Towards real-time monitoring of pig welfare status using heart rate and thermography

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MSc Thesis Adaptation Physiology

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List of abbreviations

AF	After feeding
ANS	Autonomic Nervous System
BF	Before feeding
Bpm	Beats per minute
ECG	Electrocardiogram
HRV	Heart Rate Variability
IR	Infrared
IRT	Infrared Thermography
Iso	Isolated, pigs are housed in physical isolation, while being able to smell, hear and see each other
NS	Nervous System
Paired	Pigs are housed in pairs
PLF	Precision Livestock Farming
PNS	Parasympathetic Nervous System
PPG	Photoplethysmography
ROI	Region of Interest
SNS	Sympathetic Nervous System

Abstract

In our current housing and management systems for livestock animals, these animals can experience stress from stressful situations. In order to subjectively assess the level of stress the animals experience, technology seems promising. Precision Livestock Farming can monitor physiological, behavioural and environmental 'cues' automatically and continuously. A heart rate belt and infrared thermal camera can collect cardiac and body surface temperature data in real time. That data can be used to assess the welfare status of the animal. This study aimed to investigate the potential of using wearable and non-contact sensors, namely heart rate monitors and thermal cameras, for real-time monitoring of pig welfare status. Five pigs were followed during different situations, namely paired- and separated housing, before- and after feeding. On the last day, during transport, the heart rate and velocity were measured. Each start of a new situations was accompanied by raised heart rates and heart rate variability for a few minutes. Feeding and eating did have the longest period of raised heart rates. Transport related events also showed increased heart rates, which decreased again after a few minutes. However, these findings did not seem significant in this study. The body surface temperatures did not differ between the situations, and there was no correlation between the body surface temperatures and the rectal temperature. Continuous monitoring of heart rate, heart rate variability and body surface temperatures of pigs seems promising. However, before future use, the sensors probably still need more research and development for better accuracy.

1 Introduction

Animals are kept by humans for a variety of reasons; work, hobby, or their products, such as milk and meat. Keeping animals also means that we, as humans, are responsible for their welfare. In 1998 the European Commission published the Council Directive 98/58/EC on animal welfare that includes the protection of animals based on the Five Freedoms. The Five Freedoms mean that animals should be:

- Free from thirst and hunger
- Free from discomfort
- Free from pain, disease, and injury
- Free from fear and avoidable stress
- Free to express normal behaviour (European Commission, n.d.).

Over the years, different definitions of animal welfare have been published with even more approaches to assess animal welfare. Fraser (2008) found that the approaches generally covered three main points; "*1) Basic health and biological functioning of animals, 2) natural behaviour and natural living conditions for animals, and 3) affective state of the animals.*" These three points overlap and interact with each other (Figure 1). 'Affective states' i.e., emotions, is the most difficult of these three points to assess in animals because the measured or observed changes in physiology (e.g., increased heart rate) can have both a positive (e.g., excitement or activity) or negative (e.g., fear) emotional state (Paul *et al.*, 2005). 'Basic health and functioning' and 'natural living' are easier to measure and assess in animals when indicators have been established. In this thesis, the focus is on the physiological reaction of pigs to different activities and conditions that could cause the animal to experience some stress or excitement.

