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Recording methods of respiratory parameters in cattle

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cattle; respiration; respiration rate; energy exchange; sensors; pressure; lung function; diagnostic techniques

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List of abbreviations

AOI	Area of interest
ATB	Leibniz Institute for Agricultural Engineering and Bioeconomy e.V
Bpm	Breaths per minute
BRD	Bovine respiratory disease
IOS	Impulse oscillometry system
pO_2	Partial pressure of oxygen
pCO_2	Partial pressure of carbon dioxide
RR	Respiration rate
RTQ	Respiratory time quotient
Vmin	Respiratory minute volume
Vt	Tidal volume

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1. Introduction

1.1 Why is it important to record respiratory parameters in cattle?

Respiration rate (RR) is a fundamental parameter to evaluate the health status of cattle since it is a very sensitive indicator for animal discomfort at an early stage. However, it is not specific for the diagnosis of respiratory dysfunctions in animals (Reinhold 2023) as it is an indicator of stress and painful processes in general (Knickel et al. 2000; Stöber 1990) as well as heat exposure (Pinto et al. 2020; Schütz et al. 2014). Since other influencing factors such as pregnancy, body size, and age also lead to deviations in the RR, an altered RR must always be assessed under the given circumstances regarding cow-based factors and ambient conditions (Atkins et al. 2018; Reece and Rowe 2018). The great advantage of the RR is that it can be measured objectively by using numerical values (breaths per minute (bpm)) like for body temperature. Whereas in the case of other specific respiratory symptoms such as dyspnea or cough, many studies often lack a clear demarcation between physiological and pathological symptoms and depend on the observer (Ferraro et al. 2021).

Nevertheless, the RR is highly susceptible for interference und can rapidly change during the day; therefore, continuous recording of respiratory parameters by visual devices would be valuable in order to obtain less biased data (Atkins et al. 2018). However, to measure the RR automatically a standardized reference method is needed. The current gold standard method for measuring the RR is the visual observation of the flank movement of the cow over a short period of time. Currently various methods can be found in literature how to count the RR in cattle (Lowe et al. 2019; Milan et al. 2016; Maia et al. 2014). Therefore, the first paper of the dissertation focused on the investigation of different counting methods of the RR and aimed to determine the most reliable method. In order to diagnose respiratory diseases more accurately, additional lung-specific parameters such as biomarkers in the blood, lung function, and ultrasonic testing are required (Ider and Maden 2022; Puig et al. 2022; Porter et al. 2021). For lung function testing in calves a facemask is currently needed, which limits the natural behavior of animals and can therefore only be applied over a short period of time. Consequently, the second paper of the dissertation focused on the evaluation of a new measurement method of the tidal volume (Vt) in cattle.

In summary, this dissertation compared various recording methods of RR and investigated a possible new measurement method of the Vt in cattle as well. The long-term goal is to measure these parameters automatically and to use them as an early indicator for respiratory diseases, heat stress, and discomfort. The investigations were carried out on calves as well as on cows.

a) Anatomical conditions

Especially in cattle, an early detection of respiratory diseases is advisable because cattle are predisposed to respiratory tract diseases due to their anatomical conditions (Robinson 1982). The bovine lung is divided into 6 lobes: four on the right (cranial, middle, caudal, and accessory) and 2 on the left side (cranial and caudal) (Weekley and Veit 1995). One of the anatomical peculiarities of the bovine lung is its strong segmentation, which means that each segment is supplied by only one bronchus and is clearly demarcated from the other segments by connective tissue. In addition, there are – as in sheep and pigs – no interbronchial connections in cattle (Reinhold 1997). Therefore, obstructive lung diseases lead to an interruption of oxygen supply of this lung segment resulting in atelectasis. This pulmonary system has the evolutionary advantage that an infection of one lung segment can be spatially well delimited. In addition, the number of pulmonary capillaries per alveolar unit is lower in cattle than in other species resulting in a lower gas exchange capacity (Veit and Farrel 1978). Furthermore, since cattle only have 25 % of the lung volume per unit body weight compared to the mammalian mean, they need to ventilate a larger percentage of their lungs already at rest (approximately twice the mammalian mean). This leads to a 3 times higher RR at rest than other mammals. These low respiratory capacities contribute to cattle being less tolerant to heat and more susceptible to respiratory tract infections (Koch and Reinhold 2015; Weekley and Veit 1995).

Moreover, differences between breeds exist; for example, it could be shown that Blue Belgians in particular have smaller lungs compared to their body mass than other cattle species. In addition, in this breed, lower amounts of surfactant-associated proteins could also be detected. These proteins serve to reduce the surface tension of the alveoli allowing an effective gas exchange and preventing alveolar collapse. This explains why Blue Belgian calves suffer more frequently from respiratory diseases than Holstein Friesian calves (Danlois et al. 2003).
Furthermore, calves do not reach lung maturity until they weigh 300 kg (which is when they are approximately 1 year old), which makes them particularly susceptible to lung diseases (Reinhold 1997).

In summary, due to their anatomical peculiarities, cattle are predisposed to atelectasis and are more susceptible to respiratory diseases as they are often unable to compensate for diseased lung areas (Gustin et al. 1987). Therefore, monitoring of respiratory parameters especially in cattle is of great relevance.

b) Respiratory infectious diseases

Respiratory infectious diseases in cattle are summarized under the term bovine respiratory disease (BRD) complex. BRD is a multifactorial disease involving environmental factors, immune status, infectious agents as well as management mistakes as triggers (Fiore et al.

2022; Smith et al. 2020). BRD plays a fundamental role especially in calves, due to the immune system that is not yet fully developed, but can also lead to pneumonia in maternal cows (Burrows et al. 2022). More than 20 different types of viruses cause bronchitis in cattle, which takes a mild course in very good environmental conditions (including a good barn climate and high antibody titer of calves). Unfortunately, the germ load in the barn is often very high, especially in calves (Koch and Reinhold 2015), due to high occupancy, poor ventilation, and the purchase and sale of calves at large collection points, which come from various farms bringing along a varied germ and immune spectrum (Özbek and Ozkan 2020). This often leads to secondary infections of the lungs with bacteria such as Mannheimia haemolytica, Histophilus somni or Pasteurella multocida, which can cause pneumonia due to the weakened immune system (Santos-Rivera et al. 2021; Timsit et al. 2017). In addition, there are many negative long-term effects in calves with clinical or subclinical pneumonia such as lower daily gains, poorer reproductivity, and shorter longevity (Poulsen and McGuirk 2009; Donovan et al. 1998). An early detection of respiratory symptoms can therefore promote animal health and welfare as well as reduce economic losses. Furthermore, new restrictions in the veterinary medicine regulations in Germany (BMEL 2022) also require mandatory reporting of antibiotic use in dairy cows since 2023. Therefore, a precise selection of the animals to be treated is necessary in order to prevent an overuse of antimicrobials (Ferraro et al. 2021).

Consequently, a thorough recording of respiratory parameters in the stable is fundamental in order to start a therapy quickly, efficiently, and selectively. The main signs for the BRD complex in cattle are nasal discharge, cough, dyspnea, increased RR, ocular discharge, and open mouth breathing (Ferraro et al. 2021). As clinical examination on its own is often not sufficient to diagnose BRD (White and Renter 2009) and to determine the degree of lung lesion with certainty, further diagnostic tools as pulmonary function testing, ultrasonography or analysis of blood gas are beneficial (Baruch et al. 2019; Chernitskiy et al. 2019). Currently also sensor systems, which record the calves behavior to automatically detect sick animals (Carslake et al. 2021), as well as technical breath analysis systems detecting potential biomarkers for BRD (Spinhirne et al. 2004) are gaining in relevance.

c) *Heat stress*

The optimal temperature zone for cows ranges approximately between - 0.5 and + 20 degrees Celsius. In this temperature range, cows do not have to spend extra energy to cool or heat the body and can easily maintain their physiological body temperature (Herbut et al. 2019; West 2003). However, the exact comfort temperature zone varies individually, as it depends among other factors on lactation and gestation stage, age, and species (Thornton et al. 2022; Atkins et al. 2018; Gauly et al. 2013). Due to predicted climate change, the exposure of cows to heat load will further increase (Gauly et al. 2013) since cows already experience heat stress –

depending on breed and species – at temperatures above 20 degrees Celsius (Thornton et al. 2022) or 25 degrees Celsius (Gauly et al. 2013). Heat stress occurs, when the animal produces more heat than it can release (St-Pierre et al. 2003). Due to heat stress, the milk yield of cattle drops, fertility decreases, due to a reduction in feed intake, daily gains also decline, and the animal becomes more susceptible to diseases (Thornton et al. 2022; Vitali et al. 2009; Silanikove 2000). Generally, multiparous cows are more affected than primiparous (Bernabucci et al. 2014; Holter et al. 1996). Even calves of cows that were in high gestation during heat period can be diagnosed with negative late effects like a different immune response (Strong et al. 2015), lower birth weight, and poorer daily gains (Dado-Senn et al. 2020a) than other calves.

However, in addition to the risks for animal welfare due to heat stress (Kim et al. 2022; Pilatti et al. 2019); the associated global financial losses due to lower milk yield production under a high greenhouse gas emission scenario by the end of the century are enormous (Thornton et al. 2022). Therefore, it is fundamental to develop a reliable heat stress warning system, which activates shading, ventilation, and cooling measures to reduce heat stress at an early stage (Schütz et al. 2009; West 2003).

At the current stage, the temperature-humidity index (THI) is used as a heat stress threshold for the classification of heat stress, originally developed by Thom (1958). The THI takes the ambient temperature and the relative humidity into account (Schüller et al. 2013). An THI threshold of 70 for standing cows and 65 for lying cows can be used as a suitable threshold for heat stress according to the RR (Pinto et al. 2020). It could be shown that at THI values >70, the RR of standing cows increased rapidly (mean RR at breakpoint: 37 bpm) and in lying cows the RR already increased sharply at THI values > 65 (mean RR at breakpoint: 39 bpm). Depending on the source, THI values between 60 and 75 are also mentioned as threshold values (Bohmanova et al. 2007; Ravagnolo et al. 2000).

However, the THI is not an animal-related parameter as it only captures ambient conditions and it has been shown that heat stress predictions based only on THI quantification have led to both under- and overestimate the effects of heat on cows (Atkins et al. 2018; Zimbelman and Collier 2011). Since lying cows experience heat stress earlier than standing ones due to a physiological higher RR at rest (physiological RR for lying cows: 24-50 bpm (Reece and Rowe 2018)), a consideration of the animal individual body posture for a classification of heat stress according to the RR would be useful. Furthermore, there is a correlation between the milk yield and RR. For each additional kilogram of milk, the RR increased by approximately 0.23 bpm. Therefore, cows with a higher milk yield experience heat stress earlier than those with a lower milk production (Pinto et al. 2019).

In summary, since the experienced intensity of heat stress is influenced by animal-specific factors such as breed, age, stage, and number of lactation and gestation as well as the body posture (Atkins et al. 2018), an animal-individual recording of respiratory parameters would be useful to allow a more precise and animal-oriented reaction to heat stress.

1.2 Which respiratory parameters are recorded in cattle and what methods are used?

a) *External examination of the respiration*

Since cattle are obligate nose breathers (Reinhold 2023), mouth breathing is always considered pathological and occurs in association with heat stress, stress, or pain (Schütz et al. 2014). The visual assessment of the bovine RR is optimally performed from obliquely posterior to the right side of the cow, because the rumen is located on the left side, which can cause further flank movements (Baumgartner et al. 2018). The RR is expressed in breaths per minute (bpm) and ranges for a standing dairy cow at rest between 26-35 bpm and for a lying cow between 24-50 bpm. A higher RR in a lying position is due to the fact that the rumen presses against the diaphragm, which leads to an increase of the RR (Reece and Rowe 2018). In addition to the frequency, the quality of breathing, the rhythm, and the type of breathing can also be determined (Baumgartner et al. 2018). Cattle breathe predominantly abdominally with inhalation occurring actively through contraction of the intercostal muscles and lasting about half as long as exhalation through passive contraction of the lungs (Stöber 1990). The ratio between the duration of in- and expiration is recorded as the respiratory time quotient (RTQ) and provides an indication of the type of ventilation disorder (Reinhold 2023). For example, in cases of deeper airway obstruction, the RTQ is increased due to a prolonged inspiration (McKiernan et al. 1993). The current gold standard methods of RR recording are the manual counting of the flank movement as well as the measurement of RR by spirometry (explained in detail under 1.2 b) pulmonary function testing).

Another new approach to record the RR in cattle is an RR sensor developed at the Leibniz Institute for Agricultural Engineering and Bioeconomy e.V. (ATB) in cooperation with the start-up company GOUNA (Strutzke et al. 2019) measuring the differential pressure in one nostril during in- and exhaling. A respiration curve can be derived from the pressure differences with positive pressure values corresponding to exhalation and negative pressures to inhalation.
The most recent approaches aim at avoiding animal contact completely in order to affect the normal behavior as little as possible. This includes, for example, infrared thermography (IRT), which is based on the idea of measuring the temperature change of the inhaled and exhaled airflow through the nostrils (Kim and Hidaka 2021; Lowe et al. 2019). Another new approach is a laser distance sensor recording the body movement of the regio abdominis by measuring the distance between laser and cow at certain points (Pastell et al. 2007).

b) Pulmonary function testing

Pneumotachography and ultrasound spirometry have established as gold standard methods in pulmonary function testing for measuring respiratory tract ventilation parameters (Reinhold 2023). These recording methods were originally developed for human medicine and measure the gas flow rate per unit time of the expiratory air. For the application in cattle, a tightly fitting facemask is necessary (Dißmann et al. 2023). These masks were originally used in pediatrics and therefore fit only calves until approximately 120 kg. Under spontaneous breathing, ventilatory parameters such as RR, Vt, respiratory flow rate, and RTQ can be recorded in animals (Reinhold and Fodisch 1993). In the following, the recording of Vt is described in more detail since this dissertation focused on the recording of Vt besides the RR.

