail harness and the actual probe (Fig. 1): a cross-linked polyethylene tube connected to the harness via a quick connect and an aluminum probe container at the other end of the tube containing a TidbiT v2 temperature logger (part UTBI-001, Onset Corp., Pocasset, USA). Similarly, Lea et al. (2008) and Lee et al. (2018) described how iButton temperature loggers (Embedded Data Systems, Lawrenceburg, USA) were attached by shrink plastic to a PVC rod connected to a rubber tube, which in turn was attached to the tail (Fig. 2). The radio frequency identification (RFID)-based digital thermometer (Debnath et al., 2017), using a DS18B20 Programmable Resolution 1-Wire Digital Thermometer (Maxim Integrated, San Jose, USA), goes one step further as it is not only an automated but also a remote measurement device. The RFID is a

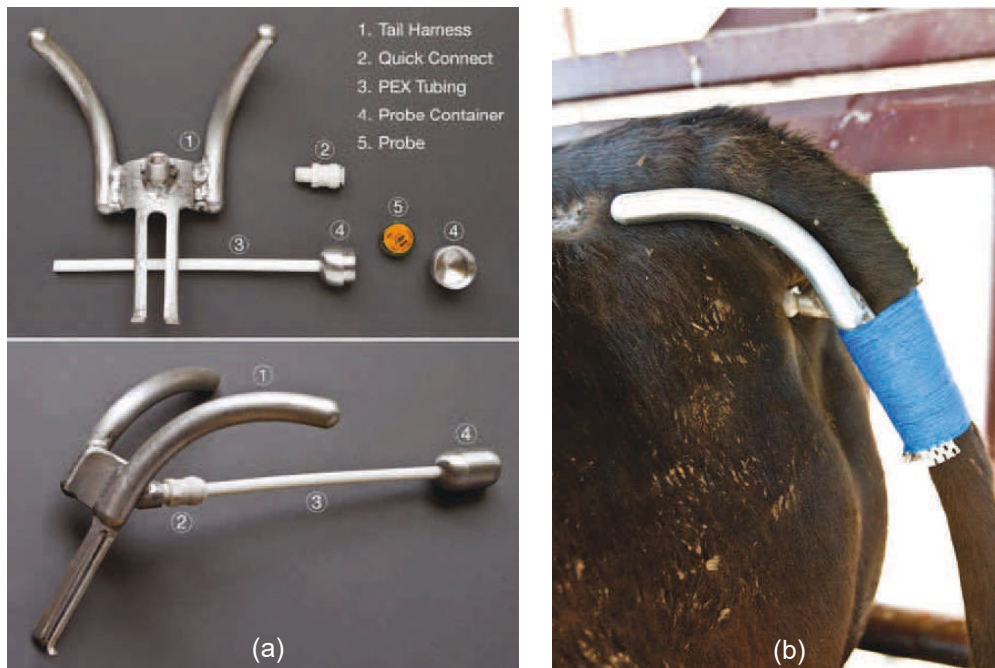


Figure 1. Device to monitor rectal temperature automatically in cattle. (a) Rectal probe with temperature logger and (b) attachment of the rectal logger probe to the tail (Reuter et al., 2010).



Figure 2. Device to monitor rectal temperature automatically in cattle. (a) Rectal probe with temperature logger and (b) attachment of the rectal logger probe to the tail (Lea et al., 2008).

technology for transmitter-receiver systems for automatic and contactless identification, localization, and data retrieval via radio wave frequency. An RFID system consists of a transponder, which is located on or in the object or living being and contains an identifying code, and a reader for reading the identification and data. Real-time rectal temperature data of an RFID rectal thermometer with rectal temperature data of a mercury bulb thermometer in dairy cattle was evaluated, aiming to develop a wireless, non-invasive RFID thermometer connected with an Android smartphone app. No significant difference

was found between RFID and mercury bulb thermometer measurements. For the authors, this confirms that body temperature records from RFID thermometers are acceptable for the real-time monitoring of rectal temperature.

Table 1. Devices of automated rectal temperature measurement by author.

Author	Device
Gaughan et al. (2008)	Rectal probe indwelling temperature logger with girth harness
Reuter et al. (2010)	Rectal probe indwelling temperature logger with tail harness
Debnath et al. (2017)	RFID rectal thermometer
Lee et al. (2018)	Rectal probe indwelling temperature logger with tail harness

The measurement of body temperature in the vagina is mostly scientific in nature. The agreement between rectal and vaginal temperature is described as high in the majority of the studies. Correlation coefficients are in the range of 0.46–0.97, with emphasis on the high positive (Table 2). Investigations to evaluate the temperature profile took place under actual field conditions (Hillman et al., 2009; Lees et al., 2018a) and during different lactation stages (Suthar et al., 2013; Vickers et al., 2010) or after lipopolysaccharide challenge (Burdick et al., 2012). The latter claims that the vaginal device allows measurement of vaginal temperature without causing a potential bias, which might be associated by a stress response as a result of handling procedures and/or the presence of humans. This is relevant for all remote measuring systems. The devices developed are essentially two different technologies. One is the much more common blank CIDR device indwelling temperature logger (Fig. 3), and the other is a temperature logger encased in a plastic anchor (Fig. 4). The manufacturers of the individual components of the devices may vary (Table 2). Tresoldi et al. (2020) point out the impact that differences in data collection devices and sampling strategies have on body temperature measurements. This must be considered when interpreting the data. Relatively small, inexpensive, and inaccurate loggers did not correctly classify animals compared to high-accuracy loggers. Vaginal temperature measurements taken as often as every 120 min (every 5, 10, 15, 30, 45, 60, and 120 min) resulted in more accurate estimates than strategies in which temperature was recorded one to three times per day. Lees et al. (2018a) compared the two commonly used devices of automatic continuous vaginal and rectal temperature recording. These were temperature loggers attached to a progesterone-free CIDR device

and a rectal probe with tail harness, respectively. For the latter, the loggers were attached to soft polyethylene tubing and fixed with heat shrink plastic to be held in place in the rectum. The exposed end of the probe was fixed to the underside of the tail with veterinary tape (Tensoplast® Vet, BSN Medical Inc., Hamburg, Germany). Recordings were collected in 20-s intervals over 18.5 h. Within this time, mean rectal and vaginal temperature followed a similar pattern, but vaginal temperatures did not appear to exhibit rapid fluctuations and were consistently lower than rectal temperatures. This is also indicated by the coefficient of variation, which was on average lower ($p < 0.001$) for vaginal (0.38%) than rectal temperature (0.44%); when using the Bland-Altman comparison method, vaginal temperature was on average $0.22 \pm 0.01^{\circ}\text{C}$ lower than rectal temperature. The authors concluded that vaginal temperature may be less affected by temperature changes due to factors such as defecation or laying down but is more sensitive to changes in core body temperature than rectal temperature. Burdick et al. (2012) support this thesis by attributing it to the greater blood flow to the vaginal cavity than to the rectum. It is noticeable that the relationships established in the studies by Lees et al. (2018a), Burdick et al. (2012), and Suthar et al. (2013) are greater than those established by George et al. (2014), Vickers et al. (2010), and Kaufman et al. (2018). The latter used intravaginal data loggers, which recorded temperatures continuously, and handheld thermometers, which only allowed time point measurements. The former measured rectal and vaginal temperature equally continuously using indwelling temperature data loggers. This suggests that simultaneous continuous measurements may improve the observed relationship (Lees et al., 2018a). An approach of measuring vaginal temperature remotely is also described (Sakatani et al., 2018). The device used consists of a temperature sensor embedded in an anchor-like plastic shell (Gyuonkei, Remote Inc., Oita, Japan), sending data immediately to the server by WIFI connection. This monitoring system is based on the detection of two temperature changes and the time between them and could be effective for predicting calving time.

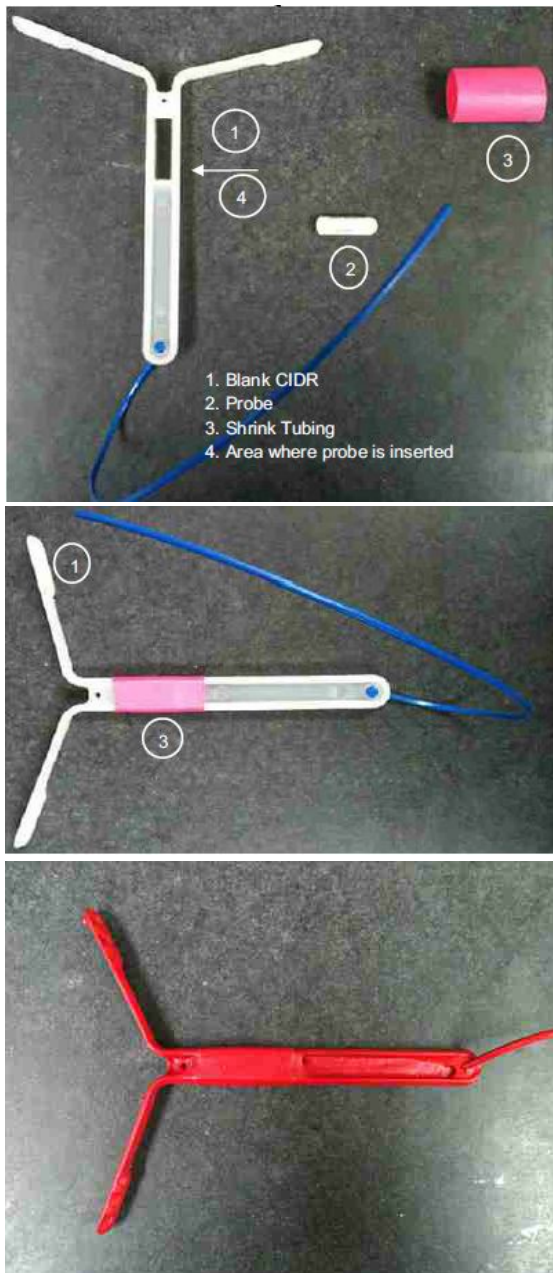


Figure 3. Device to monitor vaginal temperature automatically in cattle. Blank CIDR device containing the temperature logger, which is fixed with a heat-activated shrink tube. The entire device is then coated in multi-purpose rubber coating (PlastiDip) to seal all components (Burdick et al.,2012).

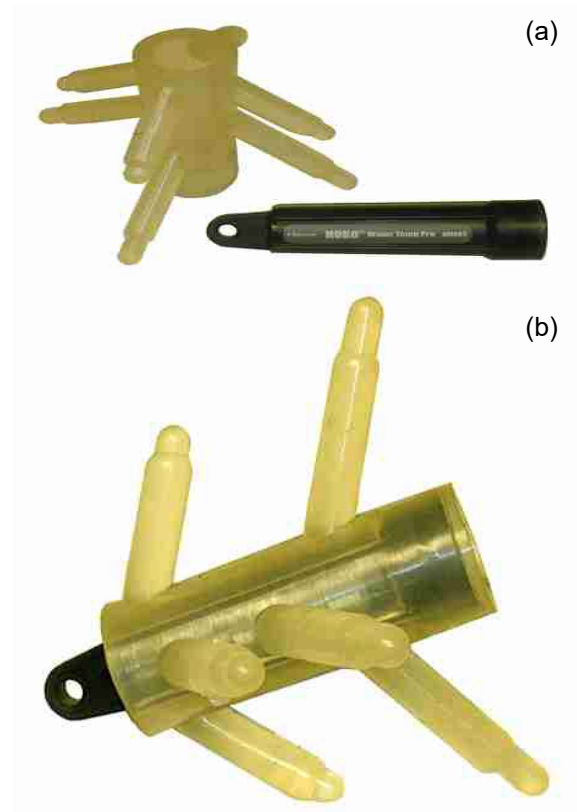


Figure 4. Device to monitor vaginal temperature automatically in cattle. Temperature logger on the side (a) and encased (b) in a plastic anchor (Hillman et al.,2009).

Table 2. Correlation coefficients and coefficients of determination of vaginal temperature and rectal temperature as gold standard.

Author	Technology	Agreement
Hillman et al. (2009)	Temperature logger (Model HOBO Water Temp Pro, Onset Computer Corporation, Bourne, USA) encased in plastic anchor	$R^2 = 0.90$
Burdick et al. (2012)	Blank CIDR ¹ device (Pfizer Animal Health, Sterling, USA) indwelling temperature logger (Star-OddiDSTmicro-T; MeterMall USA, Marysville, USA)	$r = 0.97$ ($P < 0.0001$)
Suthar et al. (2013)	Blank CIDR ¹ device (Pfizer Animal Health, Berlin, Germany) indwelling temperature logger (Minilog 8, Vemco Ltd., Halifax, Canada)	$r = 0.92$ ($P < 0.001$) and $r = 0.94$ ($P < 0.001$)
Vickers et al. (2010)	Blank CIDR ¹ device indwelling temperature logger (Minilog 8, Vemco Ltd., Halifax, Canada)	$r = 0.81$ ($P < 0.001$) for post-partum cows $r = 0.46$ ($P < 0.001$) for peak-lactation cows
Lees et al. (2018a)	Blank CIDR ¹ device (InterAg New Zealand, Hamilton, New Zealand) indwelling temperature logger (iButton DS1922L, Thermochron iButton Device; Maxim Integrated, San Jose, USA)	$R^2 = 0.72$ ($P < 0.0001$)
Burfeind et al. (2012)	Blank CIDR ¹ device indwelling temperature logger (Minilog 8, Vemco, Ltd., Halifax, Canada)	$r = 0.85$ ($P < 0.001$)
George et al. (2014)	Intravaginal temperature logger (Stowaway Tidbit, Onset Computer Corp, Bourne, USA)	$r = 0.78$ ($P < 0.001$)
Kaufman et al. (2018)	Blank CIDR ¹ device (Zoetis, Florham Park, NJ, USA) indwelling temperature logger (DS1922L Thermochron iButton Device; Maxim Integrated, San Jose, USA)	$r = 0.63$ ($P < 0.01$)

¹ Controlled internal drug release

The measurement of body temperature in the rumen takes place primarily with the aid of radiofrequency rumen boluses, which are also already used for health monitoring in practice and are available on the market. For example, the systems from samXtec (smaXtec Animal Care GmbH, Graz, Austria), Phase IV (Phase IV Engineering Inc., Boulder, USA), and SmartStock (SmartStock, LLC, Pawnee, USA) should be mentioned here. Rumen boluses are administered orally by an applicator and are then placed in the reticulum. Two different data delivery systems have been described in the literature. In the first, one or more barn receivers are installed in the cow pen to collect the data transmitted by the boluses (active transponder) with radio frequency. A base receiver collects the transmitted data and then directs it to a personal computer with software for data storage (Alzahal et al., 2011; Cooper-Prado et al., 2011; Ammer et al., 2016; Lees et al., 2018b; Liang et al., 2013; Rose-Dye et al., 2011; Šematoviča et al., 2017). In the second system, the sensor data collection differs in that radar sensors detect the animal as it passes a reader. Then, a magnetic field induces an electrical charge in the bolus, activating the transmitter (passive transmitter). The bolus responds to this interrogation signal by transmitting its unique identification number and temperature on a coded radio frequency. This information is then transmitted to an on-site computer as described before (Adams et al., 2013; Bewley et al., 2008a). In addition to temperature recording, however, the focus is on determining rumen activity and rumen pH as health markers. Alzahal et al. (2011) investigated the changes in rumen temperature caused by systemic diseases associated with febrile responses on the one hand and subacute pancreatic acidosis on the other hand. The bolus system (SmartStock LLC, Pawnee, USA) was able to detect the increase in body temperature associated with infectious diseases. However, it could also detect changes in rumen temperature associated with feed composition, which allows conclusions about rumen pH using rumen temperature. The correlation between rectal and ruminal temperature is mostly described as low positive to moderate. Only one paper showed a high correlation. The correlation coefficients ranged from 0.34 to 0.89 (Table 3.). According to Bewley et al. (2008a), the correlation between ruminal and rectal temperature is greater when ruminal temperatures are higher in spring and fall. Temperature-recording radio frequency ruminal boluses are used in diagnostics and for infectious disease detection and profiling (Rose-Dye et al., 2011; Carroll et al., 2009), production disease detection (Adams et al., 2013), cow behavior (Šematoviča et al., 2017), animal reproduction (Cooper-Prado et al., 2011; Antanaitis et al., 2018), and heat stress (Liang et al., 2013; Lees et al., 2018b). Although Prendiville et al. (2002) have

already shown that the rumen bolus is an effective means of continuously measuring body temperature, the efficiency of the bolus still needs to be improved (Table 3). Fluctuations in ruminal temperature are associated with eating but especially drinking events and reflect the cows' feed and water intake habits. The consumption of large amounts of either body-warm, warm, or cold water affects rumen temperature differently. The negative impact of water consumption on temperature could be clearly demonstrated (Bewley et al., 2008b). Approaches to address these variations include monitoring the amount and timing of water intake (Carroll et al., 2009) and editing the data to remove ruminal temperatures influenced by recent drinking bouts (Bewley et al., 2008a). Cooper-Prado et al. (2011) excluded a drop in rumen temperature below 37.72°C from the analysis.