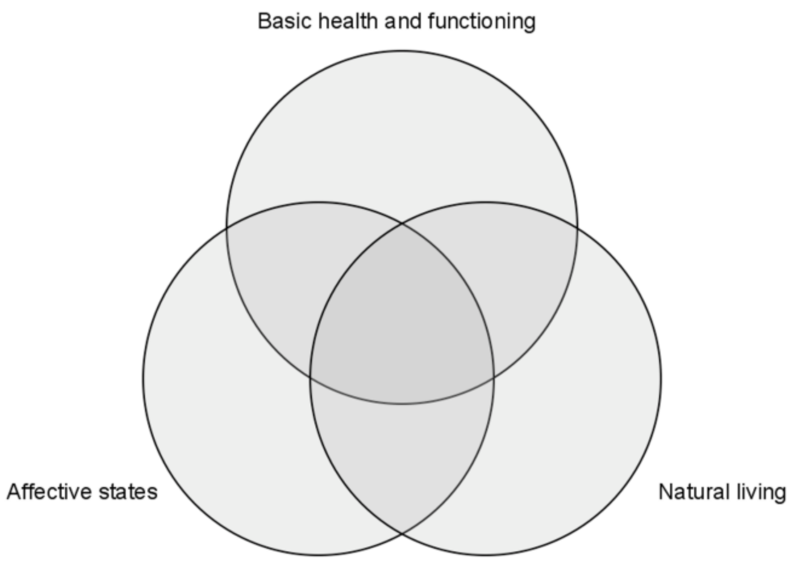

Figure 1: The three main points of animal welfare (Fraser, 2008).

For assessment of animal welfare, the Welfare Quality® Assessment protocol has been developed based on feeding, housing, health, and behaviour. This protocol is mostly based on animal-based measures with additional management- and design-based measurements (Welfare Quality®, 2009). However, it is not designed for continuous monitoring and is therefore not able to detect early warning signals of impaired animal health or welfare (Stygar *et al.*, 2021).

Animal welfare is currently a 'hot topic' regarding livestock production. In the pig industry, animal welfare is researched, and different solutions or adaptations are considered or developed to reduce the welfare issues that are currently still present.

1.1 Pig production and welfare issues

After the Second World War, the Dutch government decided that the Dutch would never have to go hungry again. Therefore, the farmers were stimulated to expand and increase production (Braakman, 2011; Mansholt Campus, n.d.; Mulder, 2014; Van der Klaauw, 2016). These stimulations have resulted in almost 11 million pigs in the Netherlands (CBS, 2022). Worldwide, more than 745 million pigs are produced yearly (USDA, 2022), of which the majority is used for meat production.

The pig production sector has been criticized and studied because of reduced animal welfare of pigs. Although the housing systems have changed over the years, there is still room for improvement. For example, the farrowing crates in which sows are housed from right before farrowing until the weaning of the piglets. The farrowing crates are about 1.90 m x 0.8 m to decrease the events of piglet crushing. However, this also limits social contact with other sows, movement, and nest-building behaviour and thus can decrease sow welfare (Sánches-Salcedo & Yáñez-Pizaña, 2022).

Next to that, the decisions of the farmers are often between animal welfare or financial loss. Sows with fertility issues or health problems may have to be culled. Even though there are regulations for transporting unhealthy animals, it is found that these regulations are not strictly followed. Options for a farmer to do with a sick sow can be euthanasia, sending the sow to a hospital pen for care, or to the slaughterhouse even when the sow is unfit to be transported. Often, the farmer chooses to send the sow to slaughter because that probably has the lowest financial loss (Cockram, 2020; Engblom *et al*., 2007).

Most fattening pigs are transported twice in their life. First from the breeding farm to a fattening farm and later to the slaughterhouse. Pigs experience stress during transport and transport-related handling (Avéros *et al.*, 2008). Mixing with unfamiliar pigs can lead to more aggressive behaviour to establish hierarchy and the eabsence of food is in contrast to the five freedoms (Barton Gade, 2004; Brandt & Aaslyng, 2015). The way of handling during loading and unloading the pigs depends on the handler or farmer (Correa *et al.*, 2010), ramps, the slope of the ramps (Brandt & Aaslyng, 2015; Goumon *et al.*, 2013), noises, darkness or light, and slippery floors can be large obstacles for pigs (Brandt & Aaslyng, 2015; Correa *et al.*, 2010). The difference in temperatures in the trucks may also be a cause of stress. Higher temperatures are associated with a higher occurrence of skin lesions, indicating more slips and falls (Arduini *et al.*, 2017; Goumon *et al.*, 2013). Further, the duration of the transport (Goumon *et al.*, 2013), loading density (Gerritzen *et al.*, 2013), and driving style of the driver (Nøddegaard & Brusgaard, 2004) can all influence the experienced stress of pigs.