The Vt is given in mL or L and is defined as the volume moved per breath during resting breathing. The Vt can be a useful parameter for diagnosing obstructive and restrictive ventilation disorders (Vt decreased). Like RR, Vt is a very sensitive parameter, but it is not specific to the respiratory tract (Reinhold 2023), therefore it must always be interpreted in consideration of other parameters as well. For example, the Vt can be helpful in diagnosing and assessing the degree of latent respiratory failure in calves. Calves with subclinical lung disease show a higher respiratory minute volume (Vmin) under load just like healthy calves, but this increase is only due to a higher RR, because subclinical lung disease calves are not able to increase their Vt under load in contrast to healthy calves (Chernitskiy et al. 2019).

Furthermore, since the Vt depends on body weight, it should always be expressed in mL/kg for enabling comparability between animals. The physiological Vt in calves is approximately 8 - 10 mL/kg (Koch and Reinhold 2015). To measure the Vt, an impulse oscillometry system (IOS) can be used, which consists of a pulse generator, a pleated hose, a pneumotachograph, a pressure sensor, and a Y-piece; the structure and operation have already been described in detail by Reinhold et al. (1998a) and Smith et al. (2005). Measurements with the IOS are particularly suitable for therapy control or for clinical studies in order to record the influence of exogenous agents on the individual animal in follow-up examinations (Reinhold et al. 1998b). For example, Ostermann et al. (2014) studied the pathophysiology of respiratory Chlamydia infection in bovine lungs using the IOS, volumetric capnography, and a re-breathing system. In Publication “B”, the IOS was used as the gold standard method to validly record the Vt.

c) *Further respiratory diagnostic methods*

Besides the external examination of the respiration and pulmonary function testing, there are also other established methods as well as new promising attempts to diagnose respiratory diseases in cattle more precisely:

- **Respiratory gas analysis** includes the capnography as well as the detection of CO, N_2O, and NO by multipass absorption spectroscopy methods. The analysis of the amount of respired gases cannot only be useful for anesthesia monitoring (Reinhold 2023), but elevated levels of CO and NO can also be detected in asthma and an altered ratio of $N_2O:CO_2$ as well as the amount of exhaled NO may be helpful in the diagnosis of BRD (Burciaga-Robles et al. 2009).

- In the **analysis of blood gases and acid-base status,** the measurements of partial pressure values such as oxygen (pO_2), carbon dioxide (pCO_2) as well as the pH value in the arterial blood and the alveolar-arterial pO_2 are mainly indicated for the quantification of respiratory insufficiencies (Reinhold and Födisch 1993). In addition, these parameters can also be used, for example, to evaluate barn ventilation systems (Sabuncuoglu et al. 2008). In order to detect latent respiratory insufficiencies, the analysis of blood gases under exercise is widely used, especially in horses, but also possible in calves (Chernitskiy et al. 2019; Höchel 2004).
- In addition**,** the **analysis of biomarkers** in blood serum is becoming increasingly popular in veterinary medicine for the detection of respiratory diseases. For example, tumor necrosis factor-a, transforming growth factor-ß1, and interleukin-17A could be suitable biomarkers in determining a systemic inflammatory response in infectious pneumonia in calves (Ider and Maden 2022).
- The **ultrasound examination** of the thoracic lung area can be a useful tool to the clinical examination to better assess the severity of an infection of calves with suspected BRD (Fiore et al. 2022; Baruch et al. 2019; Reinhold et al. 2002). Since ultrasonography findings correlate more strongly than clinical findings with pathologic findings, they have a higher sensitivity and specificity for detecting pulmonary disease in cattle. Therefore, they might be more precise in assessing the severity of infection. This is why calves that are evaluated based on clinical scoring alone may be misclassified (Porter et al. 2021).
- The **bronchoalveolar lavage fluid** can be used to identify pathogens and to determine the severity of inflammation (Ider and Maden 2022; Reinhold et al. 2005).
- **Bronchial brushing** is practiced to collect both microbes and cells from the lower respiratory tract. In cattle, it is used to investigate the microbial of the lung (Prohl et al. 2014).
- **Electric Impedance Tomography** (EIT) is a new approach to identify calves with insufficient lung expansion (Bleul et al. 2021). Therefore, a belt with electrodes is fixed at the calves 6th intercostal space measuring the surface potential after applying current to the electrode pairs. Since the electrical impedance is negatively correlated with increasing intrapulmonary gas volume, the EIT shows dynamic changes in lung ventilation by generating frames of the ventilated lung area (Bleul et al. 2021).

1.3 Objectives of this study

The overall objective of this study was to evaluate common methods for measuring the RR regarding their reliability and to evaluate a new measurement method of the Vt in cattle.

Therefore, the study was divided into two experimental designs that resulted in 2 publications. Paper "A" was focused on the evaluation of different recording methods of the RR and Paper "B" on a new recording method of the Vt.

a) *Publication "A"*

   The common method for recording the RR is to observe the flank movements of cattle for a certain period of time. There are several new approaches to automatically record the RR. However, as gold standard method for the training of an automatic recording system of the RR, the manual observation of the flank is still often the method of choice. Thereby, various recording methods lack a uniform counting system as basis. The counting of the RR for a period of 60 s (Milan et al. 2016), 30 s (Baumgartner et al. 2018) or 15 s (Maia et al. 2014) as well as the counting of 5 (Lowe et al. 2019) or 10 breaths (Stewart et al. 2017) are commonly used in practice. The objective of Paper "A" was to compare these 5 methods regarding their practicability, reliability, and validity, because a uniform recording of the RR is indispensable for a comparability of studies. The hypothesis was that a shorter period of observation increases inaccuracy.

The results of this study were published in Veterinary Research Communications (impact factor: 2.8).

b) *Publication "B"*

   The common method for recoding the Vt in cattle is a pneumotachograph integrated into an IOS (Smith et al. 2005). However, this measurement method requires a breathing mask that must be fixed to the head of the calf, for which a fixation of the calf is necessary. This influences the behavior of the animal and, in addition, the use of the IOS is limited to calves up to approximately 120 kg due to the limited opening diameter of the breathing mask. In addition, it is only possible to record data over a limited period of time. Therefore, the objective of Paper "B" was to find a more efficient method to measure the Vt in calves over a longer period of time without restricting the natural behavior. Currently, the RR sensor (Strutzke et al. 2019) only derives the RR from the pressure difference data. The hypothesis was that a Vt equivalent can be derived from the pressure of the RR sensor as well.

The results of this study were published in Sensors (impact factor: 3.9).

2. Research Publications in Peer-Reviewed Journals

2.1 Publication “A”

How should the respiration rate be counted in cattle?

Lena Dißmann, Julia Heinicke, Katharina Charlotte Jensen, Thomas Amon,
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RESEARCH

How should the respiration rate be counted in cattle?

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Abstract
Respiration rate (RR) is a proficient indicator to measure the health status of cattle. The common method of measurement is to count the number of respiratory cycles each minute based on flank movements. However, there is no consistent method of execution. In previous studies, various methods have been described, including counting flank movements for 15 s, 30 s or 60 s as well as stopping the time for 5 or 10 breaths. We assume that the accuracy of the aforementioned methods differs. Therefore, we compared their precision with an RR sensor, which was used as the reference method in this study. Five scientists from the fields of agricultural science and veterinary medicine quantified the flank movement according to each of the five methods mentioned above. The results showed that with an average RR of 30 breaths per minute (bpm), all methods showed a high correlation to the values of the RR sensor. However, counting breaths for 60 s had the highest level of conformity with the RR sensor (Lin`s concordance correlation coefficient: 0.96) regardless of the level of RR. With rising RR, the inaccuracy increased significantly for the other four investigated methods, especially when counting 5 and 10 breaths. Therefore, we would recommend that counting for 60 s should be used as the standard method for future studies due to its high precision regardless of the level of RR.

Keywords Respiration rate · Method · Respiration rate sensor · Flank movement

Introduction

Respiration rate (RR) is an important parameter to evaluate the health status of cattle since it is an indicator of stress and painful processes (Knickel et al. 2000; Rosenberger 1990) as well as heat exposure (Pinto et al. 2019; Schütz et al. 2014). There are several approaches to automatically record the RR. Some of the most innovative methods are infrared thermography (IRT) techniques based on measuring the temperature change of the inhaled and exhaled airflow through the nostrils (Jorquera-Chavez et al. 2019; Kim and Hidaka 2021; Lowe et al. 2019). Additionally, there are sensor systems such as a differential pressure sensor fixed on the nose (Strutzke et al. 2019), a respiration monitoring system strapped to the cow`s abdomen as a belt (Eigenberg et al. 2000), a laser distance sensor recording the body movement of the regio abdominis (Pastell et al. 2007) and a sensor measuring air temperature near the nostrils (Milan et al. 2016).

Nevertheless, observing flank movements is still the most common technique in practice to measure the RR and is often used as a reference method for validation of the sensor systems mentioned above. However, there is no clear gold standard in cattle, and we found various methods described in scientific papers. One method is to measure the time until 5 breaths are completed, applied by Kim and Hidaka (2021) and Lowe et al. (2019) as a reference method to analyze the breathing pattern with IRT. Lowe et al. (2019) argues that this method reduces the probability of calf movements during counting.

Another frequent technique is to measure the time until 10 breaths are fulfilled. This method was employed by Stewart et al. (2017) as a reference value for their infrared-based measurements, Schütz et al. (2014) in their study on the influence of different amounts of shade on the RR of cattle

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in relation to heat load and Li et al. (2020) with regard to the correlation between respiration and rectal temperature as well as the prediction of the RR.

In other studies, the RR measurement was accomplished through a time limit instead of a breath limit. Maia et al. (2014) counted flank movements for 15 s after putting a face mask on cattle to measure the physiological response. The textbook Clinical Propaedeutics of Domestic Animals (Baumgartner et al. 2018) recommends counting at least 30 s of flank movement, also applied by Pinto et al. (2019) in their examination of the influence of climate and circumstantial factors on RR in cows. Jorquera-Chavez et al. (2019), Milan et al. (2016) and Strutzke et al. (2019) counted the RR for 60 s as a reference method for validating their sensor systems.

In the present study, we tested and compared the various methods described (counting 5 and 10 breaths, 15 s, 30 s and 60 s) regarding their agreement with respect to the measured RR of an RR sensor, recently developed in our working group (Strutzke et al. 2019) and used as the reference method in this study. This sensor automatically calculates the RR in the nose during inspiration and expiration. We hypothesize that shorter periods of observation increase inaccuracy. Our aim is to define the most meticulous method for counting RR that should be consistently used in further studies.

Materials and methods

In total, 46 episodes were recorded (Samsung Galaxy Note 10.1, Seoul, South Korea) over one minute each in an experimental barn in Groß Kreutz (Germany) over 2 days in January 2018. In the experiment, six healthy cows differing in age, lactation stage (1st-5th lactation, days in milk: 47–196) and gestation stage (4–80 days) were filmed consistently at a 45-degree angle from behind. The dairy cows were housed in a free-stall barn, equipped with 53 lying cubicles (straw-lime mixture) and were part of an existing herd of 55 cows on the first day and 54 cows on the second day. The animals were able to move freely in the barn during the experiment so as not to restrict their natural behavior. Water and a total mixed ration were freely available. Seven to nine video sequences were taken of each cow during the lying and standing periods (60% lying, 40% standing), with and without ruminating and while dozing (eyes half-closed, without rumination). In addition, each cow was equipped with a respiration rate (RR) sensor attached to a halter (Strutzke et al. 2019). The experimental study using animals was approved by the State Office for Occupational Safety, Consumer Protection and Health (LAVG Brandenburg, Germany) under the study number 2340–1-2018.

Afterward, three veterinarians and two agricultural scientists counted the RR on the basis of the video sequences according to the five methods (5 breaths, 10 breaths, 15 s, 30 s and 60 s) in random order using a smartphone stopwatch app. By means of an LED lamp that lights up briefly at the beginning and end of the recorded minute, the period to be studied was dependably defined. The LED lamps were fixed on the halter of the cows and were synchronized with a marker in the RR sensor recordings (for further details see Strutzke et al. 2019). Only whole breaths were counted, beginning with the inspiration after the first light of each video minute. When counting by breaths, the time was recorded in seconds with two decimals. Values were then extrapolated to breaths per minute (bpm) and compared to the value of the RR sensor.