Tympanic temperature measurement is another approach for estimating the body core temperature and can also be performed remotely. Guidry and McDowell (1966) reported that tympanic temperature responded more rapidly than rectal temperature to both environmental fluctuations in the form of rising and declining ambient temperatures and to internal stimuli. The authors concluded that temperature measured at the tympanic membrane is more suitable than rectal temperature for determining the speed of the response of cattle to internal and external temperature changes. Research findings regarding the suitability of tympanic temperature measurement to identify diseased animals are conflicting. Whilst a previous study (Prendiville et al., 2002) found that tympanic measurements correlate well with rectal temperatures ($r = 0.80$; $P < 0.05$) in non-diseased cattle and that there was no difference between rectal and tympanic temperature ($P > 0.05$), McCorkell et al. (2014) showed a failure in reliably detecting diseased calves. Davis et al. (2003) and Mader et al. (2010) used a thermistor with its tip located near the tympanic membrane, attached to a data logger, which was wrapped in gauze and secured to the ear using self-adhesive tape for automated tympanic temperature logging. Over time, various remote ear temperature sensing and monitoring systems have been developed for the use in cattle. The devices consist of an ear tag-like attachment to which a temperature sensor is placed in the ear canal to measure body temperature. Data are transmitted wirelessly. In addition to temperature monitoring, some of the technologies process activity data through a 3-axis accelerometer. Next to assessing animal movements, this can also be useful for predicting rumination in cattle and, accordingly, identifying illness (Koltes et al., 2018).

Table 3. Correlation coefficients and Equations of ruminal temperature and rectal or vaginal temperature as gold standard.

Author	Technology	Agreement
Prendiville et al. (2002)	Radio frequent rumen temperature bolus (Innotek, Inc., Garrett, IN)	$r = 0.34$ ($P < 0.05$) ¹
Carroll et al. (2009)	Radio frequent rumen temperature bolus (TechTrol, Inc., Pawnee, USA)	$r = 0.39$ ($P < 0.05$) ¹
Rose-Dye et al. (2011)	Radio frequent rumen temperature bolus (SmartStock LLC, Pawnee, USA)	$r = 0.89$ ($P < 0.01$) ¹ $r^2 = 0.80$, $y = 0.858x + 5.4724$
Lees et al. (2018b)	Radio frequent rumen temperature bolus (Smartstock LLC, Pawnee, USA)	$r = 0.52$ ($P < 0.0001$) ¹
Bewley et al. (2008a)	Radio frequent rumen temperature bolus (Phase IV Engineering Inc., Boulder, USA)	$r = 0.645$ ($P < 0.0001$) ¹ Rectal Temperature = $19.23 + 0.496 \times \text{Ruminal Temperature}$ (a.m. milking) Rectal Temperature = $15.88 + 0.587 \times \text{Ruminal Temperature}$ (p.m. milking)
Ammer et al. (2016)	Radio frequent rumen temperature bolus (smaXtec Animal Care GmbH, Graz, Austria)	$r = 0.48$ ($P < 0.001$) ¹ $r = 0.53$ ($P < 0.001$) ²

¹ Rectal temperature as gold standard

² Vaginal temperature as gold standard

Technologies to be mentioned in this regard are the Cow Manager BV (Gerverscop, NL), DoggTag (Herddogg systems, Longmont, USA), SenseTag (Quantified Ag, Lincoln, USA), and the TekVet Health Monitoring System (East Palmetto, USA). The device that has been studied in this area is the Fever Tag System (FeverTags, Amarillo, USA). It is a

temperature-sensing ear tag designed to indicate elevated body temperature by a flashing LED signal (Fig. 5), so far only studied in calves. It is intended to serve as an early detection system for clinical diseases. When examining this system, McCorkell et al. (2014) demonstrated the limited effectiveness and weaknesses of the device. The shortcoming of the device was a too high threshold for activation deviating from the manufacturer's specification. The empirical



Figure 5. Fever Tag® temperature sensing ear tag (McCorkell et al., 2014).

threshold was higher than the expected threshold of 39.8°C. The detection rate was 100% only for temperatures > 40.5°C. It is expected that calves with these core temperatures will show clinical signs of disease anyway. A tag with a lower activation threshold could potentially provide the early detection of elevated body temperature. Current models are described as having multiple preset temperature thresholds, which could be more effective in early detection. However, there are still concerns that ear tags provide false-positive or false-negative signals under changing environmental conditions.

Body temperature measurement via RFID microchips is a technology that has emerged and been studied within the last decade. The microchips described were implanted subcutaneously or intramuscularly at a wide variety of sites (Table 4). Correlations between implantation sites and rectal temperature ranged from 0.02–0.76, i.e., negligible to high positive, or could not be determined (Table 4). Strong correlations between implantation sites within different animals suggest that temperature microchips have the potential to determine baseline temperatures and abnormalities associated with disease, such as fever (Woodrum Setser et al., 2020). Giro et al. (2019) pointed out that the influence of bioclimatic indicators, such as that of the temperature humidity index (THI), on measurements near body surfaces must be considered. Reid et al. (2012) found low correlations between rectal and peripheral temperature from implantable radiofrequency microchips in Holstein steers challenged with lipopolysaccharides. However, as a result of induced fever, rectal and peripheral temperature moved asynchronously after the challenge. Therefore, there is potential for the early detection of diseases that also alter basal temperature. By applying a machine-learning algorithm to measured subdermal

temperature and environmental data, the body core temperature of a cow could be reliably predicted (Chung et al., 2020). The authors studied a real-time temperature monitor design for on-farm use; the system comprised an implantable biosensor, a wearable RFID scanner, and a long-range hub, and Wi-Fi technology was used to send the data from the hub to a cloud server. The produced data showed coherent relationships between the implant and vaginal temperature (Table 4). Standard error, temperature difference, and the correlation value associated with the higher THI data were significantly better than those associated with the lower THI data. However, performance of machine learning prediction still varied among cows. The implantable wireless thermometer developed by Iwasaki et al. (2019) represents a special form in this context. It consists of a negative temperature coefficient thermistor and a Bluetooth™ 4.0 wireless module encased in a biocompatible polylactic acid housing surrounded by polydimethylsiloxane. The implantable wireless thermometers were surgically implanted at 10 different sites in the cow (Table 4). The highest agreement was obtained for the temperature of the inner flank wall. This was set as the new gold standard for estimating core body temperature from subcutaneous temperatures and ambient temperature using machine learning and resulted in a correlation coefficient of 0.93 (Table 4).

Thermography has been used in animals to detect injury or inflammation. In this context, temperature measurement of the eye (Schaefer et al., 2007; Schaefer et al., 2012), udder (Metzner et al., 2014), and other body surfaces (Hoffmann et al., 2013; Peng et al., 2019) seems to be suitable in cattle to access body temperature. Eye temperature is most likely related to the internal body temperature. Correlation coefficients with rectal or vaginal temperature range from $r = 0.26$ ($P < 0.05$) for ocular globe temperature (Giro et al., 2019) to $r = 0.58$ ($P < 0.001$) for maximum eye temperature (George et al., 2014). According to Schaefer et al. (2007), the value of the thermal orbital maximum is most sensitive to stress and disease onset. Schaefer et al. (2012) developed a RFID-driven, noninvasive infrared thermography technology to determine bovine respiratory disease complex in calves. For this, the radiated temperatures around the orbital area were monitored using an infrared camera mounted on a motorized shaft installed next to the water station. The RFID enabled the identification of the calf as well as the signal to the control system to rotate the camera if necessary and to initiate capturing images when the calf's head was visible. Compared with a defined gold standard (core temperature, white blood cell count, clinical score, neutrophil/lymphocyte ratio), sick animals had higher peak infrared thermal values

Table 4. Correlation coefficients of microchip-measured and rectal temperature, vaginal temperature, or endoceliac temperature as gold standard.

Author	Technology	Sensor position	Agreement
Woodrum Setser et al. (2020)	RFID microchip (Bio-Thermo, Allflex USA Inc., Irving, USA)	Subcutaneously in the upper scapula ¹	$r = 0.24, P \leq 0.99$
		Intramuscularly in the trapezius muscle ¹	$r = 0.17, P \leq 0.98$
		Subcutaneously by the scutulum ¹	$r = 0.06, P \leq 0.99$
Giro et al. (2019)	RFID microchip (Lifechip ®, Destron Fearing, Saint Paul, USA)	Subcutaneous temperature ¹	$r_{RT} = 0.236, P < 0.05$
Reid et al. (2012)	RFID microchip (Biothermo, Digital Angel Corp., St. Paul, USA)	Subcutaneous at the base of the ear ¹	Under thermoneutral conditions: $r = -0.19 - 0.53$
			Under thermoneutral condition and LPS challenge: $r = -0.48 - -0.40$
		Subcutaneous posterior to the poll ¹	Under high ambient temperatures: $r = 0.30$
			Under high ambient temperatures and LPS challenge: $r = -0.20$
		Subcutaneous beneath the umbilical fold ¹	Under thermoneutral conditions: $r = -0.02 - 0.61$
			Under thermoneutral conditions and LPS challenge: $r = 0.30 - 0.36$
			Under thermoneutral conditions: $r = -0.07 - 0.43$
			Under thermoneutral conditions and LPS challenge: $r = 0.03 - 0.38$

¹Rectal temperature as gold standard

Continuation Table 5. Correlation coefficients of microchip-measured and rectal temperature, vaginal temperature, or endoceliac temperature as gold standard.

Author	Technology	Sensor position	Agreement
Chung et al. (2020)	RFID microchip (LifeChip Microchip, Destron Fearing Fort Worth, USA)	SC back of the ear base, under the scutiform cartilage ³	$r = 0.58 - 0.85$
Iwasaki et al. (2019)	Implantable thermometer (LBCA2HNZY-series Bluetooth™ 4.0 wireless module, Murata Manufacturing Co., Nagaokakyō, Kyōto Prefecture, Japan)	Endoceliac and subcutaneous temperatures ¹ : abdominal cavity inner flank wall inner ventromedial wall axilla ear root withers flank ventromedial at the abdomen tail base groin	$r = 0.74^{**}$ $r = 0.76^{**}$ $r = 0.46^{**}$ $r = -0.22^*$ $r = 0.40^{**}$ $r = 0.34^{**}$ $r = 0.60^{**}$ $r = 0.62^{**}$ $r = 0.47^{**}$ $r = 0.22^*$
* ($p < 0.05$) and ** ($p < 0.01$)			
		Temperatures predicted by machine learning:	
		Subcutaneous temperatures (Ax, ER, W, F, V, TB, G) and ambient temperature ⁴	$r = 0.93$
		Subcutaneous temperatures (TB, V) and ambient temperature ⁴	$r = 0.77$

¹Rectal temperature as gold standard

²Temperature humidity index

³Vaginal temperature as gold standard

⁴ Inner flank wall as gold standard

at $35.7 \pm 0.35^{\circ}\text{C}$ than healthy animals at $34.9 \pm 0.22^{\circ}\text{C}$ ($P < 0.01$). When implementing early disease detection systems, especially if they are based on emitted infrared radiation, it is crucial to account for camera-to-object distance, wind speed, camera settings (emissivity and humidity), and solar loading. Specifically, Giro (2019) demonstrated the

influence of bioclimatic indicators such as THI on surface measurements. Thus, the correlation between THI and surface temperatures of the ear base and ocular globe temperature was $r = 0.52$ ($P < 0.05$) and $r = 0.71$ ($P < 0.05$), respectively. By using shelters or shields, these effects can be minimized. In general, environmental conditions that may negatively affect temperature results have to be considered, and these could be automatically accounted and compensated for with the help of innovations in hard- and software as well as environmental monitoring. Current research is based on automated thermal image processing and computer vision (Jaddoa et al., 2021; Jorquera-Chavez et al., 2019).

Another, albeit rare, technology to be mentioned is the automated measurement of cattle surface temperature with a temperature sensor mounted in a shell-shaped device on the inside of the metatarsus (Kou et al., 2017). Regression analysis of surface to rectal temperature considering season and time point of the samplings was performed, resulting in a moderate correlation coefficient ($r = 0.562$).

The SMARTBOW ear tag (Smartbow/Zoetis LLC, Weibern, Austria) is a dairy cow monitoring system based on a 3D accelerometer. It has been evaluated for real-time localization (Wolfger et al., 2017), heat detection (Schweinzer et al., 2019), parturition (Krieger et al., 2018), and the detection of health deviations (Gusterer et al., 2020) based on activity measurement and rumination monitoring (Borchers et al., 2016; Reiter et al., 2018). In addition to the accelerometer, a temperature sensor is installed. The aim of the study was to evaluate the practicality of the temperature sensor of this ear tag monitoring system to detect sick animals, characterized by an increase in core body temperature in the course of fever.

The aim of the present study was to evaluate the use of an ear tag-integrated temperature sensor for detecting dairy cows suffering from fever, detected by rectal thermometry. For this, the following hypotheses were tested:

1. The measures taken by the ear tag-based thermometer have a high reproducibility.
2. The measures taken by the ear tag-based thermometer have a high accuracy.
3. The transition from normo- to hyperthermia in dairy cows as determined by thermometry can be traced via the ear tag-based temperature curves.