In the current pig production system, some occurring behaviours of the pigs indicate non-optimal welfare/housing systems. Behaviours have been linked to the affective state of pigs. For example, playing is a positive and a natural behaviour that occurs when the primary needs (e.g., food, safety, etc.) are met (Newberry et al., 1988). Behaviours that have a negative association, and are thus unwanted in our housing systems, are, for example, belly nosing, tail- and ear biting, aggression, and fighting (Peden *et al*., 2018). To prevent piglets from tail biting, the farmers coup tails as a preventative measure. However, removing a functional body part of the piglets and causing pain and discomfort while doing so, was still considered to be better than the alternative in which they would bite and eat each other's tails.

With more animals, it is probably easier for the farmer to prevent or cure unwanted behaviours and diseases with a simple 'fix' such as antibiotics and tail docking. However, in recent years research has found that that is not a solution that fixes the initial problem, it only controls the symptoms. Using antibiotics as a preventative measure has been banned in the European Union due to antibiotic resistance and human health. Therefore, the farmer had to find alternatives for keeping healthy and happy pigs.

The view on animal welfare differs between farmers. Some sheep and cattle farmers believe that increasing the number of animals on their farms (even further) would decrease the ability to provide adequate care (Buddle *et al.*, 2021). The view of farmers on animal welfare differs. Intensive sheep- or cattle farmers think that the ability to monitor every animal regularly and adhere to the five freedoms indicates good animal welfare. However, extensive sheep- or cattle farmers think that limited stress and sufficient food, water, and shelter are signs of good animal welfare (Buddle *et al.*, 2021). These views could also apply to pig farmers.

It all seems to be coming down to regular monitoring for early signs of discomfort of the animals. However, when the farmer expands and keeps more animals, it is much more difficult to keep monitoring all individual animals and giving adequate care. Next to that, the consumers (and animal scientists) demand for better animal health and welfare. Therefore, the demand for precise monitoring increases. A solution for animal welfare monitoring might be found in technology, like precision livestock farming.

1.2 Precision livestock farming technologies and welfare monitoring

Precision Livestock Farming (PLF) is the automatic "*continuous real-time monitoring of health, welfare, production/reproduction, and environmental impact*" of individual animals (p. 7, Berckmans, 2017). The information about the status of the animals and their environment can help the farmer make quick and evidence-based management decisions (Berckmans, 2014). Sensors can detect (sudden) physical or behavioural changes in animals (Stygar et al., 2021), and when an animal needs (extra) care or attention from the farmer, the PLF system can give a signal to the farmer. In this way, PLF can play a significant role in ensuring better animal health/welfare status and efficient livestock production. PLF can monitor specific behavioural or physiological signs that indicate that something is wrong with the animal and, if necessary, alert the farmer. However, because all animals (living organisms) are unique and therefore do not (re)act as the mean, not all approaches of sensor technology are suitable for animal monitoring (Berckmans, 2017). To catch the (sudden) reactions of the animals, continuous monitoring is needed. Continuous monitoring can range from every second to once a day, which depends on the measured variable. For monitoring stress, every second is recommended, while monitoring weight can be done every day (Berckmans, 2017).

1.2.1 Sensor and sensing technologies for real-time animal welfare monitoring

An electronic technology device that receives physical (e.g., humidity, temperature, movement) or physiological (e.g., the response of the animal to its environment) input and in response produces an electrical signal is called a sensor (Patel *et al*., 2020). Sensors can be used for continuous monitoring of the animal's well-being, fertility cycle, feeding behaviour, possible metabolic problems and udder health, milk yield, and – quality (Abeni *et al.*, 2019).