We compared the five methods regarding their validity (agreement of the measurement results with the RR sensor) and reliability (differences between observers). The number of cases “n” was obtained from the 46 studied videos and multiplied by five (number of observers). First, we determined the mean absolute deviation of the studied methods compared to the RR of the sensor and investigated whether there were differences depending on the level of RR. For this, we used JMP (Version 16.0, SAS Institute Inc., Cary, NC, USA). To assess the agreement between the five different methods and the RR sensor, Lin's concordance correlation coefficient (CCC) (Akoglu 2018) was calculated. To detect differences regarding the reliability, we additionally calculated the CCC for each observer. For the CCC, we used R via the R Studio Interface (Version 4.0.3.; © 2020, The R Foundation for Statistical Computing) and the package “DescTools” (Signorell et al. 2021). Lin’s CCC is particularly suitable to measure the agreement of two methods or raters, as it is based on precision (degree of variation) and accuracy (degree of location or scale shift) (Barnhart et al. 2002).

Results and discussion

The results substantiate that counting respiration rate (RR) for 60 s has the lowest mean absolute deviation and therefore the highest level of agreement with the values measured by the RR sensor regardless of the level of RR. Counting for 60 s differed from the RR sensor by an average of 1.8 breaths with a standard deviation of 2.02. The second highest level of agreement can be attributed to counting breaths for 30 s, followed by stopping for 10 breaths (Fig. 1). Up to an RR of 25 bpm, counting 5 breaths was more accurate than counting breaths for 15 s. At a higher RR, counting breaths for 15 s became more accurate than counting 5 breaths. Overall, the deviation from the RR sensor was on average smaller at a low RR and increased with rising RR for all methods,

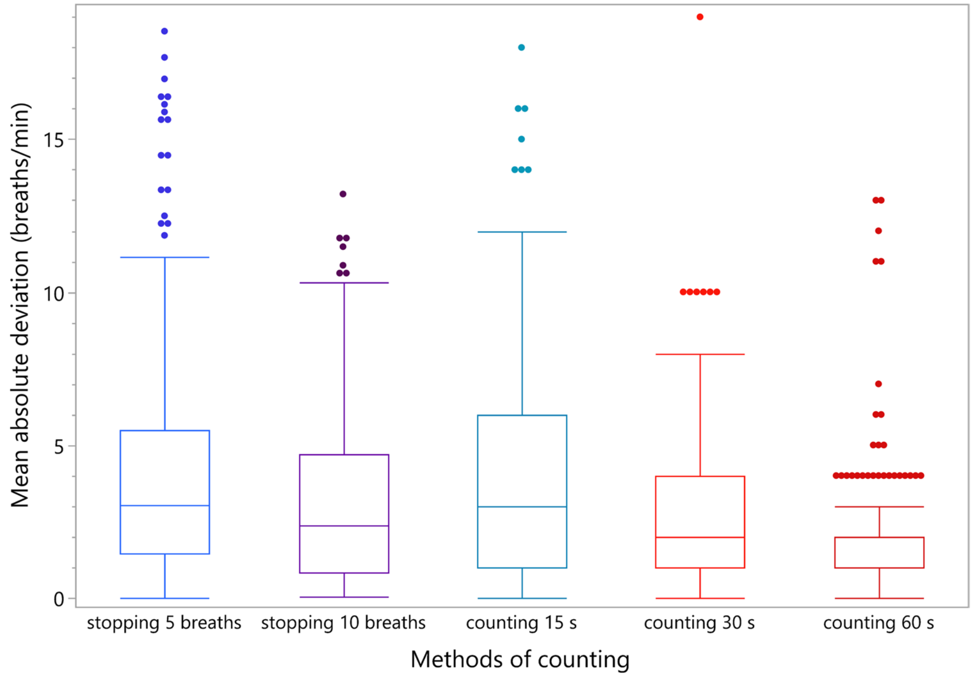

Fig. 1 Boxplot analysis of the mean absolute deviation of the respiration rate (RR) from the RR sensor by the five investigated methods of counting (n = 230)

except for counting for 60 s. At 60 s, the deviation remained approximately constant regardless of the RR (Fig. 2).

In our experiment, the average RR was 30 bpm; thus, counting 5 breaths corresponded to an average observation time of 10 s, and counting 10 breaths corresponded to an average observation time of 20 s.

Moreover, it was noteworthy that for all methods, the RR was on average underestimated at a low RR and overestimated at a high RR. However, the position of the cow (lying or standing) had no influence on the detectability of the RR, and the mean absolute deviation from the RR sensor was approximately the same for both positions.

Regarding the CCC, all methods achieved a CCC > 0.8 (Table 1). However, there were differences concerning the single methods: the level of agreement of the investigated methods proved to be in the same order as with regard to the mean absolute deviation. There are different approaches for the interpretation of Lin´s CCC (Akoglu 2018): According to McBride (2005), only the counting of breaths for 60 s achieved a substantial agreement (0.95–0.99).

Concerning the reliability, the five observers differed only slightly when comparing the CCC of the different methods (Table 1). All five observers reached a substantial agreement

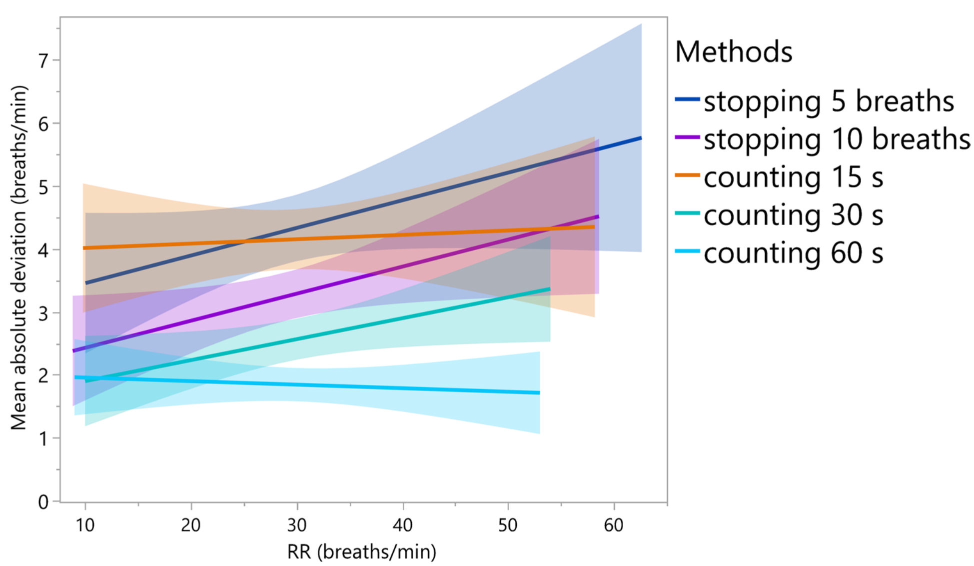

Fig. 2 Mean absolute deviation of the five investigated methods for the respiration rate (RR) sensor against the level of RR (breaths/min) from 46 recordings counted by five observers (n = 230)

Table 1 Agreement of different counting methods for the respiration rate (RR) with the measurement of an RR sensor using Lin´s concordance correlation coefficient (CCC) for all observers (n = 230) and for the best and worst of five observers (n = 46)

Method	All observers CCC (LCI-UCI)	Best observer CCC (LCI-UCI)	Worst observer CCC (LCI-UCI)
Counting RR for 15 s	0.84 (0.80–0.88)	0.87 (0.79–0.93)	0.79 (0.66–0.87)
Counting RR for 30 s	0.93 (0.91–0.95)	0.95 (0.91–0.97)	0.90 (0.83–0.94)
Counting RR for 60 s	0.96 (0.95–0.97)	0.97 (0.94–0.98)	0.95 (0.92–0.97)
Stopping time for 5 breaths	0.82 (0.77–0.86)	0.88 (0.80–0.93)	0.78 (0.64–0.87)
Stopping time for 10 breaths	0.90 (0.87–0.92)	0.93 (0.87–0.96)	0.86 (0.77–0.92)

LCI: lower confidence interval, UCI: upper confidence interval

with the RR when counting breaths for 60 s. Therefore, we conclude that the reliability of this method is sufficient.

Overall, the hypothesis that longer observation times result in a more accurate RR measurement was confirmed. Therefore, we would generally recommend using counting for 60 s as the standard method in future studies because it is the most accurate method regardless of the level of RR. An exception are very restless animals, where a longer observation period would distort the results due to cow movements and make counting flank movement more difficult, for example, in calves (Lowe et al. 2019).

Although the average RR in cattle is between 24 and 36 bpm (Rosenberger 1990), RRs of 78 bpm are not unusual in summer (Ruban et al. 2020). At these high RRs, we consider counting 5 or 10 breaths to be too inaccurate due to their short observation time. The accuracy of counting for 15 s and 30 s deteriorated less in our experiment at higher RRs than counting by breaths (Fig. 2). Consequently, for a basic acquisition of RR in daily work in practice, counting breaths for 30 s can be a good alternative to counting for 60 s considering validity, reliability and feasibility (less work).

Nevertheless, when counting 5 and 10 breaths, it is necessary to consider the reaction time in counting the last breath and stopping the stopwatch of the person evaluating the video. Even with rigorously trained researchers, human physical limitations will inevitably influence the study results. Furthermore, when extrapolating up to one minute, the number of breaths must be rounded down as well as up to obtain whole breaths.

In fact, when counting by time, different initial conditions must be considered; when counting for 15 s and then rounding up to one minute by multiplying by 4, logically from the outset, only every fourth value can be obtained as an end result, and when counting for 30 s, only every second value can be obtained. Therefore, we conclude that the counting of breaths for 60 s is the most valid method.

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Author contributions All authors contributed to the study conception and design. Material preparation, data collection and analysis were performed by L. Dißmann, G. Hoffmann, K. C. Jensen and J. Heinicke. The first draft of the manuscript was written by L. Dißmann and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

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Data availability The data that support the findings of this study are not openly available due to reasons of sensitivity but are available from the corresponding author upon reasonable request.

Declarations

Competing interests The authors have no relevant financial or non-financial interests to disclose.

Ethics approval The experimental study using animals was approved by the State Office for Occupational Safety, Consumer Protection and Health (LAVG Brandenburg, Germany) under the study number 2340–1-2018.

Consent to participate Informed consent was obtained from all individual participants included in the study.

Consent for publication Not applicable.

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2.2 Publication "B"

Evaluation of a Respiration Rate Sensor for Recording Tidal Volume in Calves under Field Conditions

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Article

Evaluation of a Respiration Rate Sensor for Recording Tidal Volume in Calves under Field Conditions

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Abstract: In the assessment of pulmonary function in health and disease, both respiration rate (RR) and tidal volume (Vt) are fundamental parameters of spontaneous breathing. The aim of this study was to evaluate whether an RR sensor, which was previously developed for cattle, is suitable for additional measurements of Vt in calves. This new method would offer the opportunity to measure Vt continuously in freely moving animals. To measure Vt noninvasively, the application of a Lilly-type pneumotachograph implanted in the impulse oscillometry system (IOS) was used as the gold standard method. For this purpose, we applied both measuring devices in different orders successively, for 2 days on 10 healthy calves. However, the Vt equivalent (RR sensor) could not be converted into a true volume in mL or L. For a reliable recording of the Vt equivalent, a technical revision of the RR sensor excluding artifacts is required. In conclusion, converting the pressure signal of the RR sensor into a flow equivalent, and subsequently into a volume equivalent, by a comprehensive analysis, provides the basis for further improvement of the measuring system.

Keywords: tidal volume; pressure; respiration rate sensor; spirometry; impulse oscillometry system

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1. Introduction

Cattle are predisposed to diseases of the respiratory tract due to the morphological and anatomical peculiarities of their lungs [1,2]. This is mainly due to the strong segmentation of the lungs compared to other animal species, the lack of collateral ventilation pathways, and the reduced gas exchange capacity of the lungs, as there are fewer pulmonary capillaries per alveoli unit. In consequence, a larger part of the lungs must be ventilated at rest, which leads to less available ventilatory reserves. Thus, a failure of individual lung areas will result in shortness of breath more rapidly than in other animal species [3].

Furthermore, in relation to their body mass, cattle possess a lower total lung volume and a smaller alveolar surface area compared to other animal species. These peculiarities in lung anatomy have significant consequences for pulmonary functions in terms of spontaneous ventilation. For example, a cow of 500 kg has a tidal volume (Vt) of approximately 3800 mL (which corresponds to a Vt of approximately 8 mL/kg body weight), while a horse of a similar weight (550 kg) has a Vt of approximately 6000 mL or 11 mL/kg body weight [4]. Vt is the volume of air inspired and expired during one respiratory cycle, and its measurement allows conclusions about the functionality of the lungs [5]. To compensate for physiologically based lower Vt, cattle must have a higher respiration rate (RR) at rest than other animal species [1,6].

Since bovines do not reach postnatal lung maturity before they have gained a body weight of approximately 300 kg, young animals are particularly susceptible to diseases of the respiratory tract [2]. Therefore, a lot of research has been published on the study of pulmonary diseases in calves and to diagnose them early [7–10]. Besides the analysis of biomarkers in the blood [11,12], also ultrasonic methods [13–15] and different sensors [16,17] have been tested as diagnostic methods.

To detect pulmonary disorders in calves at an early stage, it is fundamental to monitor the spontaneous breathing pattern. In addition to recording RR (i.e., breaths per minute (bpm)), Vt is a vital parameter in pulmonary function diagnostics. That Vt is a parameter worth measuring in calves has been proven in studies to quantify respiratory symptoms [18,19] or to assess the effects of therapies [20]. It is a well-known phenomenon that calves suffering from pneumonia show significantly lower Vt and higher RR than healthy calves [21,22]. Since Vt is inevitably associated with RR, a higher RR usually compensates for a lower Vt to avoid hypoventilation and to maintain the supply of oxygen to the organism [5].