2. MATERIAL AND METHODS

This study was approved by the State Office of Agriculture, Food Safety, and Fisheries Mecklenburg-Vorpommern, Germany (7221.3-2-013/21) and noted by the Ethics Committee of the University of Veterinary Medicine, Vienna. The temperature sensor in question is part of an accelerometer-based monitoring system in the form of an ear tag device. To verify the practical applicability of this temperature sensor for the detection of elevated core body temperature of dairy cows, the study was divided into consecutive sections. First, to check the repeatability and accuracy of the sensor's temperature measurement, it was necessary to investigate whether the ear tags measured the same temperatures or whether the change in a reference temperature could be represented by the ear tag. An experiment was carried out in which an increasing temperature gradient was generated with the aid of a water bath. Finally, to test whether the transition from normo- to hyperthermia in dairy cows can be traced via the ear tag-based temperature curves, it was necessary to investigate whether the transition from nonfebrile to febrile conditions could be represented by the temperature of the ear tags. In this context, it was also investigated whether a correlation between measured ear tag temperature and environmental or rectal temperature could be detected.

The experiment to validate the repeatability and accuracy of the ear tag temperature measurement was conducted at the teaching and research farm of the University of Veterinary Medicine Vienna (VetFarm), Lower Austria, Austria. Data collection to address the research question of identifying febrile animals took place from February 2021 to March 2021 at a commercial dairy farm in Mecklenburg-Vorpommern, Germany. Approximately 1,900 Holstein-Friesian dairy cows were housed in free-stall barns. The average energy-corrected milk yield (ECM; based on 4% butterfat and 3.4% protein) was 10,301 kg per cow in 2021.

2.1. Accelerometer-based Monitoring System and Temperature Sensor

All animals on the farms in Lower Austria and Mecklenburg-Vorpommern were equipped with an ear-attached accelerometer SMARTBOW (SB; Smartbow/Zoetis LLC). In addition to the acceleration data, temperature was recorded via a sensor mounted in the housing. The data were sent in real-time to receivers installed in the barn and then processed on the farm server. The SB systems used specific algorithms, the operating principles of which are the intellectual property of the company, to generate alerts, e.g., for declining rumination and detecting estrus events. For example, rumination alerts are based on deviations in animal-related rumination

behavior compared to individual rumination times in the days prior. Temperature data were transmitted per minute and stored in tabular form on the farm server. The sensor system was already established on the farms and had already been used to support herd management, more specifically for heat detection, localization, and the use of deviation in rumination time as part of health monitoring.

2.2. Validation of the Reproducibility and Accuracy of the Ear Tag-Based Thermometer

New ear tags, which were captured by the SMARTBOW system but not attached to animals, were used for the evaluation in the water bath. In total, eight ear tags were placed, four at a time, on a small Styrofoam plate. These were then vacuum-sealed in a plastic bag (CASO Profi-Folienbeutel 16 x 23 cm, 150 µm, CASO Design Braukmann GmbH, Germany) using a Vacuumizer (CASO VC 10, CASO Design Braukmann GmbH, Germany) so that no liquid could enter, and the largest possible contact surface was created without an insulating layer of air (Fig. 6). Cable ties were used to secure the packages to the racks in the water bath (SW23 Shaking water bath, JULABO GmbH, Seelbach, Germany). The temperature-measuring device (VOLTCRAFT DT-300, Conrad Electronic SE, Hirschau, Germany) with a freely movable NTC (Negative Temperature Coefficient) thermistor temperature sensor, the tip of which was positioned at the level of the ear tags, served as the reference temperature. The water bath was positioned and filled in a way that the ear tags were covered with water but still connected to the SB receivers (Fig. 7). Temperatures of 20–50°C were simulated in steps of 2°C, mainly to cover the physiological range of cattle from 36–42°C. The lowest temperature limit was 20°C, from which a constant temperature gradient was generated. To avoid data gaps after reaching the next target temperature, it was kept constant for 8 minutes to obtain sufficient recorded values by the SB ear tag. The reference temperature was read and noted manually, and the ear tag temperature was obtained via the software. The time of the software was synchronized in advance with the observation time.



Figure 6. Ear tags positioned in a water bath, attached to Styrofoam plates and vacuum sealed.



Figure 7. Position and arrangement of the water bath.

2.3. Tracing of the Transition from Normo- to Hyperthermia on the Ear Tag-Based Temperature Curves

2.3.1. Animals, Housing, and Feeding

The animals of the commercial farm were grouped by parity and lactation status and kept as follows: First, lactating and multiparous cows were kept in free-stall barns with a concrete floor and manure scraper in pens for up to 200 cows. The pens were equipped with cubicle manure separation material as bedding. Milking was carried out three times a day in a 48-unit side-by-side milking parlor. The same housing system was used for dry cows in the far-off period (approximately 60 to 21 days antepartum) as well as for lactating and fresh cows. Cows in the close-up and fresh cow period (i.e., from about day 21 antepartum up to approximately 10 days in milk) were moved to a “special-need” barn for more intensive (veterinary) care and surveillance. This barn is shown schematically in Figure 8. The small boxes represent the lying pens and the large boxes the calving pens or sick pens. The animals were kept in groups of about 150 animals. The barn was equipped with a 12-cow milking parlor (right bottom in Fig. 8), where fresh cows were milked twice daily at 5:30 a.m. and p.m. Animals that were new to the barn and thus in the close-up phase were stalled in the compartment to the

left of the feed bunk, which was equipped with lying pens (Fig. 8). As soon as the cows showed signs of impending calving, they were moved to straw pens with up to five cows for calving. In addition to the calving pens, the barn had compartments for sick cows and cows that could be kept on straw shortly after birth. Cows that were considered healthy after calving and colostrum collection were moved to the “fresh-cow” pen (i.e., from day 1 up to approximately 10 days in lactation). The fresh-cow pen, on the right-hand side of the feed bunk (Fig. 8), was equipped with 93 feed fences (Twist&Lock Headlocks, GEA Farm Technologies GmbH), solid concrete

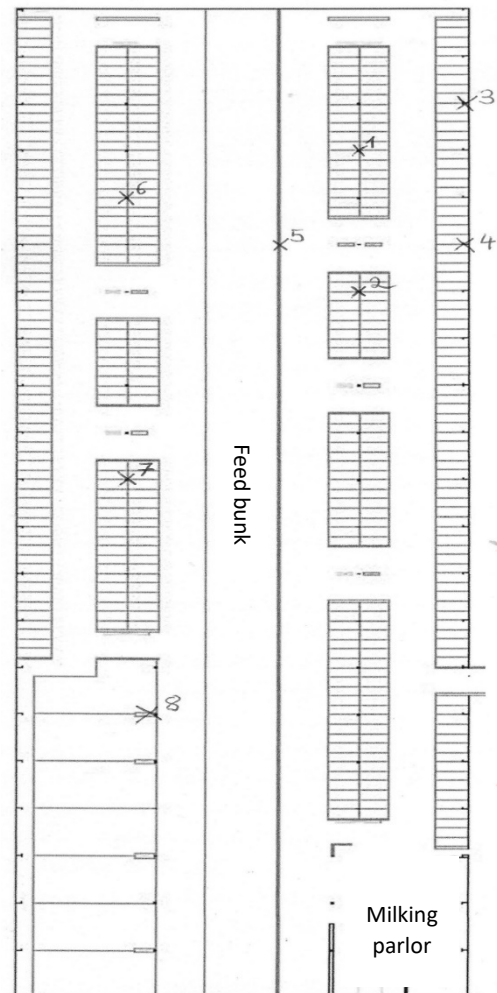


Figure 8. Stable plan of the “special-need” barn, the loggers installed are marked with a cross and a number.

floors, and 93 cubicles with bedding material consisting of horse manure and chopped straw. A total mixed ration (TMR) was fed *ad libitum* once daily in the morning at 4:30 a.m., with forage pushed up six times per day. The TMR consisted of the following components: Grass silage (28.5%), corn silage (24.7%), feed rye silage (10.0%), corncob mix and corn mix (10.0%), potato pulp, soybean (4.6%) and canola (5.6%) extraction meal, canola meal (3.6%), wheat straw (2.2%), soybean hulls (1.2%), vinasse (2.8%), and minerals (6.8%). *Ad libitum* access to water in six water troughs (3.2 m or 1.6 m per trough) was provided.

2.3.2. Temperature Logger and Weather Station for Ambient Temperature

The fresh-cow barn was equipped with temperature-humidity loggers (HOBOnet Wireless Temp/RH Sensor, Onset Computer Corporation), representing five different areas to account for differences (Table 5). The loggers were wirelessly connected to an outdoor weather station (HOBOnet RX3000 Remote Monitoring Station, Onset Computer Corporation) installed in proximity to the barn. Both the loggers and the weather station logged temperature and humidity data in 1-minute intervals. These measurements by the remote device were therefore continuous. Data were transferred automatically via WIFI/Internet and stored on a cloud system. Pandas for Python (pandas 1.0.3, Python 3.7.9) was employed to merge and resample the data to hourly and daily recordings.

Table 5. Temperature-humidity loggers and area.

Logger	Area
1	Barn, inner cubicles (1), far from the milking parlor
2	Barn, inner cubicles (2), near the milking parlor
3	Barn, outer cubicles (1), far from the milking parlor
4	Barn, outer cubicles (2), near the milking parlor
5	Barn, feed bunk

2.3.3. Examinations in the Fresh Cow Period with Animal Rectal Temperature Collection

The daily examination routine began with the morning sighting of all SB warnings. Following this, the conspicuous animals were noted for subsequent examination in the fresh-cow pen. Based on the herd health management strategy already implemented on the farm, veterinary examinations and treatments for study purposes were performed daily after morning milking, approximately between 7:00 and 9:00 am. For this, the animals were restrained in headlocks. The initial examination procedures included measuring rectal temperature to identify the animals as febrile or not, checking ear and skin temperature, and inspecting for retained placenta and vaginal discharge for all animals. A commercial veterinary thermometer for large animals (Veterinär-Thermometer SC12, SCALA Electronic GmbH, Stahnsdorf, Germany) was used to measure rectal temperature. A marking was placed on the thermometer to ensure even and appropriate penetration of 8 cm. The exact time of rectal temperature measurement of each animal was recorded from 07.03.21–22.03.21. This time point measurement was later used to evaluate the relationship between rectal temperature and SB or environmental temperature. The time of the measurements was synchronized with both the observers watching and the SMARTBOW Software. Animals were examined more closely as soon as a rectal temperature of $\geq 39.5^{\circ}\text{C}$, a negative deviation in daily milk yield $\geq 25\%$ from the previous day (from four DIM), retained placenta or vaginal discharge, cold ear or skin surface temperature as a sign of hypocalcemia, or SB rumination alarm occurred after the initial examination.

3. STATISTICAL ANALYSIS

Statistical analyses were performed using Excel and SPSS Statistics (version 26; IBM Inc., Armonk, NY). Data were checked for normality applying the Kolmogorov-Smirnov test, and descriptive analyses were made. Precision for the ear tag was investigated in a standardized setting using Pearson's correlation coefficients (r) and linear regression coefficients of determination (R^2). Following Hinkle et al. (2004) and Mukaka (2012), correlation coefficients and linear regression were categorized as follows: 0.00 to 0.30 = negligible; 0.30 to 0.50 = low; 0.50 to 0.70 = moderate; 0.70 to 0.90 = high; and 0.90 to 1.00 = very high. Temperature measurements were considered precise if r and R^2 were high (>0.70). To assess the limits of agreement between the two methods, the method described by Bland and Altman (1986) was used. To depict the deviation in the range of the physiological core temperature in dairy cows, coefficients of variation for the measured temperature levels were determined. Subsequently, the mean coefficient of variation was calculated for the temperature level from 36°C to 42°C, which includes the physiological temperature range of dairy cows. For the comparison of the temperature humidity loggers and the weather station for the daily readings, the Independent-Samples Kruskal-Wallis Test was employed, and for the hourly readings, the Independent-Samples Median Test was used. Spearman's correlation was performed to determine the nonparametric correlations between ear tag and environmental temperature, ear tag and rectal temperature, and rectal and environmental temperature. To portray daily and hourly readings of the temperature humidity loggers, weather station, and ear tag temperatures, graphic representation was chosen.

4. RESULTS

4.1. Validation of the Reproducibility and Accuracy of the Ear Tag-Based Thermometer

The reference temperature and SMARTBOW (SB) ear tag temperatures are shown in Table 6. Due to Wi-Fi connection problems, two of the SB ear tags had to be excluded from statistical analyses. Three SB tags had small data gaps with no temperature recorded, i.e., with two ear tags in only one temperature range (SB Ear tag No. 2 at 42°C and SB Ear tag No. 5 at 24°C, respectively) and one ear tag in two temperature ranges (SB Ear tag No. 6 at 26°C and 30°C). The linear regression coefficients of determination ($R^2 = 0.99$; $P < 0.001$) and Pearson's correlation coefficients ($r = 0.99$; $P < 0.001$) for the temperature of the SB ear tag against the reference temperature were very high (Fig. 9). Based on the Bland-Altman plots of agreement, most measurements were located within the 95% CI of agreement for the SB ear tag and the reference temperature. The Bland-Altman plot had a mean difference of $-0.96 \pm 0.45^\circ\text{C}$ (mean difference $\pm$ SD), showing a negative trend. Zero was outside the limits of agreement (Fig. 10). Considering the coefficient of variation, the mean deviation in the physiological temperature range of dairy (36–42°C) between SB and the reference was 0.82% (Table 7).

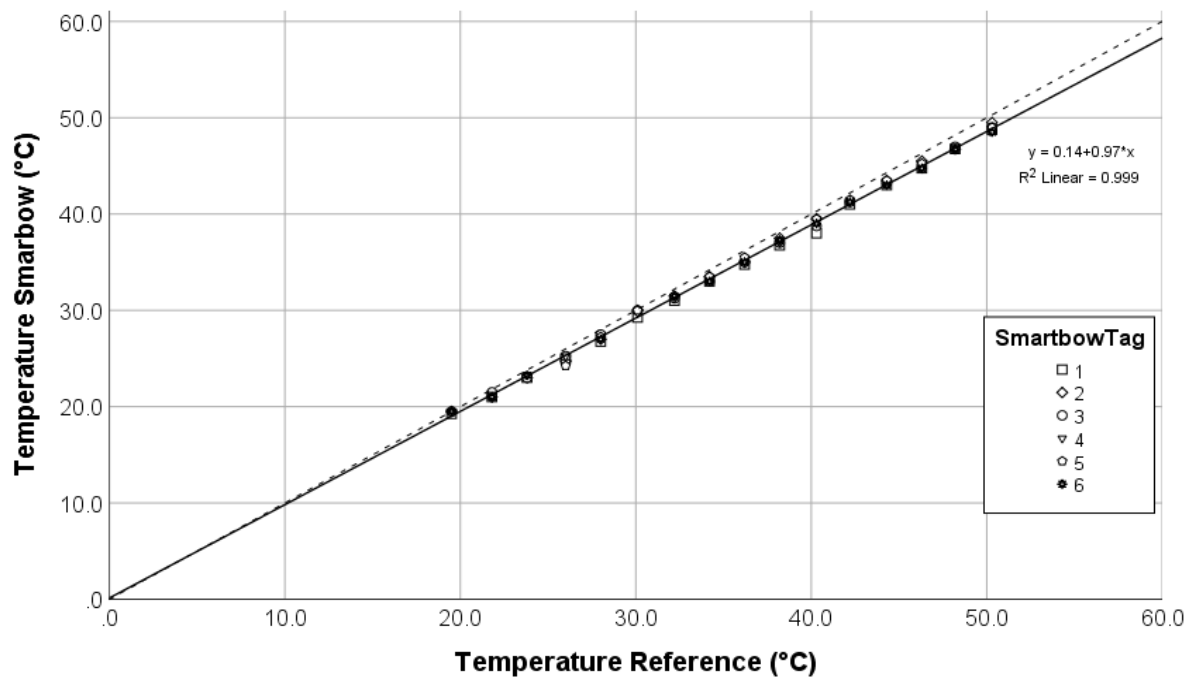


Figure 9. Regression of Temperature readings ($^\circ\text{C}$) of a temperature reference against SB ear tag readings.