There are different kinds of sensors, contact and non-contact sensors, which can be invasive or non-invasive. Contact sensors need to be in direct contact with the animal's body, for example, heart rate monitors and rectal thermometers. Non-contact sensors do not need to be in direct contact with the animal. For example, an infrared thermal camera measuring the surface skin temperature or regular camera is used to observe the behaviour of the animals. Invasive sensors can give some discomfort or cause stress for the animal during the measurement period. This is not ideal when wanting to know more about the state of the animals and it also does not improve animal welfare. Therefore, using non-invasive sensors is a better option. Non-invasive sensors do not give discomfort or cause stress during the measurement period and preferably also not much during placement. Some sensors require anaesthesia to place the sensor in the animal via surgery, like photoplethysmography, a probe is placed in a blood vessel and detects changes in blood flow (Yousef *et al.*, 2019). Other examples of non-invasive sensors are a pedometer and an accelerometer around the leg of a cow to measure activity and detect heat. An overview of available sensors can be found in Table A1 (Appendix A).

Sensors can only measure so-called measured variables. Not all measured variables are useful for detecting animal well-being. However, with certain software (models) it is possible to estimate some feature variables (e.g., number/intensity of coughing events), which can be derived, indirectly, from the easily measured variables (e.g., audio signals). These feature variables can then be used to predict some target variables (e.g., respiratory diseases) as an early warning flag for the farmer.

Indicators that can be measured for animal well-being are activity from the leg or neck (GPS or activity trackers), body temperature (thermometers and infrared thermography), lameness, hoof health, heart rate, and facial expressions (cameras). For feeding behaviour and metabolic activity, feed intake, body weight, chewing activity (camera), body score condition (camera), and milk composition (sensor in milking parlour/machine) can be measured (Abeni *et al.*, 2019). Cameras have the opportunity for individual animal monitoring. (Top-view) cameras can be used, for example, for detecting low-weight pigs (Sa *et al.*, 2015), animal distribution and activity, behaviour, and disease occurrence. Physiological indicators that might help assess the animal's affective states are cardiovascular- and thermal parameters.

1.2.2 Cardiovascular parameters as indicators for welfare status

As no tool can directly measure the emotional state of animals, there are other methods needed for assessing the affective state, like heart rate activity and body temperature. Heart beat is regulated by the autonomic nervous system (ANS). The ANS branches into the sympathetic (SNS; active) and parasympathetic (PNS; rest) nervous systems. When the SNS is active it activates (stress) hormone production and thereby increases the heart rate, cardiac output, and decreased heart rate variability (HRV). When the PNS is active the heart rate decreases and the HRV increases, which allows the body to rest and restore homeostasis (McCraty &

Shaffer, 2015). The ANS reacts to the environment of the animal. The activity of the SNS and the PNS react to and regulate the affective state of the animal. Therefore, the affective state of the animal has a large influence on the heart rate. Measuring the heart rate and thereby analysing the HRV can indicate which part of the nervous system is active and thereby if the animal is experiencing stress.

Heart rate is also dependent on physical activity. When the demand for oxygen of an organ or cell increases, the heart rate increases to increase the blood flow to that area. So, an elevated heart rate during loading is not only caused by stress. This should be considered when evaluating the heart rate (Brandt & Aaslyng, 2015). Measuring the changes in heart rate per individual animal is a more precise assessment than only measuring the heart rate. And an animal in motion can influence the measures, as it might also move the heart rate monitor (Jorquera-Chavez *et al.*, 2019).