Several noninvasive methods are available for measuring RR and Vt in animals. RR could simply be counted by observation; however, this requires personnel, time, and thorough quietness in the surrounding to avoid stress for the animals. In 2018, an RR sensor for cattle was developed at the Leibniz Institute for Agricultural Engineering and Bioeconomy that can derive the RR from the pressure difference between inhaled and exhaled breath in one nostril [23]. Most advantageous, this device is suitable for use in freely moving animals under field conditions. Moreover, a complete new non-contact approach to measure RR is infrared thermography using the temperature difference between inhaled and exhaled air to automatically record the RR in calves and adult cattle [24,25]. However, there is not yet a market-ready, universally applicable solution for this method.

Spirometry is another well-known method to assess both RR and Vt. Spirograms can easily be registered in spontaneously breathing animals wearing a tightly fitting facemask (fitting to the size of the animal's head, thus ensuring low dead space). Since a facemask is mandatory to connect the spirometer in front of the animal's head, this method cannot be used in freely moving animals. One of the best-validated methods for pulmonary function testing in calves is the impulse oscillometry system (IOS) [26,27]. The system includes spirometric measurements as well. Since the IOS was originally developed for human medicine, it has been applicable to calves with body weights comparable to those of adult humans. Under experimental conditions, IOS has been proven to measure RR and Vt in calves with high accuracy [21,22] (and can therefore be regarded as the gold standard method. The aim of this study was to evaluate whether the previously developed RR sensor could be expanded to measure Vt as well. This new measurement method would offer the possibility to assess the two main variables of the spontaneous breathing pattern continuously in the field without the necessity of connecting a facemask or fixation of the animals to be monitored. According to our conceptual goal, the high frequency pressure values (50 Hz) measured by the RR sensor should be converted into airflow and volume equivalents to ultimately compare them to the real flow and volume measured by the IOS. The physiological Vt for calves is approximately 8–10 mL/kg [6,11]. This proof-of-principle study focused on calves since IOS measurements are limited to bovines with body weights below 120 kg.

The paper is structured as follows: Sections 2 and 3 describes the methodology as well as the statistical analysis of the sensor data. Section 4 presents the results. Afterward, the results are discussed in Section 5. Finally, the conclusion is reported in Section 6.

2. Animals, Materials and Methods

2.1. Study Design

The animal testing took place at a research farm (Groß Kreutz, Germany) on two consecutive days in April 2022. The testing was approved by the State Office for Occupational

Safety, Consumer Protection and Health (LAVG Brandenburg, Potsdam, Germany) under the study number: V6-2340-12-2022 (day of permission: 25 February 2022).

Selection criteria for the calf's inclusion in the testing were clinical health and a maximum weight of 80 kg, since the mask does not fit larger animals. Clinical health was ensured by a veterinarian before the habituation process and repeated before animal testing. The clinical examination included the measurement of rectal temperature, examination of the lymph node and mucosal status, capillary refill time, lung and heart auscultation (including determination of RR and heart rate), and assessment of general condition. Ten clinically healthy calves (aged between 4 and 65 days on the first day of the experiment) were included. Using a sample size estimation tool [28], a case number of 10 calves was necessary to prove a correlation of at least 0.8 [29] of the respiratory parameters between the sensors with an α level of 0.05 and a power of 80%. These α and β levels are commonly used in other clinical studies as well [30]. Body weight ranged from 52 to 79 kg. Three weeks before the testing, the habituation process began (with the exception of the 2 calves that were 4 days old at the start of the testing) while each calf was equipped with the facemask once per week for 5 min. Measurements of spontaneous breathing were performed using two devices on each calf in a randomized order. On Day I, measurements with the RR sensor preceded IOS-measurements and vice versa on Day II. All measurements were performed in standing animals with a standardized, slightly stretched head position. Ambient conditions on the two consecutive days were comparable (Day I: ambient temperature 10 °C, relative humidity 51%; Day II: ambient temperature 11 °C, relative humidity 55%).

2.2. Measurements with the RR Sensor

First, the calf to be measured was equipped with a foal halter to which the power bank and the RR sensor were attached and connected over a USB cable (Figure 1). The dimensions of the RR sensor with the microcontroller (Gouna, Brandenburg, Germany) were 46 mm × 15 mm × 25 mm (length × width × height). The RR monitor included a sensor, microcontroller, and silicon tube, with a total weight of 45 g, plus a power bank (capacity 2600 mAh) weighing 60 g (Varter Consumer Batteries GmbH, Ellwangen, Germany). The power bank allows continuous recording of the pressure for about 6 h. However, the RR sensor is not waterproofed, so it must be removed after a short measurement period [23]. A flexible silicone tube with an inner diameter of 2 mm lead from the sensor to the nostrils where it was fixed with a nose ring and intruded 1 cm into the nasal cavity of the right nostril to transmit the recorded pressure to the RR sensor (Figure 1). The pressure was recorded continuously during inspirations and expirations over a period of 5 min per calf and the data were stored on a secure digital memory card. Since it was the first measurement with the RR sensor in calves, there were no prior comparable studies, which could have been used as a guide regarding the length of the measurement. The aim was to measure as close in time as possible with both measuring devices, but still collect valid measurement data. Experiences from individual test measurements before the actual testing have shown that 5 min is sufficient to collect valid data from the calves. Afterward, the device was cleaned with particular attention to ensure that the tube of the RR sensor was free of contamination from the inside before each new test to avoid possible measurement distortions due to liquids in the tube (e.g., from sneezing).

2.3. Measurements with the IOS

The application of the IOS to the head of a calf is shown in Figure 2. The technical design of this device and the careful evaluation of pulmonary function testing in this animal species have been described elsewhere [26,27]. For IOS data, a heated pneumotachograph (Lilly-type, mesh resistance: 36 Pa L^{-1} s) connected to a differential pressure transducer (SensorTechnics SLP 004D, Puchheim, Germany) ensured continuous measurement of airflow. At flow rates < 15 L s^{-1}, the pneumotachograph is linear within 2%. Volume was gained from the flow signal by calculating an integral from airflow during each inspiration or expiration. The results are given as volume curves over time (Figure 3).

Three consecutive measurements were registered per calf per day (each included at least 10 regular breathing cycles free of artifacts) lasting on average 24 s per measurement. The number of measurements was based on previous performed studies with the IOS in calves [21]. Read-out variables were: RR (IOS), Vt (IOS), and the minute volume of respiration (Vmin (IOS)) calculated with the formula:

$$Vmin(IOS) = RR(IOS) \times Vt(IOS)$$

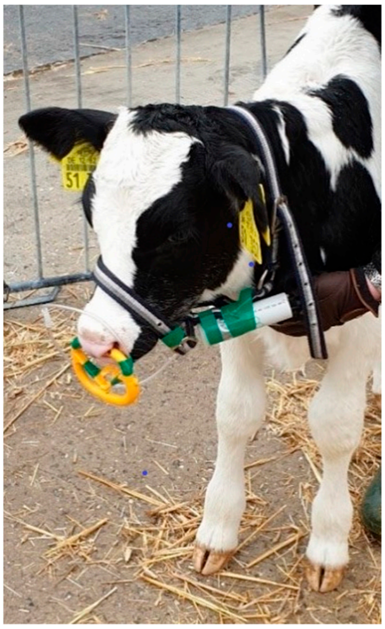

Figure 1. Respiration rate sensor fixed on the calf's nostrils with a nose ring.

Figure 2. Impulse oscillometry system adapted to a tightly fitting rigid facemask.

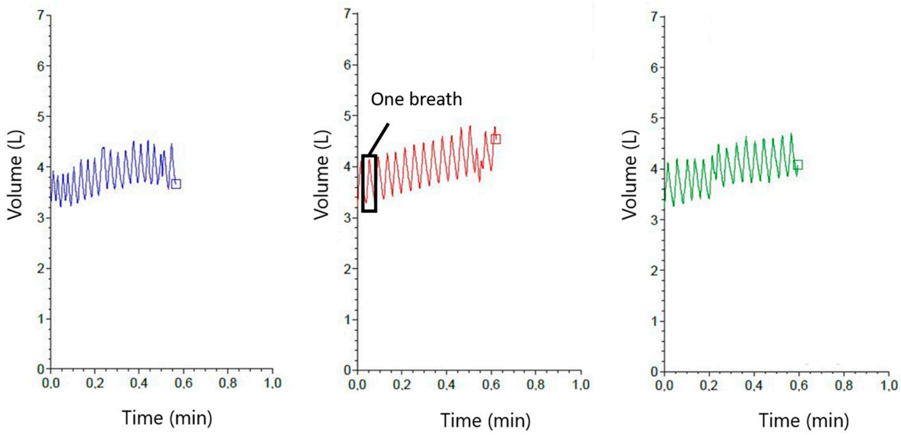

Figure 3. Impulse oscillometry system (IOS): Three consecutive measurements (each measurement is represented by a different color) of the tidal volume (difference between maximum and minimum of the Volume in L) and the respiration rate (in breaths per minute) of one calf registered by the IOS.

2.4. Data Evaluation (RR Sensor)

First, we selected sequences of the recorded 5 min periods where the calves had breathed regularly and evenly for at least 5 breaths in succession (constant frequency and constant amplitude). Since one dataset of the RR sensor from one calf was not readable, 19 datasets from both devices were included. At least 2 to a maximum of 5 sequences of the RR sensor per calf and day were selected for the evaluation, resulting in a total of 59 selected periods included in the evaluation.

Subsequently the pressure data were adjusted according to an exponential smoothing formula [31]. Afterward, the inhalation cycle was adjusted to body temperature and pressure saturated (BTPS) conditions converting inhaled pressure samples measured under ambient conditions to the condition in the lung. This kind of conversion is required to exclude measurement distortions due to ambient temperature and humidity [32]. Consequently, the obtained pressure represented the flow equivalent, with exhalation generating positive pressure and inhalation generating negative pressure (Figure 4).

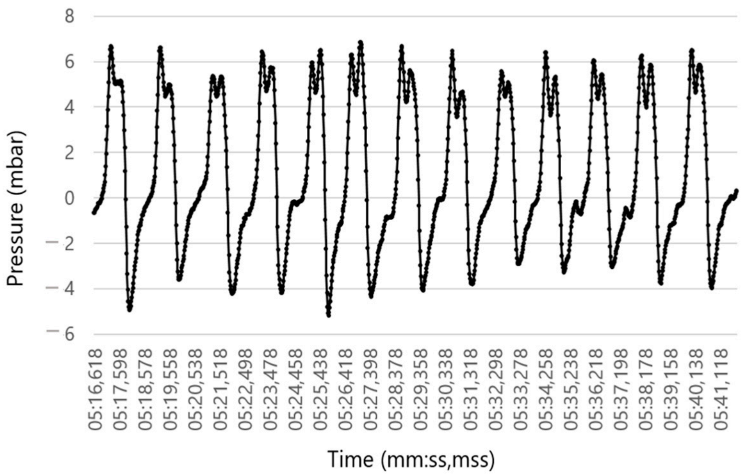

Figure 4. Respiration rate (RR) sensor: Pressure registration of the RR sensor corresponding to the flow equivalent after smoothing and adjusting the inhalation cycle to body temperature and pressure saturated (BTPS) conditions. Exhalation is associated with a positive pressure and inhalation with a negative pressure.

Second, we integrated the pressure values to obtain the volume equivalent [33] (Figure 5). In addition, a baseline correction was performed to compensate for the shift of the zero point (Smith, personal communication) which is caused by an insufficient BTPS-approximation.

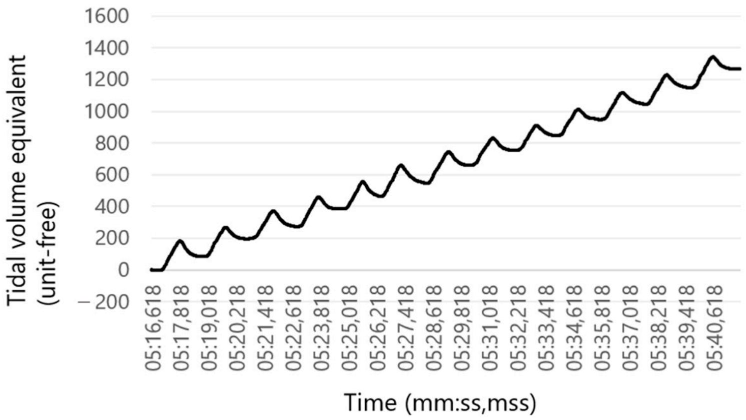

Figure 5. Respiration rate sensor: Integrated pressure curve corresponding to the tidal volume equivalent.

Afterward, the difference between the maximum and minimum pressure of each in- and exhalation was calculated representing the Vt equivalent (Figure 6). Subsequently, since the data were not normally distributed, the median of all Vt equivalents for each of the 59 selected periods was calculated for the RR sensor. Moreover, the RR for each data period as well as the respiratory minute volume (Vmin) equivalent was calculated by multiplying the Vt equivalent by the RR.