Table 6. Mean SB temperatures of the SB ear tags at set reference temperatures.

Set Ref. Temp.	SB Ear Tag No.					
	1	2	3	4	5	6
	Temp. SB Mean	Temp. SB Mean	Temp. SB Mean	Temp. SB Mean	Temp. SB Mean	Temp. SB Mean
20.00	19.25	19.50	19.50	19.50	19.50	19.50
22.00	21.00	21.00	21.50	21.25	21.00	21.00
24.00	23.00	23.00	23.00	23.25	-	23.25
26.00	25.00	24.75	25.25	25.25	24.25	-
28.00	26.75	27.00	27.50	27.25	27.25	27.00
30.00	29.25	30.00	30.00	29.75	30.00	-
32.00	31.00	31.50	31.25	31.25	31.50	31.50
34.00	33.00	33.50	33.00	33.00	33.50	33.00
36.00	34.75	35.00	35.00	35.00	35.50	35.00
38.00	36.75	37.50	37.00	37.00	37.25	37.25
40.00	38.00	39.50	38.75	39.25	39.50	39.00
42.00	41.00	-	41.25	41.25	41.50	41.25
44.00	43.00	43.50	43.25	43.00	43.50	43.00
46.00	44.75	45.50	44.75	44.75	45.25	44.75
48.00	46.75	46.75	47.00	47.00	46.75	46.75
50.00	48.75	49.50	49.00	48.75	49.00	48.50

Table 7. Coefficients of variation for measured temperature levels.

Temperature Reference	Mean SB Temperature	Std. Deviation	Variation Coefficient
19.5	19.46	0.10	0.52
21.8	21.13	0.21	0.99
23.8	23.10	0.14	0.59
26.0	24.90	0.42	1.68
28.0	27.13	0.26	0.97
30.1	29.80	0.33	1.09
32.2	31.33	0.20	0.65
34.2	33.17	0.26	0.78
36.2	35.04	0.25	0.70
38.2	37.13	0.26	0.71
40.3	39.00	0.57	1.46
42.2	41.25	0.18	0.43
44.3	43.21	0.25	0.57
46.3	44.96	0.33	0.74
48.2	46.83	0.13	0.28
50.3	48.92	0.34	0.70

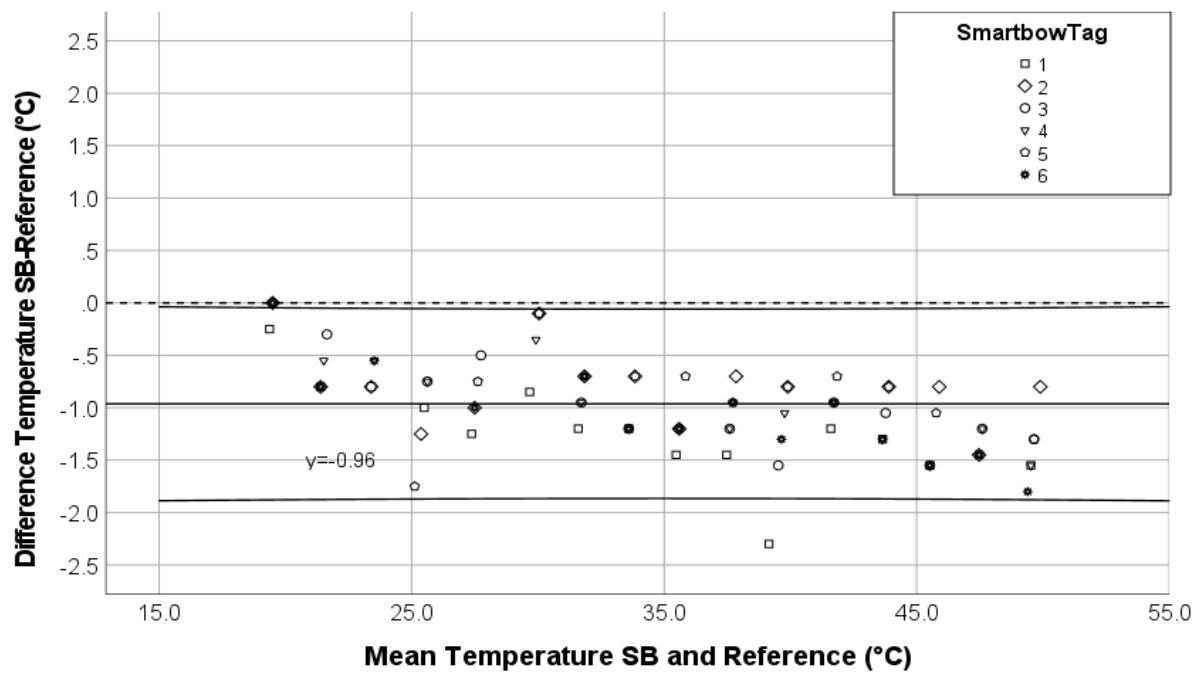


Figure 10. Bland-Altman plots of agreement comparing the differences in mean temperature readings (°C) of a reference temperature against Smartbow ear tag readings. The middle solid line represents the mean difference, and the upper and lower solid lines the upper and lower limits of agreement (2 SD from the mean difference).

4.2. Tracing of the Transition from Normo- to Hyperthermia via the Ear Tag-Based Temperature Curves

4.2.1. Analysis of Environmental Data in the Barn and Outdoor Area

4.2.1.1. Comparison of the Daily Temperature Readings between Loggers and Weather Station

The study period from 01.02.21 to 31.03.21 consisted of 59 days. With six daily readings (five temperature-humidity loggers and outdoor weather stations), the total N was 354. The following values are presented in °C. The outdoor weather station as a reference for climatic conditions measured an average temperature of 3.62 ± 5.59 (mean $\pm$ SD). Here, the minimum was -8.83 and the maximum 13.93. The mean values of the temperature of loggers 1–5 installed in the barn followed the order 4.68 ± 4.93 , 4.57 ± 4.94 , 4.19 ± 5.14 , 4.32 ± 5.08 , and 4.40 ± 4.88 . The minima and maxima were analogously -4.57 and 14.85, -4.73 and 14.67, -5.99 and 14.85, -5.58 and 14.71, and -5.30 and 14.24, respectively. The Kruskal-Wallis test for independent samples revealed no significant differences between samples ($H = 0.909$, $P <$

0.05), although numerical differences between outdoor and barn areas were evident. For the weather station values, protruding temperature minima were clearly visible, whereas these, and those in the barn, were more attenuated. This can be seen in Figure 11. According to this, the minimum temperature was about 4 K lower outdoors. In addition, temperatures in the barn were subject to less fluctuation, as evidenced by a lower standard deviation of loggers 1–5. Based on the lower deviation, it can be concluded that the barn fulfills its protective function against temperature extremes and provides an equal temperature within all measured areas.

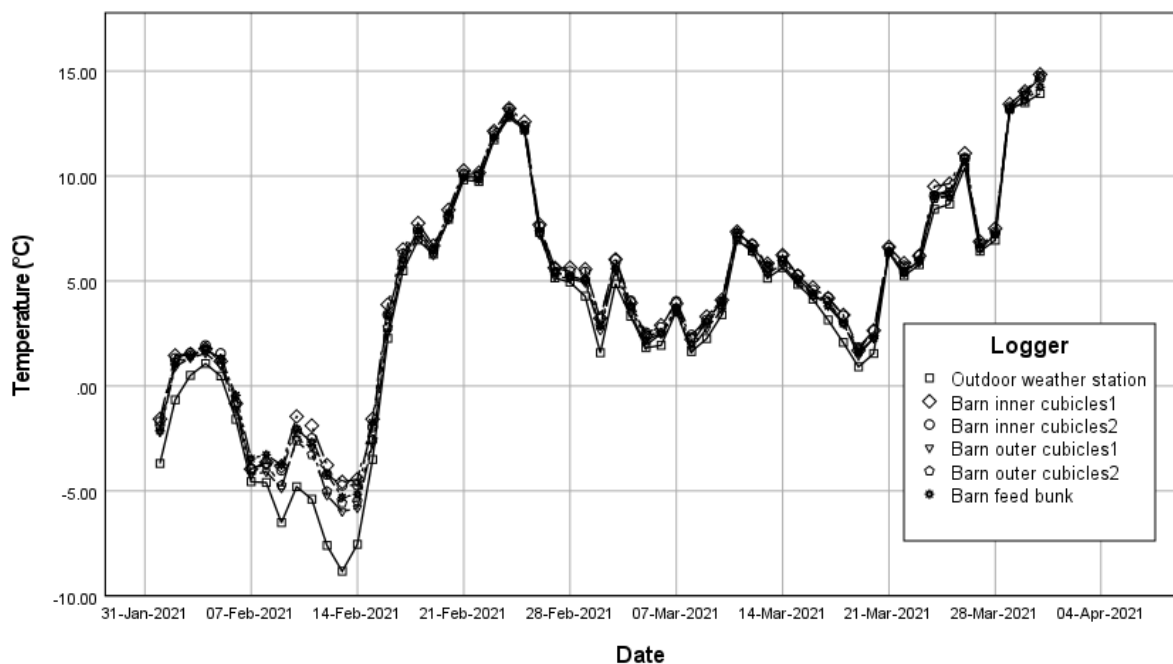


Figure 11. Graphical representation of the recorded temperatures (°C) of the temperature loggers and outdoor weather station during the study period (01.02.21–31.03.21).

4.2.1.2. Comparison of the Hourly Temperature Readings between Loggers and Weather Station

The following shows the comparison of the temperature-humidity loggers located in the fresh-cow barn to map their diurnal rhythm and, thus, that of the area of the cows under study. For the statistical analysis of the hourly readings, the first day of the study (01.02.2021) was chosen exemplarily. A complete day with 24 h for all five loggers results in a total N of 120. The Independent-Samples Median Test did not show significant differences across the samples ($H = 0.988$, $P < 0.05$). Looking at the graphical progressions of the selected days in the course of the study, a diurnal rhythm can be seen, with its low point in the early morning hours, a

subsequent rise until beyond midday, with the maximum value in the early afternoon, and a subsequent continuous drop over midnight until the morning of the next day (Figs. 12–19) The exceptions to this are 01.02.21 and 07.02.21 (Figs. 12 and 13), where the temperatures fluctuated from maximum to maximum in the afternoon to evening or remained constant during this time.

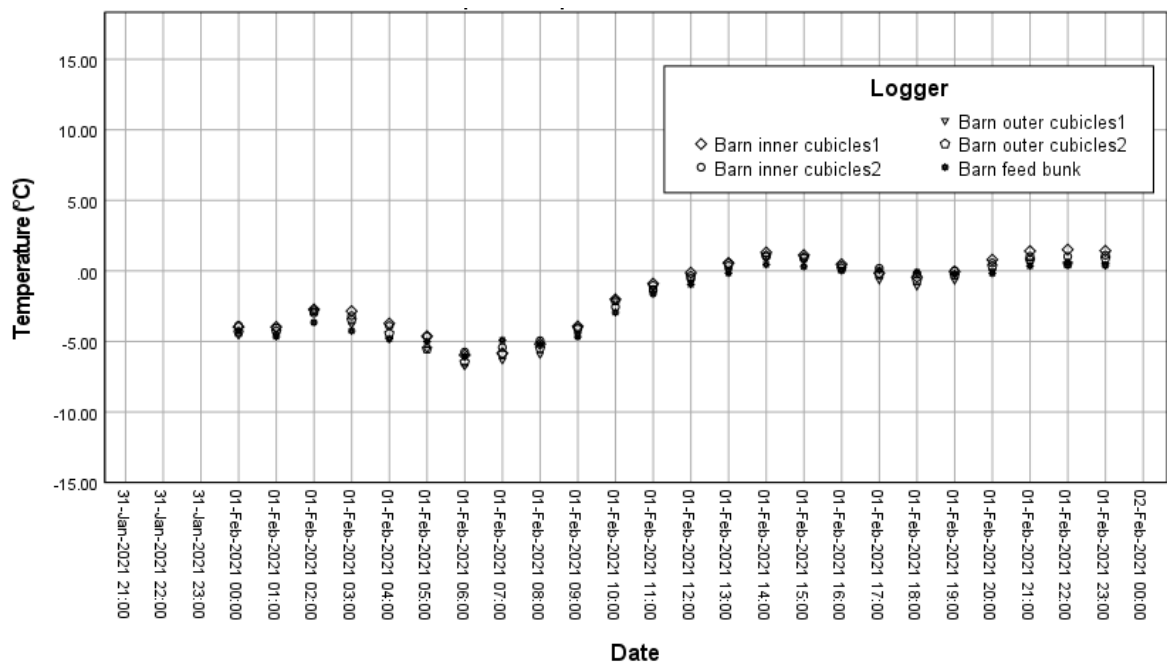


Figure 12. Temperature profile on an hourly basis – 01.02.21.