- *Transport*

A higher heart rate as found during loading and unloading of pigs might be due to handling (Correa *et al.*, 2013). This is also found in the studies of Brandt *et al.* (2017) and Brandt *et al.* (2015). The heart rate is higher during loading and unloading (97-190 bpm and 97-170 bpm).
During loading, the heart rate of pigs in winter is lower than in summer (120-134 bpm over 125-132 bpm). The pigs on an 18-hour transport (long transport) have a higher heart rate in winter than in summer (134 bpm). Right before transport, the heart rate is higher in winter than in summer (130-136 bpm over 120-130 bpm). During transport, the heart rate of pigs in winter was higher during 6- and 18-hour transport than in summer (121-125 bpm over 115-125 bpm), while during 12-hour transport the heart rate was lower (115 bpm over 130 bpm). During loading and right before transport the heart rate of pigs is higher dan during transport (Goumon *et al.*, 2013). Gerritzen *et al.* (2013) found that the heart rate in pigs was highest during loading (123-140 bpm) and lowest during the first part of the journey and the pause of the driver (103-122 bpm).

The heart rate increases during loading (130-145 bpm) and decreases gradually during the waiting period (110-120 bpm) before driving starts, to increase a little during the first transport phase (115-120 bpm). During the first stop, the heart rate decreases again (105 bpm) and stayed low during the 2nd transport phase (Correa *et al.*, 2014).

- *Measuring cardiovascular parameters*

Recording the ECG (electrocardiogram) using correctly placed electrodes is seen as the gold standard to measure heart rate, based on RR-intervals. Heart rate can be monitored using a wearable device, some are more invasive, but most can be put on like a belt around the chest and secured with an extra band. A short overview of used heart rate sensors is shown in Table A2 (Appendix A). To improve contact between the skin and the wearable sensor, often the skin where the electrodes or probe makes contact with the animal is cleaned and shaved when necessary and electrode gel is applied for better signal transmission. The main difference between these heart rate sensors is the collected data. Some, like the Zephyr Bioharness 3.0 can collect the ECG signal at a rate of 250 Hz. Others only collect the bpm every second. That makes not all devices suitable for assessing specific variables, like the HRV. The data saving also differs. Some sensors have memory space for saving data. Others need a (Bluetooth) connection to an external device (e.g., mobile phone, pc, laptop, or watch) to store data.
Studies are examining the use of (infrared thermal) imaging for non-contact monitoring of the heart rate in pigs (Barbosa Pereira *et al.*, 2019; Wang *et al.*, 2021) However, this still needs to be developed further before actual use in practice because the animal has to sit perfectly still to be able to register the small differences in the facial area and animals do move, especially during more stressful situations.

Polar heart rate monitors have been used to measure the heart rate of pigs on the day of slaughter, during transport (Brandt *et al.*, 2017; Brandt *et al.*, 2015; Gerritzen *et al.*, 2013; Goumon *et al.*, 2013; Rocha *et al.*, 2019), and novel object tests (Zupan *et al.*, 2016). Polar has different types of monitors, but in general, they all work the same. Most studies shaved the spots where the electrodes were placed in addition to cleaning and applying lubricant or electrode gel to improve connectivity. The time between putting on the Polar heart rate monitor and the start of the experiment, to let the animals get accustomed to the devices and be sure that the handling of the animals does not interfere with the experiment, ranged from one hour (Brandt & Aaslyng, 2015; Brandt *et al.*, 2017), four hours (Goumon *et al.*, 2013) and 24 hours (Correa *et al.*, 2014; Correa *et al.*, 2013; Correa *et al.*, 2010). The monitors of Goumon *et al.* (2013) were protected by a leather pouch that was connected to the rubber belt that was fitted around the pigs' chests. Brandt *et al.* (2015) and Brandt *et al.* (2017) used a specially designed nylon strap to secure the Polar Team2 Pro equipment (Polar, Helsinki, Finland). The downside of using Polar heart rate monitors is that there is a need for an external storage device that can store the data, for example, a T31 transmitter and a wristwatch receiver (Accurex Plus™, Polar Electro Oy, Kempele, Finland) as used by Gerritzen *et al.* (2013). However, with this combination, only 14 of the 32 measurements were complete and successful, the other 18 were unusable. Next to that, the monitors have a limited recording period, so it is not always possible to record the whole transport. Therefore, Goumon *et al.* (2013) divided the group, so that the first half of the transport could be recorded by the first group, and the second group could record the second half of the transport. During use on cattle, it is found that Polar heart rate monitors show inconsistent performances. This could be affected by the position of electrodes as well as a delay in heart rate changes between monitor data and ECG (Jorquera-Chavez *et al.*, 2019; Marchant-Forde *et al.*, 2004).