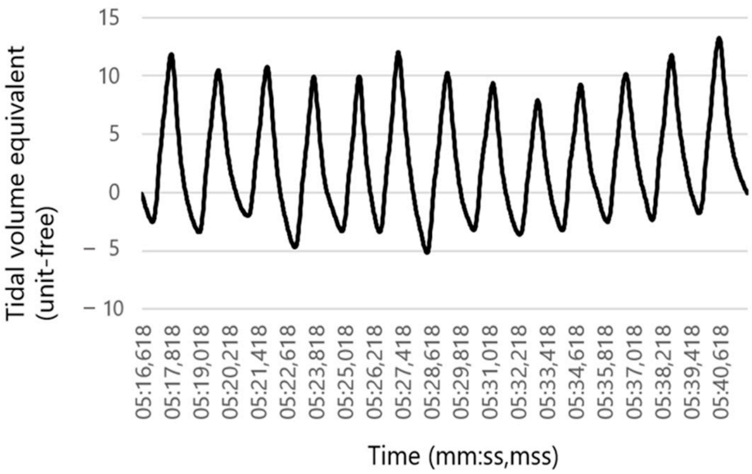

Figure 6. Respiration rate (RR) sensor: Tidal volume equivalent of the RR sensor after baseline correction.

3. Statistical Analysis

The dataset consisted of two different sensor systems, on the one hand the IOS as the gold standard and on the other hand the RR sensor as the new application method. For the statistical evaluation, we analyzed three parameters of each sensor system: RR, Vt, and Vmin. In total, we had 59 selected sequences for the RR sensor (2–5 measurements per calf and day) and 54 observations for the IOS (2–3 measurements per calf and day). We tested the raw variables for normality using the Shapiro Wilk test from "scipy" python3 package and the alpha level was selected as 0.05 [34]. Since most parameters (except Vt equivalent (RR sensor)) were not normally distributed, the median, minimum, as well as maximum and quartiles were calculated for each parameter.

3.1. Sensor Calibration

Since the RR sensor uses a different algorithm of measurement, which likely causes a systematic mistake in the results, the systematic mistake was removed by performing a sensor calibration. This mistake can be easily found in the plots with raw results (Figures 7–12). The observations, taken from the same animal, however, were slightly moved along one of the axes (along RR or IOS sensor), which gave an idea of a systematic mistake in the device. Therefore, an individual correction factor based on the mean values across the two days of observation was calculated to remove the systematic mistake [35]. For example, if the mean values across one calf were equal to 25 bpm in the RR sensor and 27 bpm in the IOS sensor, for this calf, the correction factor was 25/27, which is approximately 0.93. Then, in all the RR observations from the RR sensor, we would multiply the raw RR values by 0.93, and this new value is calibrated. We applied the same procedure to each measured parameter.

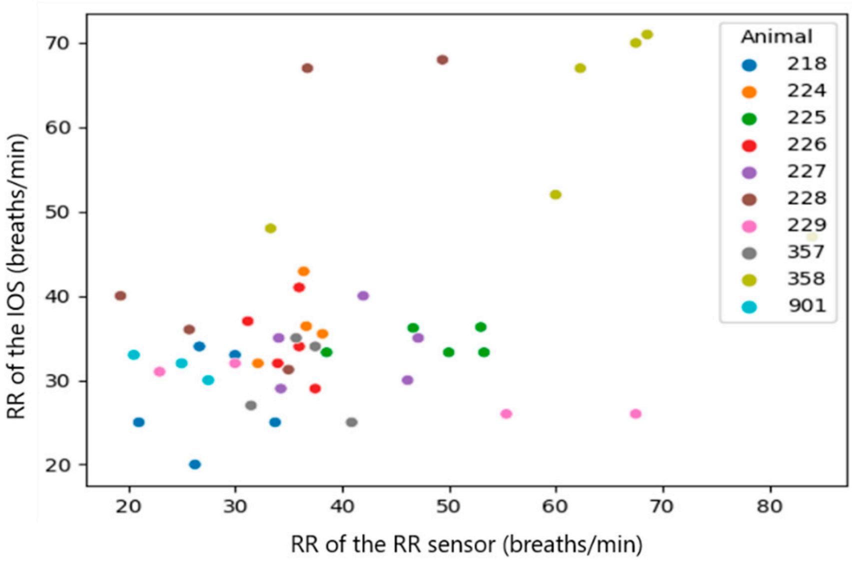

Figure 7. Scatter diagram showing the raw respiration rate (RR) measured by the impulse oscillometry system (IOS) and the RR sensor (n = 46). The data of the individual calves are marked with different colors.

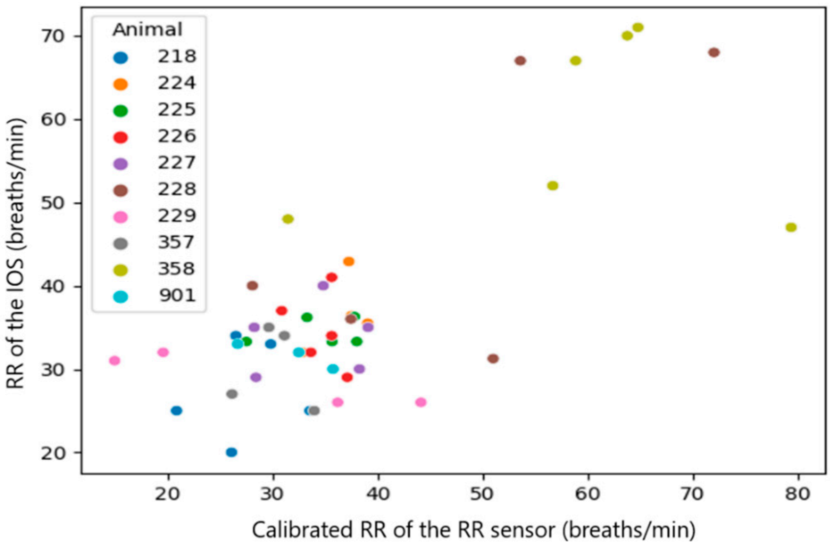

Figure 8. Scatter diagram showing the calibrated RR measured by the IOS and the RR sensor (n = 46). The data of the individual calves are marked with different colors.

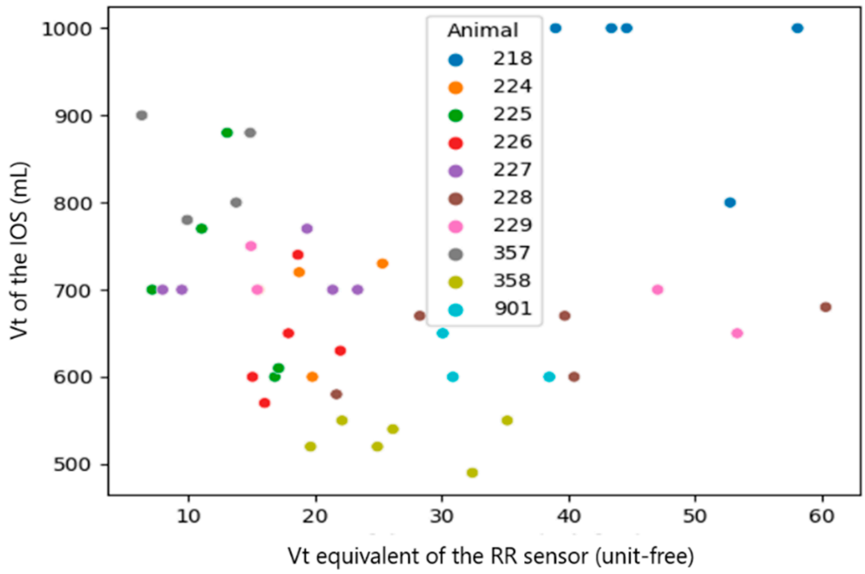

Figure 9. Scatter diagram showing the tidal volume (Vt) measured by the IOS and the raw Vt equivalent of the RR sensor (n = 46). The data of the individual calves are marked with different colors.

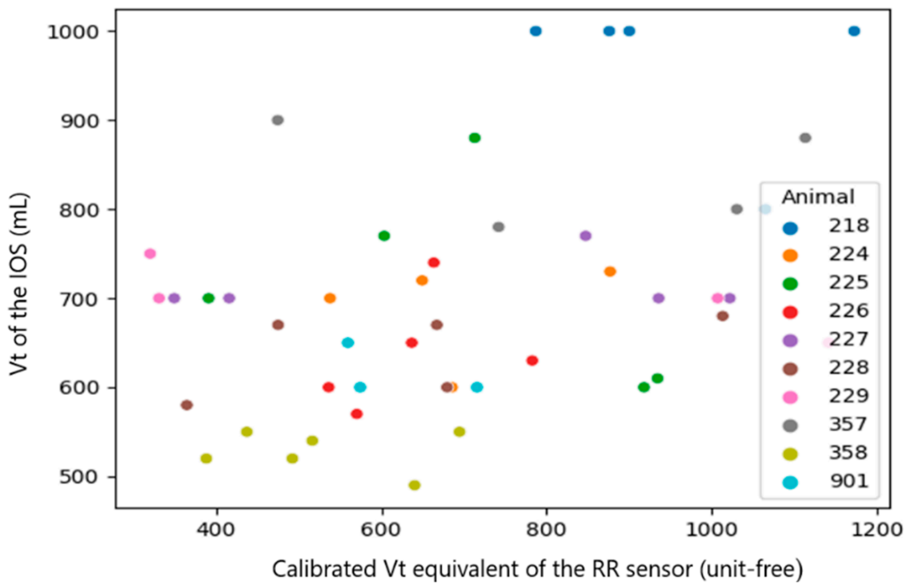

Figure 10. Scatter diagram showing the Vt measured by the IOS and the calibrated Vt equivalent of the RR sensor (n = 46). The data of the individual calves are marked with different colors.

The formula below describes the calibration process, where x is the RR sensor observation, μRR is the mean value of the RR sensor parameter, and μIOS is the mean value of the IOS parameter:

$$correction\ factor = \frac{\mathrm{x} \cdot \mu\mathrm{IOS}}{\mu\mathrm{RR}}$$

After the calibration was done, the plots started to show some correlation pattern, which were further analyzed.

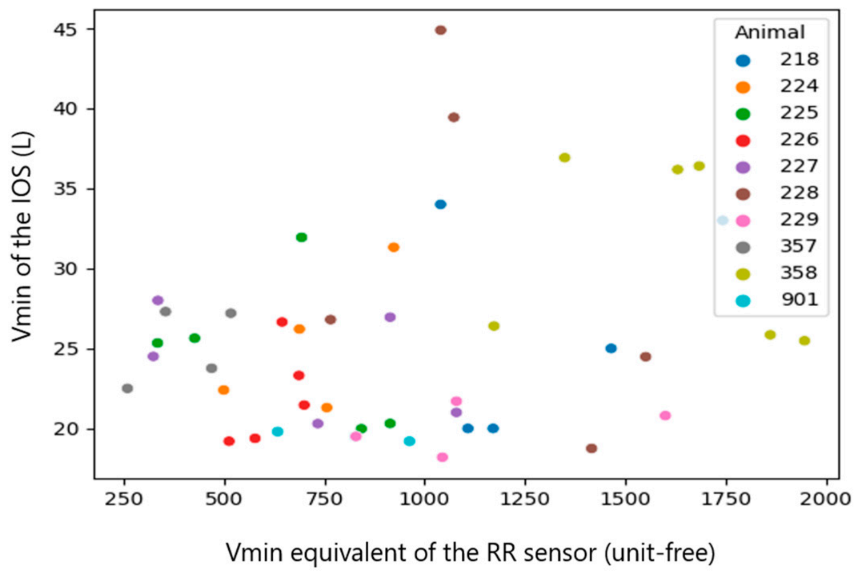

Figure 11. Scatter diagram showing the minute volume (Vmin) measured by the IOS and the raw Vmin of the RR sensor (n = 46). The data of the individual calves are marked with different colors.

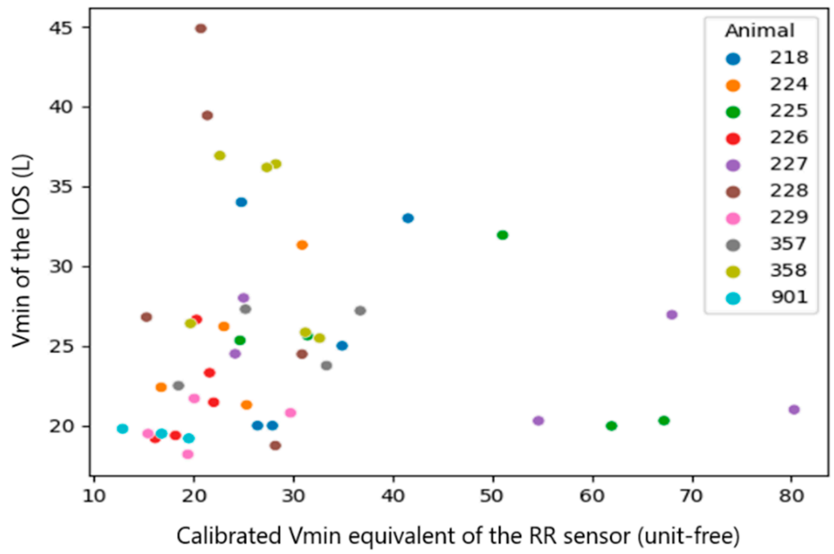

Figure 12. Scatter diagram showing the Vmin measured by the IOS and the calibrated Vmin of the RR sensor (n = 46). The data of the individual calves are marked with different colors.