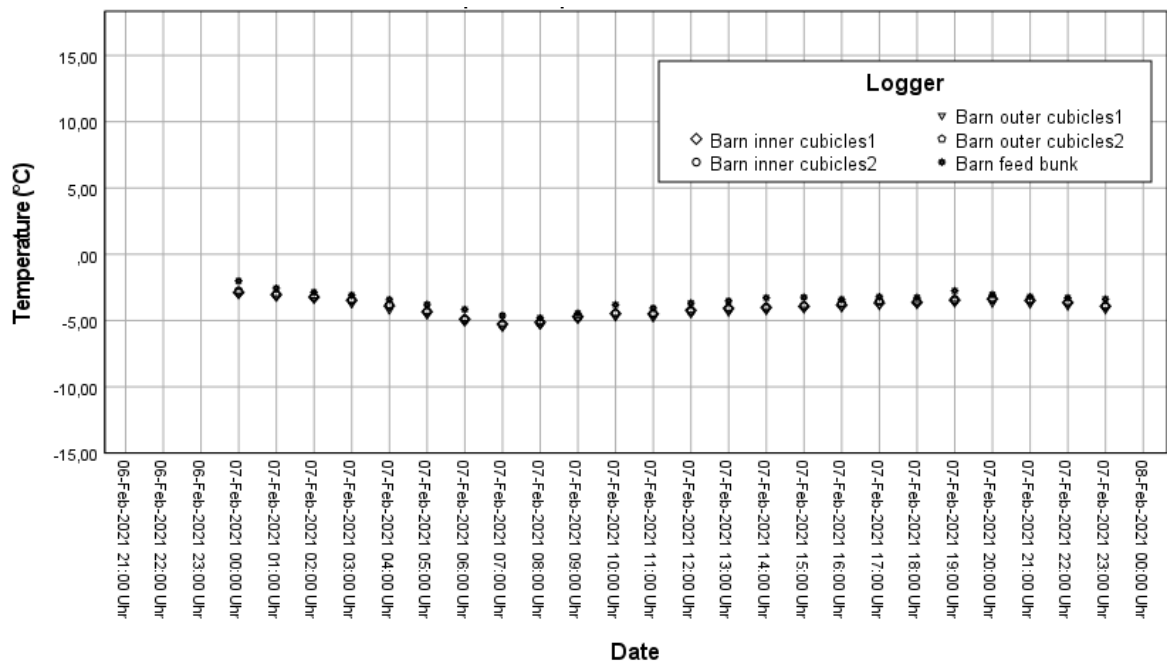


Figure 13. Temperature profile on an hourly basis – 07.02.21.

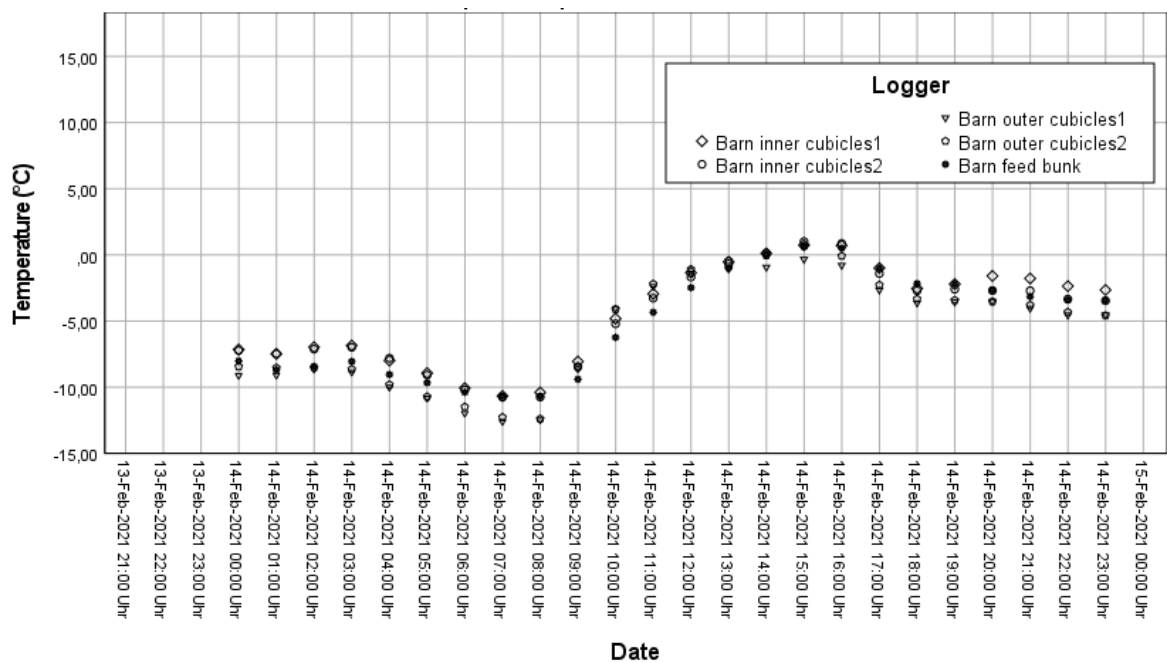


Figure 14. Temperature profile on an hourly basis – 14.02.21.

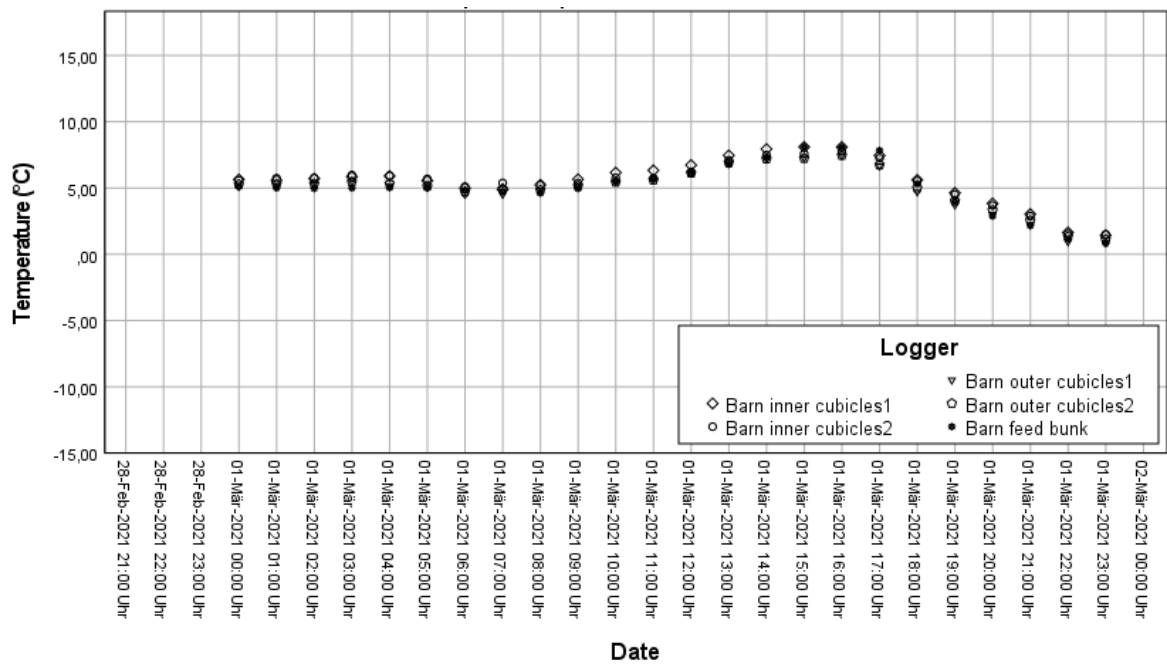


Figure 15. Temperature profile on an hourly basis – 21.02.21.

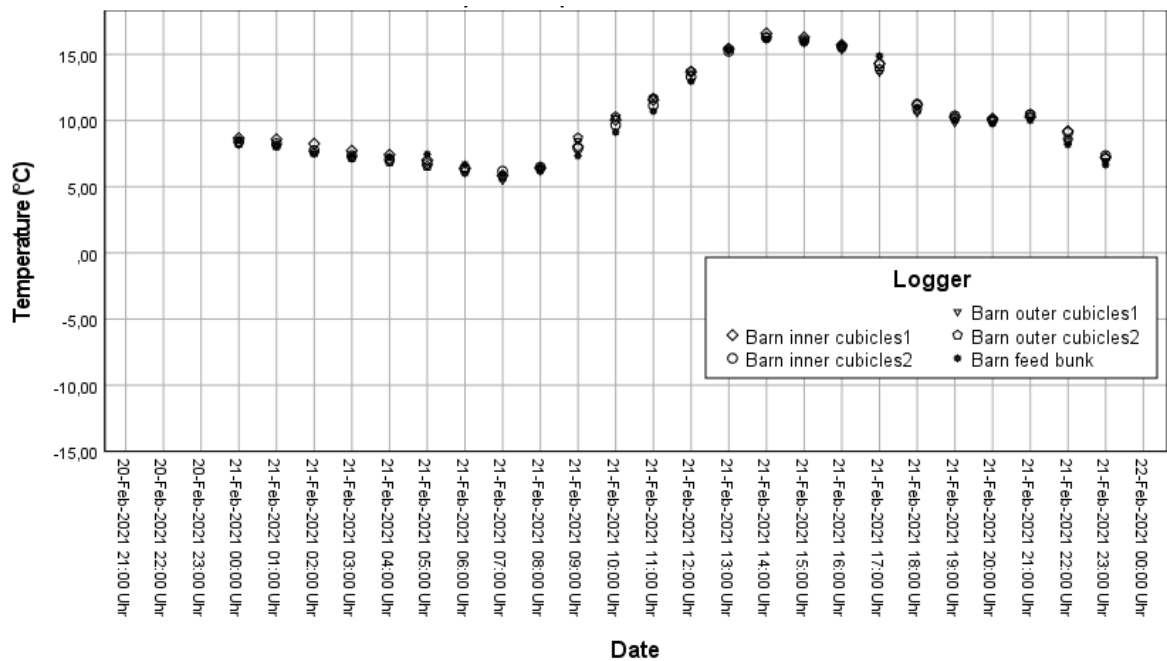


Figure 16. Temperature profile on an hourly basis – 01.03.21.

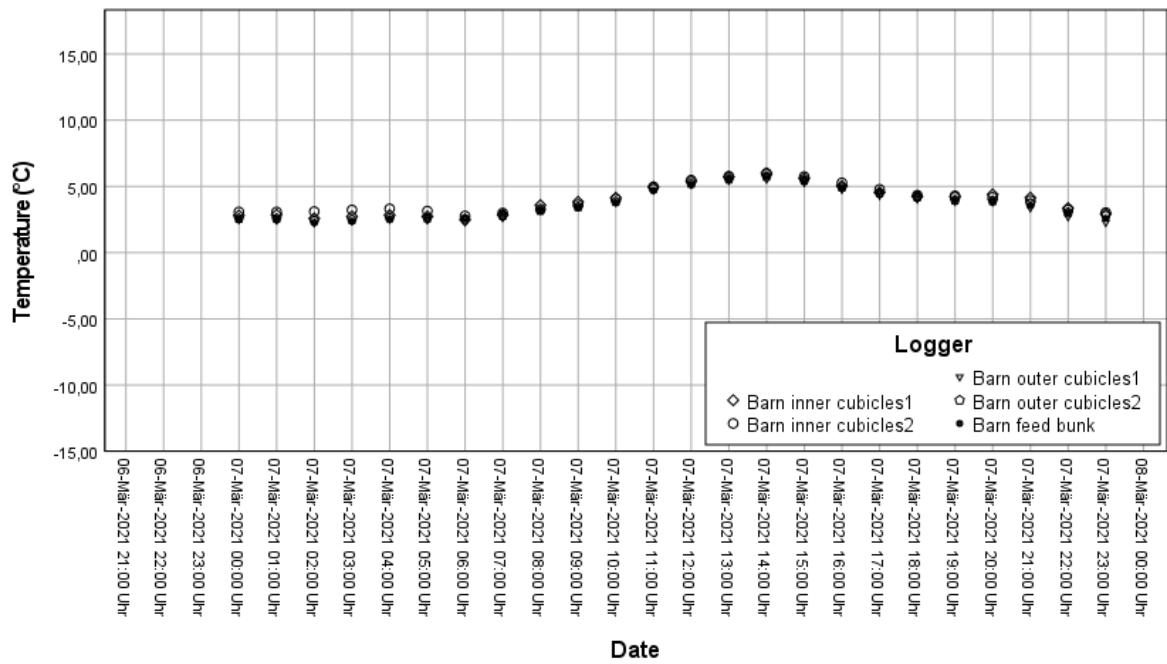


Figure 17. Temperature profile on an hourly basis – 07.03.21.

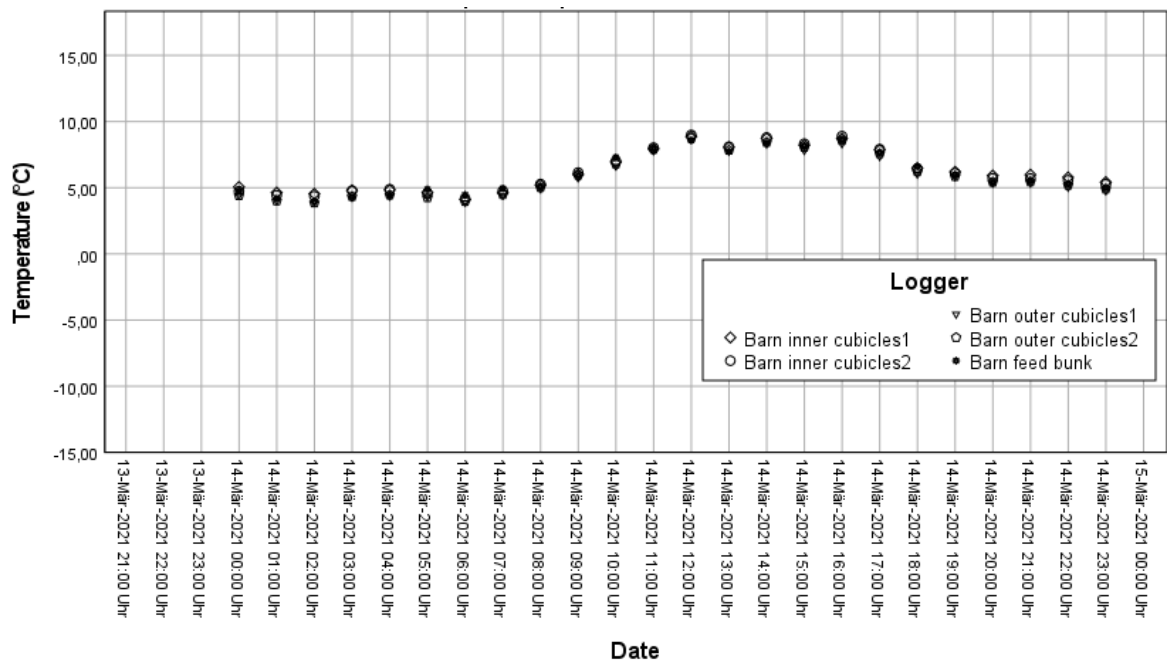


Figure 18. Temperature profile on an hourly basis – 14.03.21.