Gerritzen *et al.* (2013) also used remote telemetric loggers to trace the ECG of the pigs during transport. Therefore the pigs were shaved at the spots where pad electrodes were attached to the skin after the spot was cleaned with 70% alcohol. The data logger was placed on the back of the pig, protected by a metal box in a leather pouch, and held in place by an elastic belt. Duct tape was used as an extra measure to secure the belt and pouch.

Some sensors need to be applied under sedation or anaesthesia. For example, the Shimmer Optical Pulse sensing probe (Yousef *et al.*, 2019) and the Cortrium C3 (Bøgh *et al.*, 2020). Photoplethysmography (PPG) optically detects the heartbeat through changes in the blood flow and volume in the microvascular bed of tissues (Challoner, 1979 as reviewed by Allen, 2007). Therefore, there is no need for electrodes (for an ECG) or a device around the chest of a pig. The downside of using PPG technology is that it is very sensitive to voluntary and involuntary movements. Compared to the gold standard ECG, the accuracy level of heart rate collected via PPG is 91-97% (Yousef *et al.*, 2019). The Cortrium C3 is a device that can collect ECG, respiration curves, surface temperature, and accelerometer data. It seems to be a device for non-invasive monitoring of ECG, respiration, surface temperature, and movement in live pigs (Bøgh *et al.*, 2020). The C3 was placed when the pig was sedated. It was placed with adhesive tape on the left of the spine and covered with a bandage.

- *Heart rate variability*

The number of beats per minute is called heart rate. When the heart rate is 60 bpm it does not mean that the heart beats every second. It can mean that sometimes the heart beats every 0.9 seconds and sometimes every 1.1 seconds. This fluctuation is called HRV. Heart rate variability is "*the variation in time between adjacent heartbeats (known as RR-intervals)"* (Byrd, Johnson, *et al.*, 2020). Heart rate variability can be influenced by many things, such as metabolism, circadian rhythms, and core body temperature (McCraty & Shaffer, 2015).

The heart can respond fast due to the interplay of both SNS and PNS. Heart rate variability is, therefore, "*considered a measure of neurocardiac function that reflects heart-brain interactions and autonomic nervous system (ANS) dynamics*" (p. 47 McCraty & Shaffer, 2015). Even with the same heart rate, there might be a difference in physiological arousal (Farnsworth, July 2019). During stress, the HRV is lower than during rest (McCraty & Shaffer, 2015).

Heart rhythm oscillations are divided into four primary frequency bands: ultra-low-frequency (ULF), very-low-frequency (VLF), low-frequency (LF), and high-frequency (HF). The ultra-low-frequency is below 0.0033 Hz, VLF ranges between 0.0033 and 0.04 Hz, LF ranges between 0.04 Hz and 0.15 Hz, and HF ranges between 0.15 Hz and 0.4 Hz (McCraty and Shaffer, 2015).

The parasympathetic nervous system is reflected by the HF bands. The lower the frequency band, the higher the heart rate. The VL- and UL-frequencies can only be measured when the recordings take longer than five minutes. As the variations only occur every 300 seconds or longer.
When assessing stress and welfare of animals one can look at the autonomic regulation of cardiac activity by analysing the HRV (Byrd, Radcliffe, *et al.*, 2020; Jonckheer-Sheehy *et al.*, 2012).