3.2. Z-Score Transformation

Due to a different unit system in the sensors, we faced the problem of measurements incompatibility and applied Z-score transformation (standardization) to all measurements. We calculated the mean value and standard deviation for every measured parameter separately (RR, Vt, and Vmin) and transformed the raw values to the Z-scores, so that 95% of observations were placed between −2 and +2. That helped comparing the values in the Z-Score unit system [36].

The Formula below describes the Z-score standardization (x_z), where x is the observation value, μ is the mean value of the parameter, and σ is the standard deviation of the parameter:

$$x_z = \frac{x - \mu}{\sigma}$$

3.3. Normality Testing

To decide which statistical approach (parametric or non-parametric tests) to use, we also tested the calibrated variables for the normality using the Shapiro Wilk test from "scipy" python3 package [34] and selected α level as 0.05. Since only the calibrated Vt (RR sensor) was normally distributed, we chose the non-parametric approach further.

3.4. Non-Parametric Test

Due to lack of normally distributed parameters, we used two-tailed Wilcoxon and two-sample Kolmogorov–Smirnov tests to investigate whether the observations from two sensors (RR sensor and IOS) were taken from the same distribution or not [37,38]. Therefore, the null hypothesis was that the observations had the same source. The alternative one was that they had different origins.

We analyzed all values we had (standardized by z-score transformation 54 observations for the IOS and 59 for the RR sensor) from all calves and both days. We compared the parameters as pairs (RR, Vt, and Vmin from both sensors), resulting in 46 observations for every parameter; after removing not completed observations, we also compared both raw and calibrated values. The two-tailed Wilcoxon and two-sample Kolmogorov–Smirnov tests were performed using "scipy" pyhton3 package with basic parameters.

The α-level was selected using Bonferroni correction from "statmodels" python3 package with "not-sorted" setting, and it was 0.0167 [39].

3.5. Correlations

Since the data were not normally distributed (see Section 3.3 Normality Testing), we performed the Spearman correlation test using "scipy" python3 package for both raw and calibrated data. In addition, we performed the Spearman test for medians per animal and medians per animal per day [38].

Moreover, we also analyzed the correlation between Vt and RR for each sensor separately to analyze whether the sensor systems showed the same characteristics with changing RR.

4. Results

The calf's recorded RR was similar for both devices ranging between 19 and 82 bpm, while the Vt of the IOS varied from 6.64 mL/kg to 14.29 mL/kg body mass and the Vt equivalent of the RR sensor ranged from 6 to 60 (an equivalent is unit-free) (Table 1). After the calibration process, the range of the RR sensor values was closer to the values of the IOS and therefore, better comparable to each other.

Table 1. Raw parameters of respiration rate (RR), tidal volume (Vt), and minute volume (Vmin) of RR sensor (n = 59) and impulse oscillometry system (IOS) (n = 54).

Parameter	Unit	Min	0.25	Median	0.75	Max
RR (RR sensor)	breaths per min (bpm)	19	30	36	47	82
Vt equivalent (RR sensor)	unit-free	6	15	19	30	60
Vmin equivalent (RR sensor)	unit-free	260	529	762	1079	1946
RR (IOS)	bpm	20	31	34	40	71
Vt (IOS)	mL	425	600	700	773	1000
Vmin (IOS)	L	18	21	24	27	45
Vt/kg (IOS)	mL/kg	6.64	7	11.11	12.38	14.29

Since the calibration coefficients, calculated from the median per calf of the RR sensor and IOS, were inhomogeneous between the single calves (RR: 0.66–1.27, Vt: 19–71 and Vmin: 0.02–0.06), we used the median in the following statistical evaluation.

The two-tailed Wilcoxon test did not show a significant difference neither between raw values nor between calibrated values (Table 2). Therefore, we cannot reject the null hypothesis, that both sensors took their measurements from the same distribution. The Kolmogorov–Smirnov test was used as a second method, which also did not show any significant difference between RR and IOS sensors (p-value for all parameters > 0.8). The Wilcoxon test showed that the differences inside the groups (RR sensor and IOS) did not significantly differ from the differences between groups, which can partly show that the sensor observations were taken from the same distribution.

Table 2. p-values of the Wilcoxon test for raw and calibrated parameters between respiration rate (RR) sensor and impulse oscillometry system (IOS) of all observations (n = 46).

Parameter	p-Value	Difference
Raw RR (RR sensor) and RR (IOS)	0.7291	No
Raw Vt (RR sensor) and Vt (IOS)	0.9914	No
Raw Vmin (RR sensor) and Vmin (IOS)	0.9655	No
Calibrated RR_(RR sensor) and RR (IOS)	0.8596	No
Calibrated Vt and Vt (IOS)	0.8288	No
Calibrated Vmin and Vmin (IOS)	0.768	No

Vt = tidal volume; Vmin = minute volume.

After applying the Bonferroni correction, our conclusion did not change because our p values were much higher than α level.

A correlation between the raw data of both measurement systems was not visible (Spearman correlation coefficient (CC): 0.1~0.37). However, once we added the information about systematic mistakes in the RR sensor (calibration), the correlation in RR and Vt became clearer across the whole dataset without extracting medians from them (Table 3, all observations, Spearman CC: 0.2~0.48). The most obvious correlations were found in medians per animal (Spearman CC > 0.9, p-value < 0.05 in RR and Vt). However, in Vmin, the p-value did not provide statistical significance (p-value > 0.05) [38].

Table 3. Spearman correlations between respiration rate (RR) sensor and impulse oscillometry system (IOS) for all observations (n = 46) and median per calf (n = 10) after calibration process.

Parameter	Number of Cases	Spearman Correlation	p Value
RR_calibrated (RR sensor) and RR (IOS)	all observations (n = 46) Median per calf (n = 10)	0.4813 0.9515	0.0007 0.000022799
Vt_calibrated (RR sensor) and Vt (IOS)	all observations (n = 46) Median per calf (n = 10)	0.3709 0.9142	0.0112 0.0002
Vmin_calibrated (RR sensor) and Vmin (IOS)	all observations (n = 46) Median per calf (n = 10)	0.2246 0.5636	0.1334 0.0897

RR = respiration rate; Vt = tidal volume; Vmin = minute volume.

In summary, a strong correlation (Spearman CC > 0.9) between the sensors was only achieved for the median per calf of RR and Vt, but not when comparing all raw observations [29].

In addition, we investigated the Spearman CC between Vt and RR for both sensor systems separately, to analyze if they show the same characteristics. It is already known from the literature that a high RR (shallow breathing) is associated with a lower Vt and vice versa [5]. As demonstrated in Table 4, there was a significant negative correlation for the raw and for the calibrated RR sensor data as well as for the IOS data. Thus, the Vt equivalent showed the same characteristics with changing RR like the Vt (IOS) since the Vt equivalent increased with decreasing RR.

Table 4. Spearman correlation between respiration rate (RR) and tidal volume (Vt) of the impulse oscillometry system (IOS) (n = 54) as well as between RR and Vt equivalent of the RR sensor (n = 59).

Parameter	Spearman Correlation	p-Value
RR and Vt equivalent (RR sensor)	−0.513	0.0003
Calibrated RR and Vt equivalent (RR sensor)	−0.4621	0.0012
RR and Vt (IOS)	−0.5438	0.000094007

5. Discussion

The primary objective of this study was to investigate whether a valid Vt parameter could be extracted from the pressure signal measured by the RR sensor. Our results indicate that a flow equivalent as well as a Vt equivalent could be derived from the pressure (RR sensor). In addition, a high correlation between the calibrated RR (RR sensor) and the RR (IOS) as well as between the calibrated Vt equivalent (RR sensor) and the Vt (IOS) for the median per calf was shown. However, the Vt equivalent could not be converted into a true volume given in mL or L because the correlation between the raw data when facing all observations of both measurement systems was too low [29].

In summary, this study showed in detail how the RR sensor can be used in calves and how the pressure can be converted into a Vt equivalent. The Vt equivalent performed simultaneously with the Vt (IOS) since both are negatively correlated with the RR. The study can serve as a basis for future research in the field of lung function diagnostics in calves. Furthermore, it also points out the necessary technical improvements that have to be performed on the RR sensor to reliably record the Vt. If these technical adaptions were implemented, it would also be possible to develop such a diagnostic tool for adult cattle as well. At the current state of the art, the Vt of adult cattle can only be determined with a facemask [40] or estimated by equations from respiratory data [41] in order to detect heat stress at an early stage. Measuring the real Vt in adult cattle without a facemask would be an improvement in the lung function diagnostic. Since the study took place under real field conditions and was the first attempt to determine the Vt using an RR sensor, it would be the first RR sensor capable of measuring the two main respiratory parameters in freely moving animals under field conditions without restrictions on normal behavior.

Compared to assessing pulmonary functions by using the IOS or other spirometers in individual animals, the RR sensor can be worn for a longer period without the need for a prior habituation process. Furthermore, simultaneous recording of RR and Vt would be possible in a larger number of animals due to less personnel and resource requirements. This non-invasive approach of data recording is also an advantage over other methods of lung examination, such as ultrasound [13–15] and blood sampling [11,12], where a fixation of the calf is mandatory. The IOS was used as the gold standard because (i) it had previously been thoroughly evaluated for pulmonary function testing in calves, and (ii) it is suitable for the assessment of spirometric parameters of spontaneous breathing. Respiratory impedance as the main measure provided by IOS was not taken into account in this study, which focused on the measurement of RR and Vt. In the present study, the measured Vt values of the calves were within the physiological range since the reference values for calves are approximately 8–10 mL/kg [6,21].

Limitations and Future Research

Despite the habituation process, the calves were partially nervous during the experiment, as evidenced by hyperventilation (RRs up to 82 bpm), taking into account that the physiological RR for calves ranges from 20 to 30 bpm [5,18]. Therefore, a simultaneous measurement of the two devices would be useful to allow a direct comparison between the continuous readings as well as a longer habituation period. Unfortunately, simultaneous measurement with both devices was not possible in our animal testing due to the lack of space under the facemask for the RR sensor.

Furthermore, unsteady movements of the calves as well as moisture in the hose of the RR sensor (caused by sneezing) led to an influence of the pressure because the membrane of the RR sensor reacts very sensitively to changes in the environmental conditions. As a result, it became partly difficult to distinguish between physiological recorded pressures and artifacts.

In addition, we determined that reliable measurements with the RR sensor are only technically possible with calves from approximately 1 week of life onwards. The fixation of the nose ring in very young calves (up to approximately 4 days old) was not optimal, since it slips easily which also influences the pressure. Furthermore, the membrane of the RR sensor is subject to pressure fluctuations of 0.06%, which corresponds to approximately 0.03 mbar, thus the pressure can never be recorded with 100% accuracy [42].

The statistical results indicate that the RR sensor can replace the IOS in the future. Since the data showed a high correlation between the RR sensor and IOS for the calibrated RR and VT for the median per calf, the RR sensor seems to be a promising replacement. Regardless, the two-tailed Wilcoxon and Kolmogorov–Smirnov tests showed no significant difference between the two sensors; we did not have enough evidence to talk about sensors replacement validity yet because only the median per calf showed a high correlation between the two sensors. Therefore, at least 2–5 observations per calf of the RR sensor as well from the IOS were necessary to find the calibration coefficient, which provided a high Spearman CC and good significance.

Moreover, to replace the gold standard method, it is necessary to find the accurate algorithm, which would recalculate the results of the RR sensor in the unit-free system into mL. Therefore, it must be taken into account that the data in our study were very specific for this age and breed, as it is influenced, among other factors, by the diameter of the nostrils. For an accurate and valid approximation of the calves' Vt across all breeds and ages, it is necessary to expand the number of cases with calves differing in age, breed, weight, and environmental conditions.

However, a prior revision of the RR sensor would be required to reliably record the pressure without artifacts and to finally achieve a possible higher correlation in the raw observations. Therefore, the following technical improvements to the RR sensor would be necessary: an installation of a position sensor to ensure that only measurements are recorded when the calf is at rest and does not make restless head movements. This technical adaption would have the advantage that artifacts would automatically be absent. In addition, it would be useful to be able to record the pressure in both nostrils to exclude pressure changes due to calves' movements and pathological unilateral nasal constriction due to fluid accumulation. Moreover, it would also contribute to the precision of the data (as well be a huge reduction in workload) if the evaluation steps that we have manually performed to convert the pressure into a Vt equivalent were automatically integrated by the RR sensor. This would practically mean to automatically take into calculation the BTPS correction based on temperature and humidity measurements, smoothing of the data, as well as automatic integration of the pressure values and finally calculating the difference between maximum and minimum pressure in each breath.

Furthermore, a waterproof cover with a longer battery life (currently approximately 6 h) would be useful, enabling the RR sensor to be worn during drinking and thus over an even longer period of time. There is already a market-ready RR sensor from Gouna [43] available, which is water-resistant and allows RR measurement in cows over approximately 6 months. However, this is only adapted for cows and would need to be made smaller and lighter for calves. In addition, the market ready RR sensor only records the RR and no longer outputs the individual pressure differences, which is why it is not suitable for recording the Vt equivalent without complete revision.

The outlined technical adaptions of the RR sensor would essentially simplify the measurement method of Vt for the future because the RR sensor does not impair the normal behavior of the animals (no mask is required) and can be used at all ages from young calves to adult cattle.