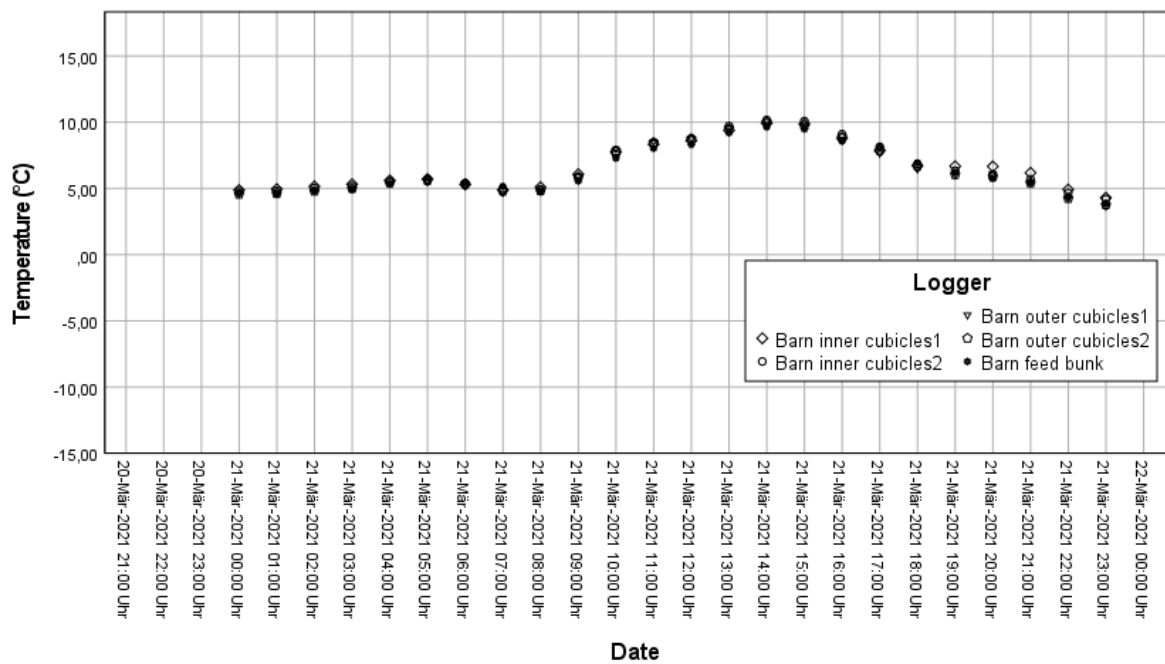


Figure 19. Temperature profile on an hourly basis – 21.03.21.

4.2.2. Graphic Comparison of the Daily Temperature Readings Between One Selected Logger and Selected SMARTBOW Ear Tags

Because no significant difference was detected when comparing the temperature-humidity loggers, only one logger was used for graphical representation. For this, Logger 5, which was located at the feed bunk, was chosen. During the study period of approximately 8 weeks, the temperature courses of the respective diseased animals were compared with those of the healthy animals. Comparison weeks were selected, in which as many animals as possible had rectal temperatures of 7 consecutive days measured to represent a transition from non-fever (physiological body temperature) to fever (increased body temperature) and vice versa. Diseased animals were defined as showing a rectal temperature of $\geq 39.5^{\circ}\text{C}$ from one day to the next and beyond. In addition to the temperature-humidity logger, diseased cows were selected, and randomly selected healthy cows were added up to a total number of 15 animals. In the following, we look at comparison week 1 (17.02.21–23.02.21), 2 (26.02.21–04.03.21), 3 (07.03.21–13.03.21), and 4 (16.03.21–22.03.21), respectively.

In comparison week 1, three different animals (cows no. 14, 67, and 6,026) showed an increase in temperature; the explicit days are marked with an arrow (Fig. 20). More precisely, cow 14 showed an elevated temperature on 23.02.2021 (39.7°C). Cow 67 showed an elevated temperature on 20.02.2021 (39.6°C), then recovered for 1 day before the temperature rose again

to $> 39.5^{\circ}\text{C}$ for 2 consecutive days (22.02.2021 and 23.02.2021 with 39.5°C). Cow 6,026 showed an elevated temperature on 20.02.2021 (39.7°C). As seen in the graph, the changes in temperature measured at the ear of the sick animals were within the limits of the healthy animals. There were no peaks or clear deviations compared to the ambient temperature.

In comparison week 2, four different animals (cows no. 106, 4,744, 5,465, and 6,484) showed an increase in temperature; the explicit days are marked with an arrow (Fig. 21). More precisely, cow 106 showed an increased temperature on 3 consecutive days (28.02.2021–02.03.2021, with 39.6°C , 39.8°C , and 40.1°C , respectively). Cow 4,744 as well as cow 6,484 showed an elevated temperature on 04.03.2021 (41.0°C and 39.5°C , respectively). In cow 5,465, the temperature was increased in 2 consecutive days (28.02.21 and 01.03.21, with 39.9°C and 40.3°C , respectively) before it dropped again to a temperature within the physiological range. The temperatures measured at the ear were, overall, lower for this cow. This could possibly be due to the greater exposure to environmental influences; i.e., the ear tag was perhaps less protected in the pinna and located more towards the outer edge of the ear. As seen in the graph, the changes in temperature measured at the ear of the sick animals were within the limits of the healthy animals. The exception was cow 5,465, which generally showed a lower level. There were no peaks or clear deviations compared to the ambient temperature.

In comparison week 3, three different animals (cows no. 4,959, 6,570, and 6,639) showed an increase in temperature; the explicit days are marked with an arrow (Fig. 22). More specifically, cow 4,959 showed a 1-day increase on 08.03.21 (39.6°C), as did cow 6,639 on 13.03.21 (39.5°C). An increase in temperature over 3 days (10.03.21–12.03.21) was measured in cow 6,570 (39.5°C , 40.4°C , and 39.9°C , respectively). As seen in the graph, the temperature changes measured at the ear of the sick animals were within the limits of the healthy animals, and there were no peaks or significant deviations compared to the ambient temperature, with the exception of cow 6,639. A negative peak compared to the healthy animals and the ambient temperature could be observed on the day prior to the temperature increase.

In comparison week 4, cow no. 6,683 showed an increase in temperature; the explicit day is marked with an arrow (Fig. 23). More precisely, the cow showed a 1-day increase on 17.03.21 (39.5°C). It can be seen in the graph that the changes in temperature measured at the ear of the sick animal were within the limits of the healthy animals. There were no peaks or clear deviations compared to the ambient temperature.

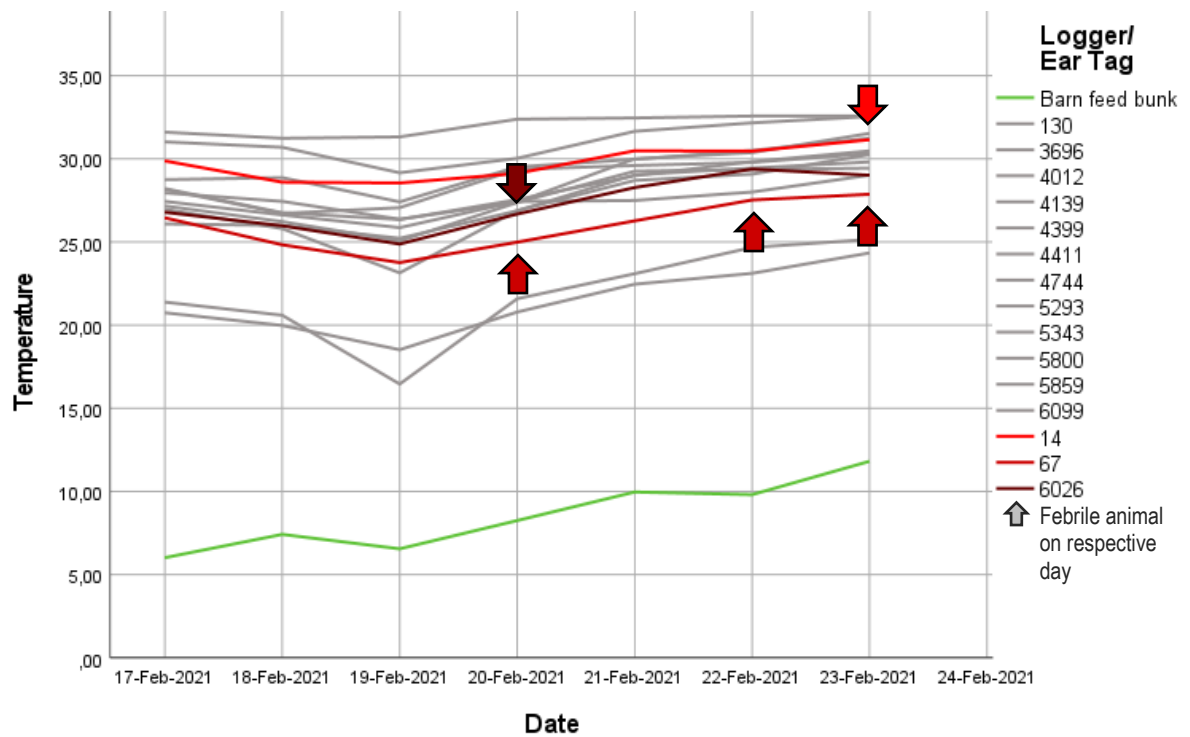


Figure 20. Comparison week 1 (17.02.21–23.02.21). The arrows show the febrile animals on the respective day.

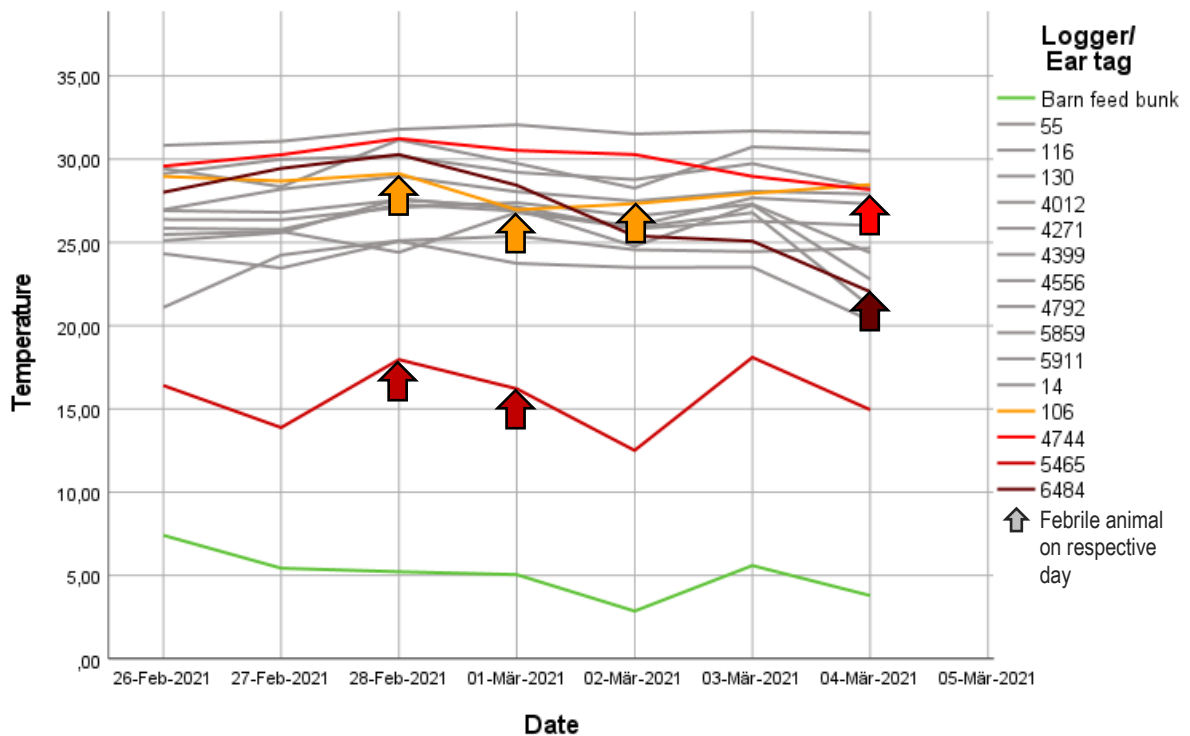


Figure 21. Comparison week 2 (26.02.21–04.03.21). The arrows show the febrile animals on the respective day.

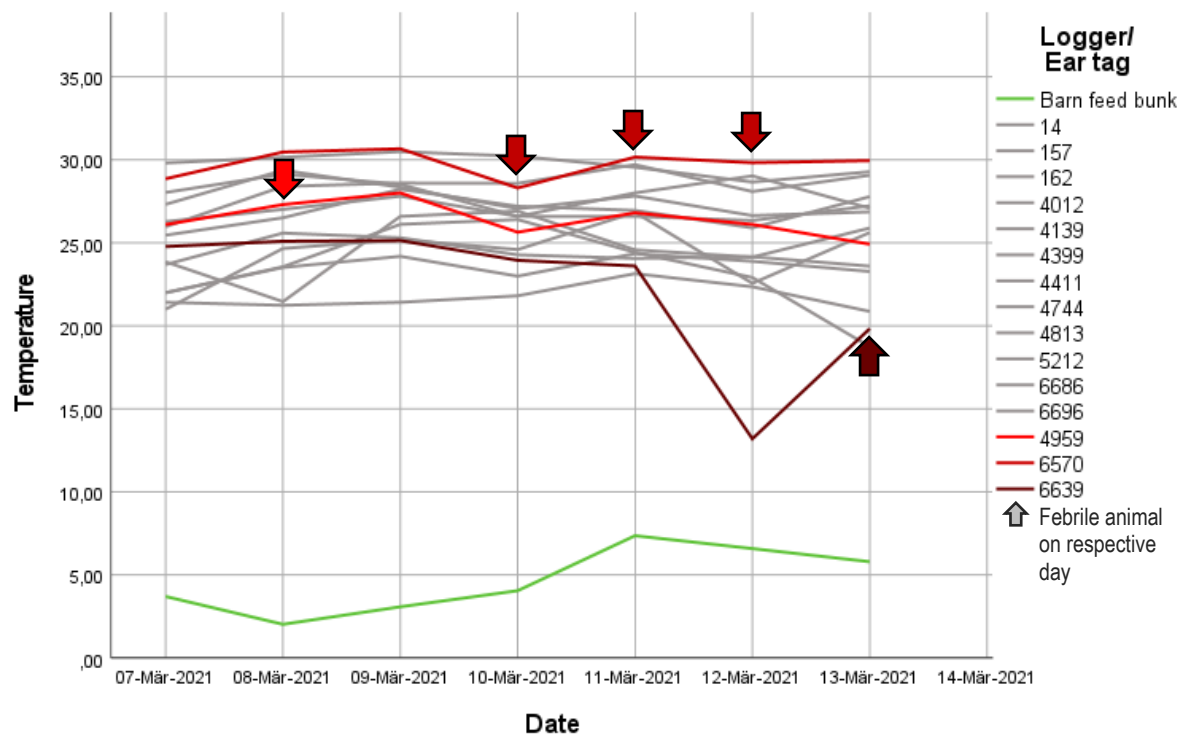


Figure 22. Comparison week 3 (07.03.21–13.03.21). The arrows show the febrile animals on the respective day.