A lower LF/HF ratio was used as an indicator of the activity of the PNS. However, that should be considered cautiously, as the balance between the PNS and SNS is not as first thought, it does not 'compete' to regulate the heart rate (McCraty & Shaffer, 2015). The emotion of worry is associated with a lower HF power (Thayer et al., 1996). During the night, LF power and LF/HF ratio have lower values and HF power has higher values. While during day time, LF power and LF/HF ratio have higher values and HF power has lower values (Bilan *et al*., 2005; Huikuri *et al*., 1994; Li *et al.*, 2019).

Short-term analysis is, on average, a couple of minutes, while long-term analysis is somewhere between 1 and 24 hours. Long-term analysis is more stable than short-term analysis, but is more time-consuming and might be more challenging to analyse due to noise. However, the long-term analysis may be better to describe the autonomic functioning of a specific population, due to constant fluctuations of cardiovascular autonomic function (Li *et al.*, 2019). Heart rates collected over a longer time may be used for more complex statistical time-domain measures. The variables that are calculated from data collected in time frames of less than five minutes can be used to compare the HRV during different activities throughout the recording period. For example, eating, sleeping, exercising, etc. (American Heart Association Inc, 1996).

1.2.3 Thermal and infrared thermography parameters as indicators for welfare status

Body temperature is the reflection of the activity in the animal's body and indirectly also reflects the health status of the animal. The changes in body temperature can be very informative and helpful in diagnosing diseases in pigs. However, one must keep in mind that the body temperature also changes within the normal rhythm of the pigs, for example, the reproductive cycle in sows. Measuring body temperature occurs mainly with a contact sensor, putting some thermometer into the mouth, rectum, or vagina. It would be easier and more comfortable for the animal when temperatures can be taken in a non-contact way (Zhang *et al.*, 2019).

Physiologically, body temperature is controlled by the central nervous system, using autonomous, endocrine, and behavioural mechanisms. Thermogenesis, rate of heat exchange between air and skin, movement, and evaporation are the most important mechanisms for thermoregulation. Vasodilation and vasoconstriction (expansion and contraction of veins to direct the blood flow) help to maintain the body temperature (Mota-Rojas *et al.*, 2021).

An animal (or object) radiates energy (electromagnetic waves) outward, which is different for each object. The difference between emissivity and temperature between the surrounding and the target is used to generate thermal gradients, which are shown in the infrared radiation energy density distribution map (thermal image). Different temperatures are shown by different colours in the infrared (IR) image. The higher the temperature, the lighter the colour. The cooler the temperature, the cooler/darker the colour (Zhang *et al.*, 2019). Infrared thermography (IRT) has been used in veterinary medicine to detect (early) pain or stress in animals because it is non-invasive. After surgery IRT can also be used to detect differences in blood flow of the surgical area and inflammations. Infrared thermography is being used as a tool to help diagnose diseases. Multiple animal species have already been assessed when not feeling comfortable; cattle, poultry, goats, rabbits, laboratory animals, and so on. Infrared thermography is a great tool to help detect diseases, injuries, and other problems that can influence animal welfare (Mota-Rojas *et al.*, 2021).

- *Transport*

Body temperature of pigs during transport can be measured by using iButton devices. The iButton device was used by both Goumon *et al.* (2013) and Gerritzen *et al.* (2013). However, in the study of Goumon *et al.* (2013), the pigs had ingested the device, while Gerritzen *et al.* (2013) placed the device in the vagina of the pigs, kept in place by a rubber ring. Looking at different loading densities, Gerritzen *et al.* (2013) found that the body temperature of pigs decreased within two to three hours after departure by a maximum of 1 ºC. During the break of the driver, the body temperature increased slowly and increased faster right after the break. Body temperature ranged from 37.6 to 39.2 ºC. The higher body temperatures recorded before the transportation could be due to the handling, as restraining can be stressful for pigs (Gerritzen *et al.*, 2013).
During loading, right before transport, and during transport, the gastrointestinal tract temperature of pigs was in winter lower than in summer (39.2-39.6 ºC vs 39.6-40.5 ºC). During transport, the temperatures were slightly higher than during loading (Goumon *et al.*, 2013).