In summary, it can be stated that before further animal testings are performed, a technical revision of the RR sensor is necessary (in particular: an installation of a position sensor, a waterproof cover, measurements in both nostrils, and a longer battery life). Moreover, the development of a larger facemask would be required to install the RR sensor under the facemask and therefore allow a simultaneous measurement with both devices. If these technical improvements were implemented, a testing on calves differing in age, breed, weight, and environmental conditions would be useful to develop a reliable algorithm converting the Vt equivalent into volume in mL.

6. Conclusions

This study demonstrated how to convert the pressure of the RR sensor into a flow equivalent and subsequent volume equivalent by a comprehensive analysis. It could be shown that the pressure (RR sensor) behaved synonymously with the flow (IOS) and that the median of the calibrated Vt equivalent of the RR sensor per calf showed a high correlation with the Vt of the IOS. Thus, the RR sensor appears to be fundamentally suitable as a measurement parameter of the Vt. However, the Vt equivalent (RR sensor) could not be converted into a true volume in mL or L because the correlation between the raw data was too low. This proof-of-principle study provided the basis for further research focusing on technical adaptations of the RR sensor to determine reliable Vt data in parallel to RR and to develop a market-ready device.

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Informed Consent Statement: Not applicable.

Data Availability Statement: The data that support the findings of this study are not openly available due to reasons of sensitivity but are available from the corresponding author upon reasonable request.

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3. Discussion and Outlook

3.1 Application of Sensor Systems for the recording of respiratory parameters

a) Respiration rate

The RR is a very sensitive parameter closely related to the physiological health status of the cow (Shu et al. 2021), but it can also change quickly (Tresoldi et al. 2019) and is susceptible to short-term variability. Therefore, continuous recording methods of the RR are more reliable than short-term recordings (Atkins et al. 2018).

In human medicine, commercially available RR monitors (e.g., SP1, Spire, San Francisco, Cal.) are already obtainable for long term observation, which are placed around the patient's chest like a belt and record pressure changes during inspiration. However, these are of limited use for cows as they measure a maximum of 30 bpm (AL-Khalidi et al. 2011) and the RR of cattle can rise to values above 70 bpm in summer (Ruban et al. 2020; Pinto et al. 2019). A similar system has already been developed for the application in cattle by Eigenberg et al. (2000) and Atkins et al. (2018). In addition, the previous mentioned RR sensor developed by Strutzke et al. (2019) is another new measurement method to detect the RR continuously in cows.

However, non-contact methods are currently gaining more relevance, because they do not restrict the animal’s normal behavior and one sensor may be used for several cows. Moreover, no battery charging is needed, the loss of devices is limited and no fixation of the animals is necessary reducing stress for cows as well as the risk of work accidents for farmers (Shu et al. 2021; Neethirajan 2020; Halachmi et al. 2019). Visual images of the flank are mainly used (Wu et al. 2020), but a laser distance sensor (Pastell et al. 2007) or a frequency modulated continuous wave radar based system (Tuan et al. 2022) are also suitable for non-invasive recording of the RR.

Similarly, in our current research project “Artificial intelligence for measuring respiration in dairy cows” (KAMI), we are investigating in cooperation with the University of Hildesheim, the Dida Data Science GmbH and the Educational and Experimental Center for Animal Breeding and Husbandry (LVAT, Groß Kreutz, Germany) whether it is possible to automatically measure the RR in cows using artificial intelligence. On the one hand, we use an infrared camera (FLIR A65, 45°, Wilsonville) measuring the temperature change between in- and exhaling in the area of the nostrils. On the other hand, a depth camera (D455e RealSense, Intel Corporation, Santa Clara, USA) records the flank movements of the cow by continuously measuring the distance between flank and camera. A successful outcome of one of these new automatic measurements, would significantly simplify the recording of the RR over a longer period of time.

In human medicine, depth cameras have already been successfully tested for RR monitoring on infants (Cenci et al. 2015). However, a major advantage when monitoring infants is that they remain in the supine position, which means that artefacts caused by movement occur less frequent than in cattle. In cows, it is a challenge to distinguish between flank movements generated by the RR and other movements of the cow. In addition, the camera is fixed in one place, which is why the distance between cow and camera can vary depending on the cows position (Tuan et al. 2022). In cows, depth cameras have been successfully tested to record the reticulo-ruminal motility (Song et al. 2019), but not for RR recording to my knowledge.

Infrared thermography (IRT) has already been proofed as possible RR measurement tool for cows as well as for calves (Lowe et al. 2019; Stewart et al. 2017), but there is still a lack of a market-ready variant that automatically records the RR and integrates the information directly into an existing herd management systems. The fundamental principle is to measure the temperature change during in- and exhalation by recording the maximum temperature in the area of Interest (AOI), in this case, the region of the nostrils. Afterwards, the maximum values are extracted in a curve and the RR is calculated by the time duration between two peaks (Figure 1). One challenge for the development of a market-ready device is currently the positioning of the IRT camera. In our study, one infrared camera can cover a maximum of 3 cubicles and therefore a large number of cameras would be needed to cover all cows in the barn. The high costs per camera (over EUR 10,000) and the feasibility of installing that many cameras (data linkage, power consumption, number of power sockets) may prevent an implementation in practice. In addition, cows stand more during heat stress (Nordlund et al. 2019; Heinicke et al. 2018; Cook et al. 2007), which limits the recording in the lying cubicles during heat stress periods.

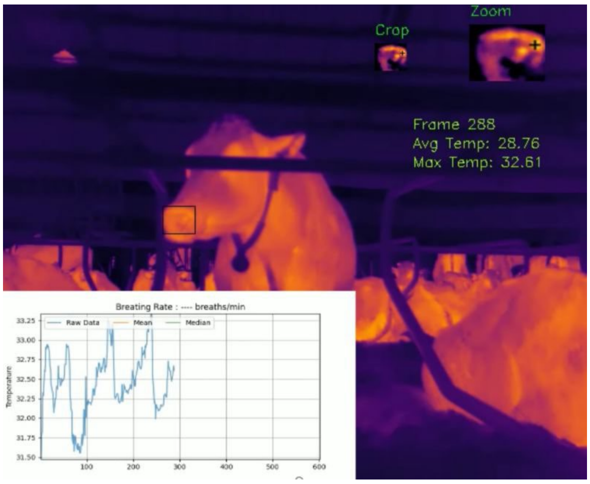

Figure 1. Infrared thermography image tracking the Area of Interest (AOI) (here nostrils) and extracting the highest temperature peaks in a breathing curve (Source: ATB and University of Hildesheim, 2023)

A recording of the RR in the milking robot was not possible. The cows are fed with concentrated feed preventing the nostrils from being visible. In addition, the representability of a recorded RR during feeding and milking is questionable, because the RR should be recorded at rest

(Baumgartner et al. 2018). As an example, Pastell et al. (2007) demonstrated that a longer milking time leads to a higher RR.

Since the RR is a good indicator for heat stress (Tresoldi et al. 2019; Gaughan et al. 2000) a completely new approach is to predict the RR based on environmental and cow related factors, that are easier to record than the RR itself (Janni et al. 2023; Dado-Senn et al. 2020b; Li et al. 2020). However, as many factors such as milk yield, lactation stage, age, temperature, health status, or position (lying or standing) have an influence on the RR (Pinto et al. 2019; Heinicke et al. 2018) predictive models depend on a great amount of reliable data from many cows over the whole day (Shu et al. 2023). Moreover, RR might even depend on the individual management system of the barn (i.e. feeding or milking time slots) (Hart et al. 2013). Since current training of prediction models do not yet use continuously recorded RR, their usefulness is limited to those certain time periods, which increases uncertainty (Li et al. 2020). Furthermore, a residual uncertainty remains due to variables that are related to heat stress but cannot be measured (Becker et al. 2021).

Regardless of whether a model aims to estimate the RR or a sensor system records the RR, as gold standard method the RR should be counted for 60 s, as this is the most accurate method according to Dißmann et al. (2022). For example Li et al. (2020) or Stewart et al. (2017) have instead determined the time of 10 flank movements, which is another possibility, but not as accurate as counting respiratory cycles for 60 s and with increasing RR it becomes even less accurate (Dißmann et al. 2022).

In summary, non-invasive visual techniques for automatic recording of the RR in cattle are currently gaining more importance as predictive models for estimating the RR to measure heat stress (Shu et al. 2021). There are already models that can estimate heat stress by predicting the RR with high accuracy (Shu et al. 2023), but their accuracy can be improved if more animal-related parameters are taken into account (Becker et al. 2021) and by continuously recording the RR as reference method (Li et al. 2020). Consequently, continuous recording methods are needed on the one hand, to train models that predict RR to detect heat stress, and on the other hand, recording the RR can serve as a useful early indicator for disease and discomfort in cattle. While animal-mounted sensors such as the RR sensor by Strutzke et al. (2019) are suitable for continuously recording of the RR of individual animals, camera systems aim to non-invasively record the RR of many cows per day in real-time by using one sensor system.

b) Tidal volume

The Vt is one of the fundamental parameters of respiratory function diagnostics and a useful indicator to evaluate lung diseases as well as necessary to measure part of the heat loss of the cow caused by the respiratory tract (Reinhold 2023; Zhou et al. 2022). Measuring the Vt, to accurately determine heat loss in cattle, is gaining more and more interest due to climate change, because it can help to adjust and to evaluate cooling mechanisms (Maia et al. 2005; Beede and Collier 1986).

As Publication “B” has shown (Dißmann et al. 2023), it is currently not possible to measure the Vt continuously and without a facemask in calves. The required technical adaptations to measure Vt reliably with the RR sensor (Strutzke et al. 2019) have been described in detail in Dißmann et al. (2023). To measure heat loss in cows, Zhou et al. (2018) and Maia et al. (2005) used facemasks adapted to the head size of cows to record the Vt, which, however, also requires prior fixation of the animal and does therefore not allow continuous measurement of the Vt.

In human medicine, there are already initial attempts to measure relative Vt by means of IRT in addition to RR (Lewis et al. 2011). The fundamental principle here is to integrate the thermal signal in order to achieve a Vt correlate. In cattle, no such attempts have yet been made to my knowledge. The challenges in cattle will probably be to find a suitable reference method measuring the Vt simultaneously and to record sufficient data of different Vt`s, since unlike in humans, it is not possible to ask a cow to breathe slowly, quickly or deeply. In human medicine, they use a respiratory inductance plethysmograph sensor that can be fixed around the thorax (Lewis et al. 2011; Wilhelm et al. 2003). This could serve as a template for similar applications in cattle in the future.

Another new approach is to predict heat loss based on mathematical models estimating besides the RR also the Vt. Thereby, only measurements of ambient conditions (Maia et al. 2005; McGovern and Bruce 2000) or additional animal specific data served as data for the prediction model (Maia et al. 2008; Berman 2005). Maia et al. (2008) showed that considering physiological parameters in a model could improve its accuracy. Furthermore, Maia et al. (2005) demonstrated in earlier studies that while RR correlates closely with ambient temperature, Vt correlates more closely with RR than with ambient conditions. Consequently, a measurement or estimate of RR is useful to calculate Vt better than by only ambient conditions. Berman (2005) did not measure the Vt for his model, but estimated it using body weight based on a formula by Hales and Findlay (1968) for Holstein cows. Although there is a strong correlation between Vt and bodyweight (Koch and Reinhold 2015), under heat stress individual animals show a different increase in RR depending on milk quantity, lactation stage, age or cow position (Heinicke et al. 2021; Pinto et al. 2019). Since Vt correlates with RR (Maia et al. 2005), it can also differ between individuals of similar weight. Therefore, measuring the real Vt as a reference method for developing a thermal balance simulation model is preferable.

To sum up, the Vt can currently only be measured with a facemask. To avoid this rather complex measurement method, especially for the investigation of heat loss in cattle, there are already approaches to estimate Vt using body weight or RR instead. However, more measurements of Vt from different breeds and body weights under heat stress are necessary to build up a reliable data set. For this approach, a simpler and more practical method to measure Vt in cattle would be useful. In Publication "B", the necessary technical improvements to reliably measure the Vt with the RR sensor are already listed. Another possibility would be to use IRT cameras for this purpose in the future, as has already been tested in human medicine.

3.2 Relevance of pulmonary function testing in future research

The milk yield per cow and year has increased on average in Germany by more than 2,000 kg, from 6,208 kg/year in 2000 to 8,499 kg/year in 2022 (BMEL 2023). Since a high amount of energy is needed for the production of milk and since, about 35 % of the consumed energy is converted into heat, the produced heat amount increases with raising milk yield (Jentsch et al. 2001). In addition, climate change also leads to an increase in heat stress in cattle due to higher ambient temperatures (Thornton et al. 2022; Gauly et al. 2013).