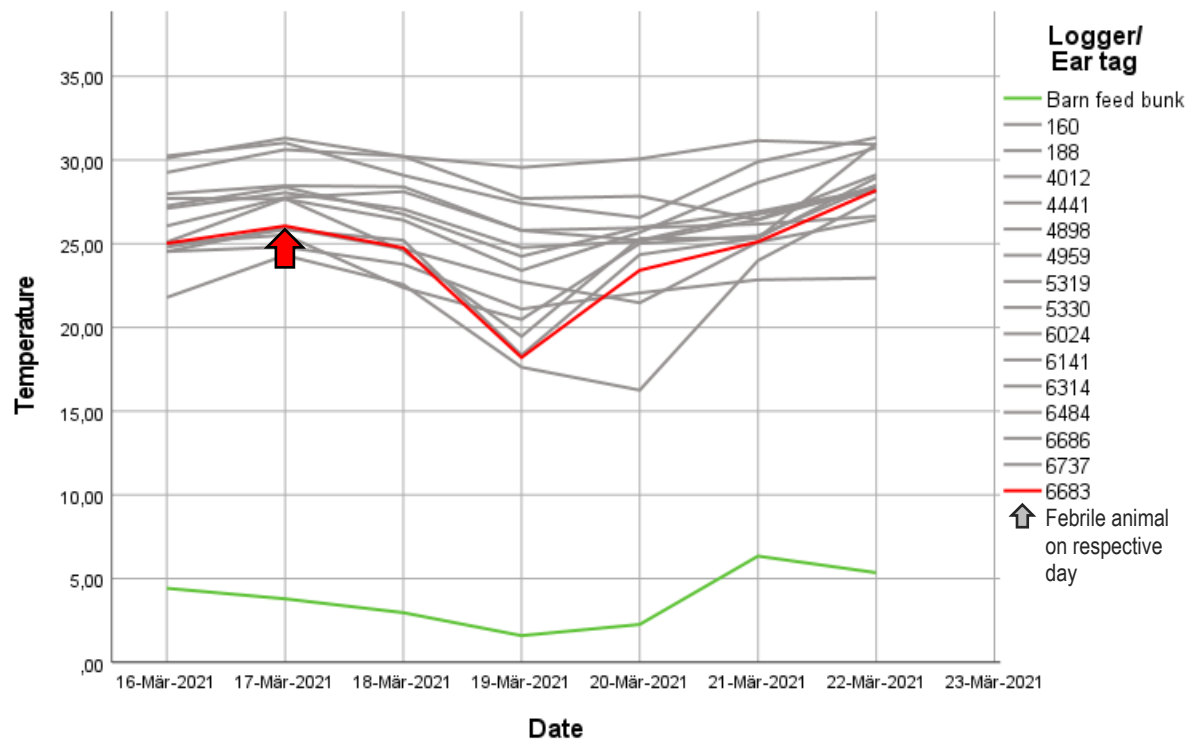


Figure 23. Comparison week 4 (16.03.21–22.03.21). The arrows show the febrile animals on the respective day.

4.2.3. Graphic Comparison of the Hourly Temperature Readings Between One Selected Logger and Selected SMARTBOW Ear Tags

For further analysis, the hourly recordings were depicted graphically, analogous to the animals selected in the previous section, to show finer differences and peak temperatures. Ambient temperature (green line) followed a diurnal rhythm, with maximum temperatures around midday or early afternoon and minimum temperatures about midnight or early morning. Here, comparison week 3 was chosen, in which, even if only one, a conspicuousness in the daily recordings could be shown. This also showed up 1 day before the temperature increased. The three different animals (cows no. 4,959, 6,570, and 6,639) that showed a temperature increase are marked with an arrow (Fig. 24). The arrow marking corresponds to the time when a temperature increase was detected using rectal thermometry. More precisely, an increased temperature was detected at cow 4,959 on 08.03.21 at 06:53 a.m., as well as at cow 6,639 on 13.03.21 at 06:49 a.m. A temperature increase over 3 days (10.03.21 at 07:01 a.m., 11.03.21 at 06:24 a.m., and 12.03.21 at 06:41 a.m.) was measured at cow 6,570. In the graph, conspicuously low values compared to the healthy animals and the ambient temperature can only be observed for cow 6,639 in the area 24 h before the temperature increase was measured.

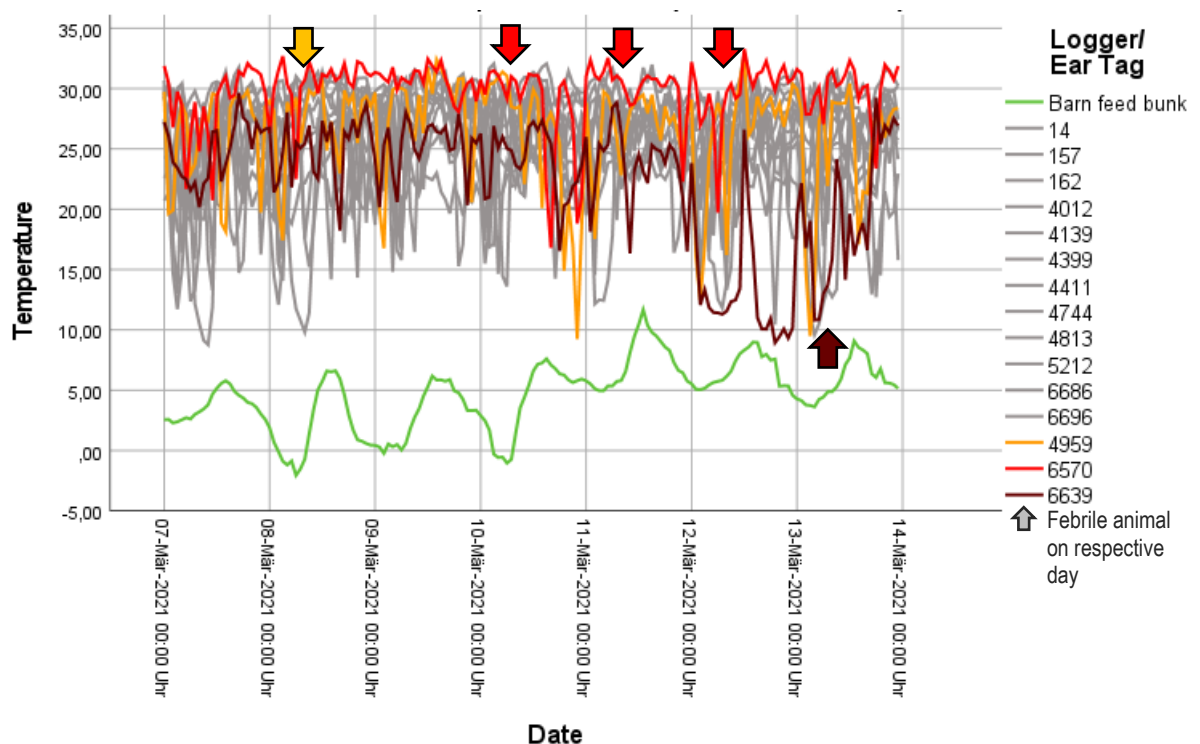


Figure 24. Comparison week 3 (07.03.21–13.03.21). The arrows mark the time when a temperature increase was detected via rectal thermometry.

In the following, the daily transitions for each sick animal of comparison week 4 are shown, i.e., from the day before the detection up to and including the day of the detection of the temperature increase. Starting with cow no. 4,959 with the day transition from 07.03.21 to 08.03.21, the animal with the increased temperature is shown in red, the environmental temperature in the form of the loggers in the area of the feeding table in green, and the healthy animals in grey (Fig. 25). For the ambient temperature, the diurnal rhythm is recognizable, with its maximum in the early afternoon and minimum in the early morning. The graph does not show any outstanding peaks or significant deviations at any time compared to the healthy animals or the ambient temperature. At the day transition from 09.03.21 to 10.03.21, the animal showing an increased temperature, cow 6,570, is shown in red, the ambient temperature again in green, and the healthy animals in grey (Fig. 26). Although the graph shows negative peaks of the sick animal at 04:00 p.m. and 10:00 p.m., there are also some negative peaks in the healthy animals. For the ambient temperature, the diurnal rhythm is recognizable, with its maximum in the early afternoon and minimum in the early morning. At the day transition from 12.03.21 to 13.03.21, the animal showing an increased temperature, cow 6,639, is again shown in red, the ambient temperature in green, and the healthy animals in grey (Fig. 27). The graph shows that on the day before the temperature increase of the sick animal, its curve was clearly below that of the other healthy animals, with some peaks. On the day of the recognized temperature increase, the curve increased again to the range of the inconspicuous animals. For the ambient temperature, the diurnal rhythm is recognizable, with its maximum in the early afternoon and minimum in the early morning.

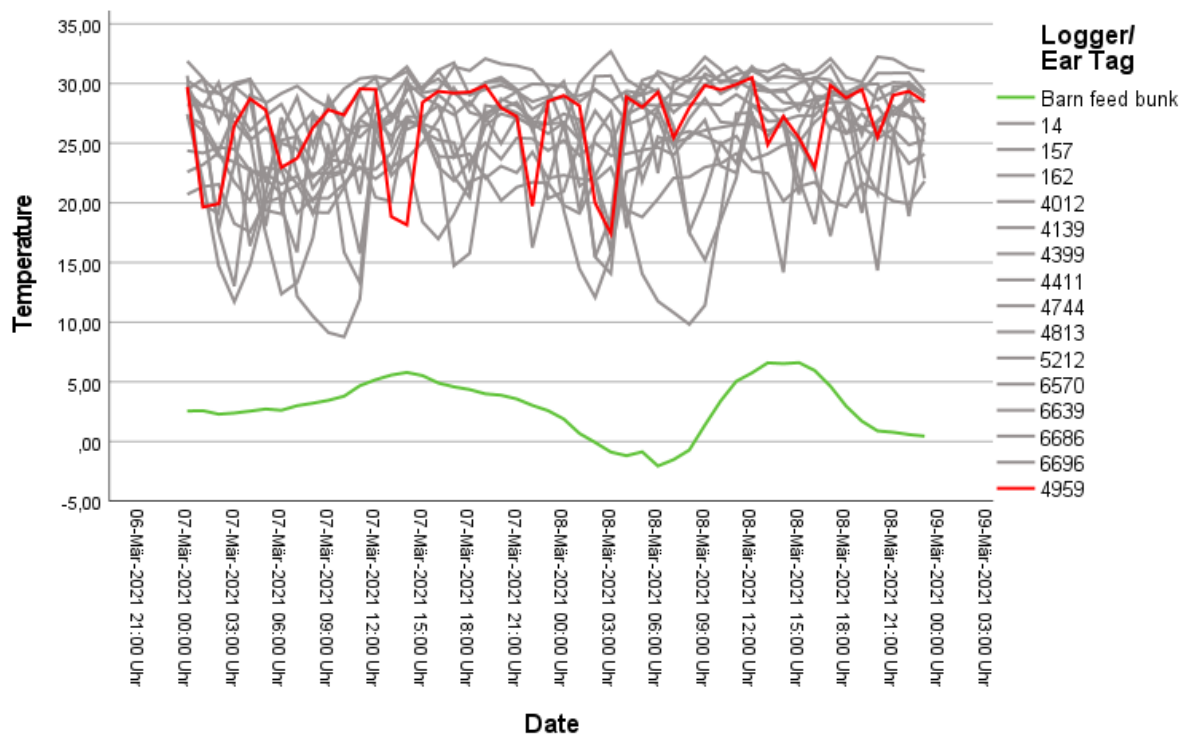


Figure 25. Comparison week 3 with day transition (07.03.21–08.03.21).

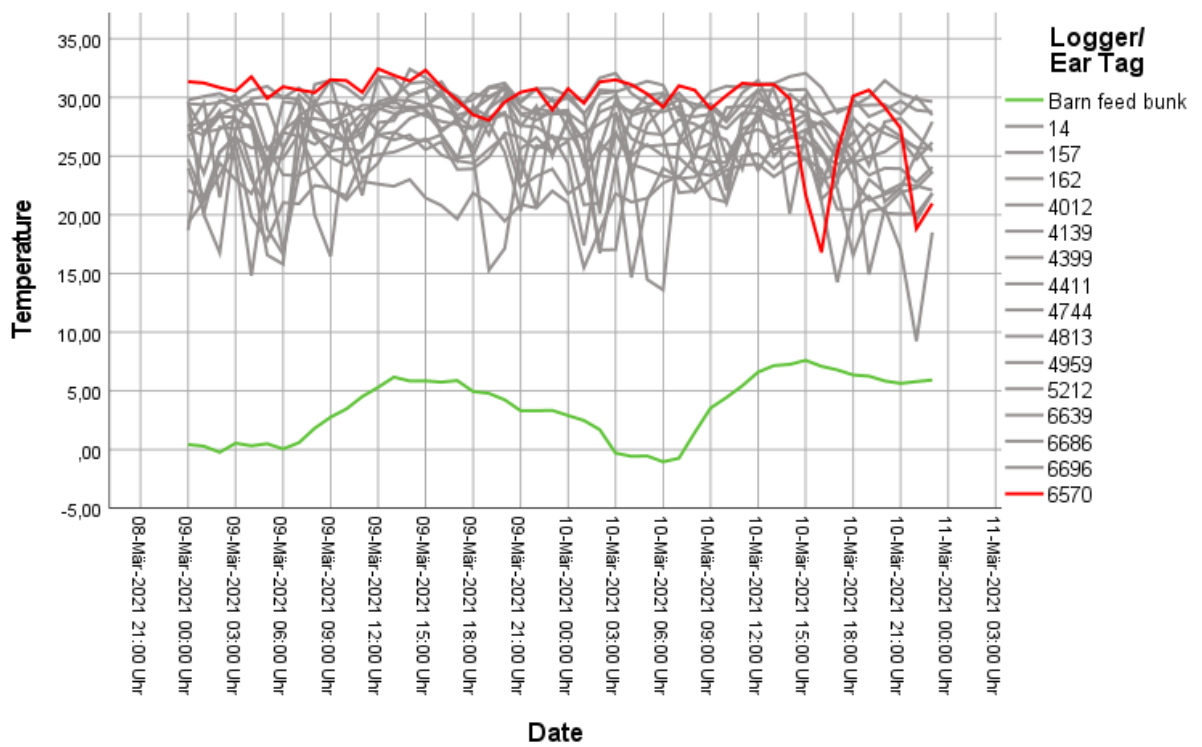


Figure 26. Comparison week 3 with day transition (09.03.21–10.03.21).

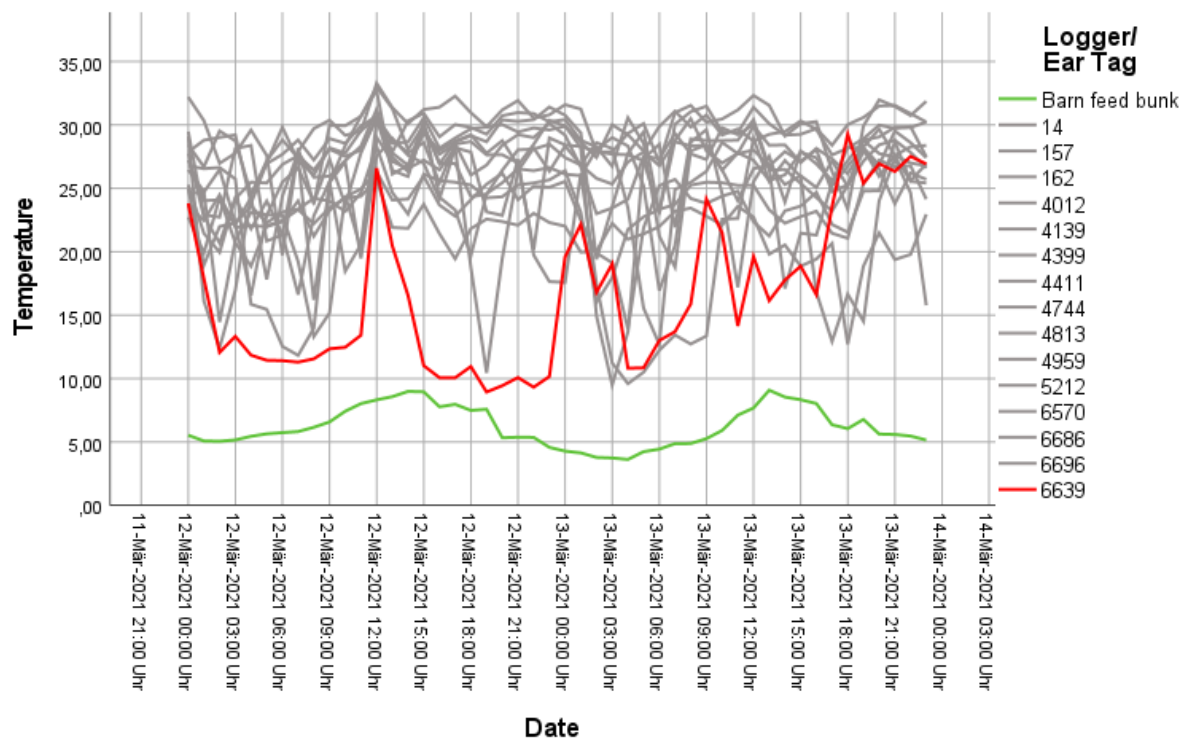


Figure 27. Comparison week 3 with day transition (12.03.21–13.03.21).

4.2.4. Relationships between Ambient Temperature and SMARTBOW Ear Tag Temperature (1), Rectal Temperature and SMARTBOW Ear Tag Temperature (2), and Ambient Temperature and Rectal Temperature (3)

- (1) Mean, standard deviation, and minimum and maximum temperature (°C) of the daily and hourly resamples for the SMARTBOW ear tag and ambient temperature represented by the Temperature-Humidity Logger 5 (feed bunk) are shown in Table 6. In the regression analysis of the effect of ambient temperature on SMARTBOW ear tag, the relationships tended to be or were negligible (daily resamples: $R^2 = 0.30$, $P = 0.01$; hourly resamples: $R^2 = 0.19$, $P = 0.01$). Spearman's correlations between ambient temperature and ear tag tended to be or were low (daily resamples: $r = 0.52$, $P = 0.01$; hourly resamples: $r = 0.41$, $P = 0.01$).
- (2) Mean, standard deviation, and minimum and maximum temperature (°C) for the SMARTBOW ear tag and rectal temperature are shown in Table 7. In the regression analysis of the effect of rectal temperature on SMARTBOW ear tag, the relationship was negligible ($R^2 = 0.02$, $P = 0.01$). Spearman's correlation between rectal and ear tag temperature was also negligible ($r = 0.11$, $P = 0.01$).

(3) Mean, standard deviation, and minimum and maximum temperature (°C) for the ambient and rectal temperature are shown in Table 8. In the regression analysis of the effect of ambient temperature on rectal temperature, the relationship was negligible ($R^2 = 0.00$, $P = 0.01$). Spearman's correlation between ambient and rectal temperature was negligible, too. ($r = 0.02$, $P = 0.01$).

Table 8. Mean, standard deviation, minimum, and maximum values of SMARTBOW ear tag and ambient temperature for the period 01.02.21–31.03.21.

Temperature	Value	°C (daily resamples)	°C (hourly resamples)
SMARTBOW Ear tag	Mean	25.38	25.36
	Standard deviation	5.00	5.97
	Minimum	4.28	-0.12
	Maximum	35.17	36.22
Ambient	Mean	4.05	4.03
	Standard deviation	3.87	4.42
	Minimum	-4.19	-7.91
	Maximum	11.81	17.46

Table 9. Mean, standard deviation, minimum, and maximum of SMARTBOW ear tag and rectal temperature for the period 07.03.21–22.03.21.

Temperature	Value	°C
SMARTBOW ear tag	Mean	24.43
	Standard deviation	5.42
	Minimum	6.71
	Maximum	33.82
Rectal	Mean	38.61
	Standard deviation	0.35
	Minimum	37.10
	Maximum	41.30

Table 10. Mean, standard deviation, minimum, and maximum of rectal and ambient temperature for the period 07.03.21–22.03.21.

Temperature	Value	°C
Rectal	Mean	38.61
	Standard deviation	0.35
	Minimum	37.10
	Maximum	41.30
Ambient	Mean	2.56
	Standard deviation	2.30
	Minimum	-2.52
	Maximum	5.76

5. DISCUSSION

The primary objective of this study was to evaluate the use of an ear tag-integrated temperature sensor to detect dairy cows suffering from fever as defined by rectal thermometry. Secondary objectives were to confirm high reproducibility and high accuracy of the measures taken by the ear tag-based thermometer to further trace the transition from normo- to hyperthermia on the ear tag-based temperature curves.

The measurements from the ear tag-based temperature sensor showed high accuracy and repeatability under standardized conditions in a water bath. However, the transition from normo- to hyperthermia in dairy cows as determined by rectal thermometry could not be reliably traced on the ear tag-based temperature curves in this study. Temperature sensors must have high accuracy with respect to the true value, which, in our experiment, was represented by the water bath temperature, and high repeatability with low deviation. This is particularly important for the detection of fever as this diagnosis depends on a single temperature measurement that exceeds the average daily body temperatures of cattle in the range of 38.0°C to 39.4°C (Hewitt, 1921; Lefcourt et al., 1999; L. C. Hicks et al., 2001). Whilst this technology has not yet been evaluated, a similar experimental setup was used in a study that examined the accuracy and precision of implantable RFID microchips using a water bath (Woodrum Setser et al., 2020). To determine reproducibility, the dispersion of measured temperatures between ear tags ("inter-device reproducibility") was determined. High accuracy was confirmed by the fact that both the linear regression coefficient ($R^2 = 0.99$; $P < 0.001$) and Pearson's correlation coefficient ($r = 0.99$; $P < 0.001$) were very high for SB ear tag temperature versus reference temperature. Bland-Altman plots of agreement showed that most measurements were within the 95% CI, and differences between measurements were close to zero. The high reproducibility was confirmed by the fact that the coefficient of variation between the ear tag-based temperature measurements was 0.82% within the physiological temperature range (36°C–42°C), which was very low. This is consistent with similar studies evaluating the accuracy and repeatability of microchips using water baths (I Huțu, F Ionescu, A Cimponeriu, C Mircea, 2009; Woodrum Setser et al., 2020). High coefficients of determination ($R^2 = 0.92$; $P < 0.001$ and $R^2 = 0.99$; $P < 0.001$, respectively) for the microchips against a reference temperature (temperature logger and rectal thermometer, respectively) are reported. Pearson's correlations between microchips and temperature loggers ($r = 1.00$; $P < 0.001$) and between microchips and rectal thermometer ($r = 0.96$; $P < 0.001$) were also very high in the *ex vivo* (water bath) study. After validating the temperature sensor under standardized conditions,

we wanted to reproduce the thermometrically determined transition from normo- to hyperthermia in dairy cows using the ear tag-based temperature curves. As a reference for this, temperature data for the environment and healthy animals were considered for comparison. With regard to the environmental temperature, there was neither a significant difference within the measurements of the five temperature loggers located at different positions in the barn nor between the measurements of the temperature loggers and those of the weather station located outside. However, it was noticeable that the barn was able to buffer temperature peaks, and hence, lower temperature fluctuations were observed compared to the outdoors. Thus, the barn fulfills its protective function against temperature extremes and ensures a uniform temperature, which could be displayed across the measured areas. While evaluating the environmental temperatures, we found a diurnal rhythm of the temperature, with a few exceptions. As a rule, the daily rhythm was characterized by a low point in the early morning hours. This was followed by a subsequent rise beyond midday and a peak in the early afternoon. Finally, a continuous decline occurred beyond midnight until the morning of the next day. The exceptions were characterized by the fact that temperatures fluctuated from one peak to another from afternoon to evening or remained constant during this period. These exceptions could be due to changing weather conditions. An influence of the barn climate management, for example by opening the curtains, is also conceivable but was not documented. A diurnal rhythm was not evident in the temperatures measured by the ear tags. Other authors, however, found a diurnal rhythm in their work on remotely measured temperatures (vaginal and ruminal, respectively). They report that several factors influenced the time of daily maximum and minimum temperatures, such as ambient conditions, housing, and season (Vickers et al., 2010; Liang et al., 2013). It could be concluded that the influence of environmental temperature on body temperature increases with increasing heat stress.

Since no significant differences were found between the values obtained by the temperature-humidity loggers placed at different locations, the measurements of one logger were used as reference for indoor temperatures. It failed to create a general graphical representation of the thermometrically determined transition from normo- to hyperthermia. In the graphical representation, only two conspicuous features could be observed. On the one hand, an animal in which fever was also detected on 2 consecutive days showed a significantly lower measured temperature level compared to other sick and healthy animals. This could have been due to several reasons, mainly related to greater exposure to environmental factors. On the one hand, the ear tag may have been less protected in the pinna and more protected on the outer edge

of the ear. Although the manufacturer provides recommendations for proper ear tag placement, proper fit should be checked in future animals participating in such studies. On the other hand, the animal itself could have actively contributed to the increased exposure to the environment through its behavior, such as by remaining in a place subjected to the outside conditions, e.g., facing part of the barn with opened curtains. This behavior could be due to a change in general behavior in the course of a febrile illness. The same applies to the drop in temperature of a feverish cow, measured by the temperature sensor on the following day compared to the temperature course of the healthy animals and the environment. In this case, an improper position of the ear tag of the affected animal can be excluded since the deviation was limited to 1 day. However, a transition from normo- to hyperthermia or vice versa could not be shown here either. For both events characterized by divergence, to clarify them, localization data, which could also be provided by the system, would have to be included in the assessment. In general, the inclusion of localization data of the cows could be useful to better classify the measured temperature values and interpret the results. Since this is an individual case, a connection to the measured temperature increase of the following day can, unfortunately, not be established, and the event is therefore to be considered random. In addition, the animal's temperature of 39.5°C was only slightly above the defined threshold. Thus, in the graphical representation, the level of fever does not seem to play a role, even though hyperthermia was measured from the threshold of 39.5°C up to 41.3°C.

The relationship between body temperature and the temperature measured by the device at the ear is significantly lower than those found in the studies on other automated and possibly remote temperature measuring devices. More specifically, the correlation of the SB ear tag temperature sensor was below the correlations of vaginal temperature measuring devices (Hillman et al., 2009; Burdick et al., 2012; Suthar et al., 2013; Vickers et al., 2010; Lees et al., 2018a; Burfeind et al., 2012; George et al., 2014; Kaufman et al., 2018) with 0.46–0.97, rumen boluses (Prendiville et al., 2002; Bewley et al., 2008a; Carroll et al., 2009; Rose-Dye et al., 2011; Ammer et al., 2016; Lees et al., 2018b) with 0.34–0.89, tympanic temperature measuring devices (Prendiville et al., 2002) with 0.80, microchips (Reid et al., 2012; Giro et al., 2019; Iwasaki et al., 2019; Woodrum Setser et al., 2020; Chung et al., 2020) with 0.22–0.76, or thermography (George et al., 2014; Giro et al., 2019) with 0.26–0.58.

To evaluate the relationship between ambient and rectal temperature, respectively, and SB temperature, as well as environmental and rectal temperature, the following needs to be considered. With a moderate to low (Mukaka, 2012) correlation, the correlation between

environmental and SB temperature was significantly higher than that between rectal and SB temperature, which was negligible (Mukaka, 2012). This means that the environment seems to have a significantly greater influence, albeit rather low, on the ear tag temperature than the temperature of the animal.

The relationship between environmental and rectal temperature was negligible, although environmental temperature may very well influence body temperature. Liang et al. (2013) demonstrated that reticulorumen temperature increased more dramatically for cows with greater milk yield as ambient temperature increased, showing that high-producing cows are more susceptible to heat stress than low-producing ones. This is explained by the fact that under heat stress, the compensation mechanisms for regulating body temperature and maintaining it in the physiological range reach their limits there. Lees et al. (2018a) highlight the advantage of continuous measurements over time point measurements. Thus, in the future, the temperature measurement of the gold standard could also be carried out continuously, automatically, and remotely via self-containing rectal devices (Gaughan et al., 2008; Reuter et al., 2010; Debnath et al., 2017; Lee et al., 2018). This would contribute to a more accurate measurement of the gold standard. As an alternative to the measurement of core body temperature via the rectum, vaginal temperature also stands out as the gold standard due to its high correlation. The described devices also impress with their practical applicability, at least in research, and should be considered in future research on this topic for the acquisition of the reference for core body temperature. Lees et al. (2018a) also emphasize the greater sensitivity of these devices to changes in core body temperature compared to rectal temperature. This is supported by Burdick et al. (2012) by attributing greater blood flow to the vaginal cavity than to the rectum. However, it is also necessary to point out possible disadvantages of vaginal measurement. Compared to a common hand-held rectal thermometer, the application is more complex. However, this is true for all self-measuring devices. As with a progesterone CIDR used in breeding hygiene, there is a risk that the device may fall out of the vagina. And while the entry of dirt does not play a role in rectal measurement, it must be taken into account in vaginal measurement.

In general, it can be said that due to the selection of the study animals, kept in the fresh-cow group, a good comparability was given. Thus, animals in approximately the same lactation state were compared. In this period, shortly after calving, a high incidence of febrile illnesses was also to be expected. A disadvantage of this was that the cows were only available for data collection for an average of 10 days as they then left in the fresh-cow group. An observation

over a longer period of lactation status, beyond the fresh-cow period, would certainly be useful. More data would then be available, first per cow over the course of her lactation and second across different climatic conditions triggered by various seasons. How this would affect regression and correlation analyses remains to be seen. Perhaps season, and thus environmental temperature levels, also have an impact on the correlation degree. Graphical analysis could benefit from more data at the individual animal level. Further research should focus on evaluating baseline temperatures of individual animals and developing algorithms for animal individual variation compared to herd and environmental variation.

6. CONCLUSIONS

The results indicate that the ear tag-integrated temperature sensors were accurate and repeatable in an *ex vivo* experiment under standardized conditions. However, the readings did not correlate with rectal temperature in the field trial, and a graphical representation of the thermometrically detected transition from normo- to hyperthermia was not possible. Consequently, the ear tags did not provide sufficient information for detecting febrile animals. Nevertheless, some correlation between environmental and measured temperature of the ear tag sensor was observed. In addition, it was shown that continuous and remote temperature measurement of the animal was possible. It is suggested that while the technology does not serve as an adequate alternative for rectal temperature, it has the potential to passively monitor temperature. Future research is needed to differentiate animal-specific variations associated with disease from other influencing factors.

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