Therefore, strategies to counteract heat stress in cattle are becoming more and more important in future: On the one hand, the measurement of respiratory parameters can be used in the future to record more precisely the heat stress load (RR measurement, determination of heat loss through respiration) and thereby improve the effectiveness of cooling measures (Zhou et al. 2022; Beede and Collier 1986). The produced heat, which can be released over the respiration (20-30 %), can be reliable determined by measuring the RR and the Vt determined (Zhou et al. 2022). On the other hand, pulmonary function testing should receive more attention in future breeding strategies (Beede and Collier 1986). In fact, since O_2 consumption correlates with energy metabolism (Gallivan et al. 1989) the continuous increase in milk yield has greatly increased the consumption of O_2. However, the number of pulmonary capillaries per alveolar unit is already at rest lower in cattle than in other species (Reinhold 1997). Therefore, an increase in performance leads to a decrease of the bovine physiological gaseous exchange reserve capacity (Veit and Farrel 1978). Besides the selection of heat tolerant breeds based on panting score (Adamczyk et al. 2013; Gaughan et al. 2010) or a slick hair phenotype (Sosa et al. 2021; Dikmen et al. 2014), the recording of RR and Vt can be additionally valuable to identify heat-tolerant breeds and genotypes within a species (Maia et al. 2008). Therefore, more research is required using pulmonary function diagnostics to examine the heat tolerance of different breeds and cross breeds (Veit and Farrel 1978).

4. Conclusion

In Conclusion, recording of respiratory parameters in cattle is fundamental to detect heat stress and respiratory disease at an early stage, because cattle are predisposed to diseases of the respiratory tract due to their lung anatomy. In addition, the RR is suitable as a general animal welfare indicator, as it reacts very sensitively to pain and stress. The Vt is also necessary to determine respiratory heat loss. Sensors are already available for automatic and continuous recording of RR, but currently non-invasive recording methods such as camera systems are becoming more important, because they do not restrict the animal's normal behavior. They are as well more efficient since one sensor may be used for more animals instead of one sensor per animal. Regardless of which method is used, the RR should be counted for 60 s, as this is the most accurate method according to Publication “A”. For the recording of Vt, a facemask is currently still necessary, requiring a fixation of the animals during recording. In Publication “B”, the necessary technical adaptions to measure the Vt by a RR Sensor, are described in detail. To avoid time-consuming recording of RR and Vt, these parameters may also be predicted based on mathematical models using other animal and environmental parameters. In order to increase the model`s accuracy, however, it is necessary to record more animal related parameters that influence the breathing and to use continuously recorded RR data as reference method. According to heat stress, the recording of RR and Vt can also be valuable in the future to identify heat-tolerant breeds and genotypes within a species.

5. Summary

The respiration rate (RR) is a suitable parameter for assessing the health status of cattle, as it is very sensitive for detecting stress or pain, and thus shows an indisposition in animals at an early stage. Furthermore, it is a suitable parameter for the early detection of heat stress, which becomes increasingly important due to climate change. In order to determine the proportion of heat emitted through respiration, an additional measurement of the tidal volume (Vt) is necessary. The measurement of Vt is also suitable to assess the performance of the lungs and allows conclusions about respiratory and lung diseases. The gold standard methods for both parameters are time consuming and can also influence the behavior of the cow. Counting the cow's flank movements is often used as the method of choice for recording RR. However, different methods are found in literature regarding duration and type of counting. While in some studies, the RR was counted for 5 or 10 breaths and then extrapolated to 60 s, other authors counted the RR for 15, 30 or 60 s. In order to determine which method is the most precise, Publication “A” compared the 5 methods mentioned above. The reference method used was an RR sensor that reliably measures the RR based on the pressure difference between inhalation and exhalation. In Publication "A", it was shown that counting the RR for 60 s is the most accurate method and that as the RR increases, the inaccuracy of the other methods continues to increase compared to counting for 60 s. Since a facemask has to be fitted around mouth and nostrils to measure Vt, the second fundamental parameter in lung function diagnostics, by a pneumotachograph, the animals have to be restrained during the entire experiment and since the behavior is severely restricted by the mask, only short measurement periods are possible. Therefore, the driving force for Publication "B" was to investigate whether it is possible to derive a Vt equivalent from the measured pressure of the RR sensor. A successful outcome would greatly simplify the measurement method, as the animals only have to be fixed for measurements with the RR sensor and can then move freely in the barn, making longer measurements possible. However, it was shown in Publication "B" that technical adaptations to the RR sensor are necessary in order to be able to reliably determine the Vt equivalent. Furthermore, there are approaches in human medicine to determine a relative Vt using infrared thermography, which can also be used to calculate the RR based on the temperature difference of the inhaled and exhaled air. The trend in livestock research is similar – sensors attached to the animal are being replaced by contactless systems for data acquisition. The main advantages of this alternative is that one sensor can record multiple animals and no animal fixation is needed to attach the sensors, which also reduces the risk of injury to humans. For this reason, we are currently testing infrared as well as depth cameras at ATB in cooperation with the University of Hildesheim, the Dida Data Science GmbH, and LVAT as part of the KAMI project (Artificial intelligence for measuring respiration in dairy cows).

The aim of future studies is to use artificial intelligence and imaging techniques to automatically record the RR and integrate this parameter into existing herd management systems.

6. Zusammenfassung

Erfassungsmethoden von Atemwegsparametern beim Rind

Die Atmungsfrequenz (RR) ist ein geeigneter Parameter zur Bewertung des Gesundheitszustandes von Rindern, da sie ein sehr sensibler Indikator zur Erkennung von beispielsweise Stress oder Schmerzen ist und somit eine Indisposition bei Tieren in einem frühen Stadium zeigt. Darüber hinaus ist sie ein geeigneter Parameter zur frühzeitigen Erkennung von Hitzestress, deren Bedeutung aufgrund des Klimawandels immer mehr zunimmt. Um den Anteil an Wärme zu bestimmen, der durch die Atmung abgegeben wird, ist ein zusätzliches Messen des Atemzugvolumens (Vt) erforderlich. Die Messung des Vt's ist außerdem geeignet, um die Leistungsfähigkeit der Lunge zu beurteilen und lässt Rückschlüsse auf Atemwegs- und Lungenerkrankungen zu. Die Goldstandardmethoden für beide Kenngrößen sind zeitaufwendig und können darüber hinaus das Verhalten der Kuh beeinflussen. Die Zählung von Flankenbewegungen der Kuh wird häufig als Methode der Wahl für die Erfassung der RR verwendet. In der Literatur finden sich jedoch verschiedene Methoden bezüglich Dauer und Art der Zählweise. Während in manchen Studien die RR für 5 oder 10 Atemzüge gezählt wurde und anschließend auf 60 s hochgerechnet wurde, zählten andere Autoren die RR für 15, 30 oder 60 s. Um zu ermitteln, welches die genauste Methode ist, wurden in der ersten Veröffentlichung die zuvor genannten 5 Methoden verglichen. Als Referenzmethode wurde dabei ein RR Sensor verwendet, der die RR anhand der Druckdifferenz von Ein- und Ausatmung verlässlich erfasst. In der Veröffentlichung A konnte gezeigt werden, dass das Zählen der RR für 60 s die genaueste Methode ist und mit steigender RR die Ungenauigkeit der anderen Methoden im Vergleich zum Zählen von 60 s weiter zunimmt. Da für das Messen des Vt`s, dem 2. fundamentalen Parameter in der Lungenfunktionsdiagnostik, eine Atemmaske um das Flotzmaul angebracht werden muss, müssen die Tiere während des gesamten Versuchs fixiert sein und da das Verhalten durch die Maske stark eingeschränkt ist, sind nur kurze Messperioden möglich. Deswegen war die Triebfeder für die Publikation „B" zu untersuchen, ob man anhand des gemessenen Drucks des RR Sensor auch ein Vt Äquivalent ableiten kann. Dies würde die Messmethode stark vereinfachen, da die Tiere für Messungen mit dem RR Sensor nur zur Anbringung fixiert werden müssen und sich dann frei im Stall bewegen können, sodass auch längere Messungen möglich sind. Jedoch konnte in Publikation „B" gezeigt werden, dass noch technische Adaptionen am Sensor notwendig sind, um das Vt Äquivalent verlässlich bestimmen zu können.

Darüber hinaus gibt es bereits in der Humanmedizin Ansätze ein relatives Vt durch Infrarotthermographie zu bestimmen. Diese kann auch genutzt werden, um anhand des Temperaturunterschieds der ein- und ausgeatmeten Luft die RR zu berechnen. Ähnlich ist auch die Tendenz in der Nutztierforschung – Sensoren die am Tier angebracht werden, werden zunehmend durch kontaktlose Systeme zur Datenerfassung ersetzt. Denn der Vorteil letzterer ist, dass ein Sensor mehrere Tiere erfassen kann und keine Einzeltierfixierung zur Anbringung der Sensoren notwendig ist, was auch das Verletzungsrisiko für den Menschen reduziert. Deswegen testen wir aktuell am ATB im Projekt KAMI (Künstliche Intelligenz zur Erfassung der Atmung bei Milchkühen) zusammen mit der Uni Hildesheim, der Dida Datenschmiede GmbH und der LVAT, Infrarot- und Tiefenkameras, um die RR automatisch zu erfassen. Ziel zukünftiger Studien ist es mittels Künstlicher Intelligenz und bildgebender Verfahren die RR automatisch zu erfassen und in bestehende Herde Management Systeme zu integrieren.

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Grand Wayne Center, Fort Wayne, Indiana, USA, 19-20 April, pp.111-126 ref.35.

8. Publications and Oral Presentations

Publications

1. **L. Dißmann**, J. Heinicke, K. C. Jensen, T. Amon, G. Hoffmann
   How should the respiration rate be counted in cattle? Vet Res Commun. 2022 Dec; 46(4):1221-1225. doi: 10.1007/s11259-022-09984-7. Epub 17 August 2022. PMID: 35976483; PMCID: PMC9684242

2. **L. Dißmann**, P. Reinhold, H. J. Smith, T. Amon, A. Sergeeva, G. Hoffmann
   Evaluation of a Respiration Rate Sensor for Recording Tidal Volume in Calves under Field Conditions. Sensors (Basel). 12 May 2023; 23(10):4683. doi: 10.3390/s23104683. PMID: 37430597; PMCID: PMC10223783

Oral Presentations

1. Measurement of respiratory characteristics in cattle.
   L. Dißmann
   PhD-Day at Leibniz Institute for Agricultural Engineering and Bioeconomy (ATB), Hybrid-Conference, 25 May 2022; Potsdam, Germany

2. Thermodynamic prediction of heat stress in dairy cattle - predictive models for barn climate optimization.
   T. Amon, S. Foroushani, G. Hoffmann, J. Heinicke, **L. Dißmann**, J David, S. Hempel, C. Ammon
   10th European Conference on Precision Livestock Farming (ECPLF), 29 August 2022; Wien Austria

3. Development and application of a respiration rate sensor in dairy cows for heat stress detection (Entwicklung und Anwendung eines Atemfrequenzsensors bei Milchkühen zur Hitzestresserkennung).
   G. Hoffmann, J. Heinicke, C. Ammon, S. Strutzke, S. Pinto, **L. Dißmann**, T. Amon
   15th Conference on Construction, Technology and Environment in Agricultural Livestock Farming (15. Tagung: Bau, Technik und Umwelt (BTU)), 15 September 2022; Soest, Germany

4. Artificial intelligence for detecting of respiration in dairy cows.
   L. Dißmann, M. Trescher, G. Hoffmann
   X-KIT kick-off event at the Fraunhofer Institute for Experimental Software Engineering (IESE), 08 and 09 February 2023 (poster presentation); Kaiserslautern, Germany

5. Artificial intelligence for measuring the respiration rate in dairy cows.
   L. Dißmann, R. Antia, L. Chinthakayala, N. Landwehr, T. Amon, G. Hoffmann
   Book of Abstracts of the 74th Annual Meeting of the European Federation of Animal Science, Book of abstracts No. 29 (2023) 26 August – 01 September 2023 pp. 758 (28 August 2023, presentation in Session 65); Lyon, France

6. Artificial intelligence for recording of respiration in dairy cows.
L. Dißmann
X-Kit Cluster Meeting “Animal husbandry” at Educational and Research Institute for Animal Breeding and Husbandry (Lehr- und Versuchsanstalt für Tierzucht und Tierhaltung (LVAT)), 26 September 2023; Groß Kreutz, Germany

7. Recording methods of respiratory parameters in cattle.
L. Dißmann
PhD-Day at Leibniz Institute for Agricultural Engineering and Bioeconomy (ATB), Hybrid-Conference, 05 December 2023; Potsdam, Germany

8. Sensor systems in dairy livestock farming based on the example of respiration rate recording.
L. Dißmann
Online presentation for agricultural students of the bachelor program Livestock & Agriculture Technology, Lakeland College, 31 January 2024; Alberta, USA

9. Artificial intelligence for detecting the respiration in dairy cows.
L. Dißmann, A. R. Muhammad, M. Trescher
Poster as part of the AI Research Atlas
https://ai-science-atlas.innohub13.de/ (accessed on 28 February 2024)

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11. Conflict of Interest

In the context of this work, there are no conflicts of interest due to donations from third parties.

12. Declaration of Independence

I hereby declare that I wrote the manuscript myself. I certify that I have used only the sources and assistance indicated. My contribution to the research project within the framework of my cumulative doctoral thesis is summarized in the following table.

	RESEARCH PROJECT 1 / PUBLICATION "A"	**RESEARCH PROJECT 2 / PUBLICATION "B"**
STUDY DESIGN	+++[1]	++
DATA COLLECTION	++	+++
DATA ANALYSIS	++	+++
MANUSCIPT WRITING	+++	+++
MANUSCRIPT EDITING	+++	++

[1]Score: + ≤ 50%; ++ = 50-70%; +++ ≥70%

Potsdam, den 28.02.2024 Lena Sophia Dißmann