- *Measuring thermal parameters*

A non-contact option to measure the pigs' body temperature is the use of thermal cameras. A wide range of thermal cameras is available. A short overview can be found in Table A3 (Appendix A). Other examples are the Avio thermoGear NEC G120 EX, Fluke TI32, FLIR T420, FLIR SC620, and IR-TCM 384. Arduini *et al.* (2017) used the Avio thermoGear Nec G120 EX thermal camera to measure the surface body temperature in pigs right after unloading. Lu *et al.* (2018) took top-view images of piglets with a Fluke TI32 to test the ear base temperature extraction algorithm. Riemer *et al.* (2016) used the FLIR T420 to study lateralization and emotional response in the ear temperature of dogs. Boileau *et al.* (2019) used a FLIR SC620 to take pictures at a 10-second interval during pig contests. And Rocha *et al.* 2019 used the IR-TCM 384 to test the ability of IRT to monitor pigs during transport and handling stress. The height at which the thermal cameras were mounted ranged from 1.7m to 5m above the ground (Arduini *et al.*, 2017; Boileau *et al.*, 2019; Lu *et al.*, 2018; Rocha *et al.*, 2019).

- *Regions of interest*

The body surface temperature can be measured in different places of the animal, so-called thermal windows or regions of interest (ROI). These can be the eyes, the ear base, or the vulva. The presence of blood vessels in the subcutaneous skin results in a higher temperature than in the surrounding skin areas (Jia *et al.*, 2020). If the thermal window is useful in an animal depends on the density of the hair or feather. The eyes (orbital region) and ears (ear region) are found to be the most reliable for assessing body temperature in pigs in response to (acute) stressors like transport and handling, and changes in the environment, with the largest variation in skin temperature observed in the orbital region (Arduini *et al.*, 2017; Rocha *et al.*, 2019). The eyes and ears are not covered by as much hair as other body parts (Jorquera-Chavez *et al.*, 2021; Jorquera-Chavez *et al.*, 2020). The orbital region has been used successfully to indicate core body temperature and is not affected by ambient temperatures (As reviewed by Jorquera-Chavez *et al.*, 2020). The ear base might be less accurate compared to the eye region, but it is still useful and might even be the best option when the eyes are not visible, due to angle (Jorquera-Chavez *et al.*, 2020; Jorquera-Chavez *et al.*, 2019; Lu *et al.*, 2018). For example, with a top-view image, the eyes are not always visible.

The thermal windows in an animal can change with age, stress, and biological state (e.g., farrowing). Ears, eyes, nose, breasts, back, foot, hoof, vulva, and head are all possible thermal windows. Some studies found that the forehead had the most correlation with the rectal temperature, others found a high correlation between the ear base and the internal body temperature. The highest temperature can be found in the anus, eyes, ear roots, and armpits (as reviewed by Zhang *et al.*, 2019).

The regions of interest in pigs for assessing body temperatures seem to be the eyes, ears, spine, back, rear, hind leg, and front leg (Boileau *et al.*, 2019). However, depending on the angle the pictures are taken not every region is suitable (Boileau *et al.*, 2019). Therefore, the thermal images will be made from the head as much as possible, including the ears and eyes.

1.3 Objective and research questions

This study aims to investigate the potential of using wearable and non-contact sensors, namely heart rate monitors and thermal cameras, for real-time monitoring of pig welfare status. Therefore, the following research questions have been formulated:

What is the potential of using wearable and non-contact sensors, namely heart rate monitors and thermal cameras, for real-time monitoring of pig welfare status?

- Whether and how can cardiovascular parameters indicate welfare status?
- Whether and how can thermal parameters indicate welfare status?
- Whether and how can cardiovascular parameters and acceleration indicate welfare status during transport?

The hypothesis is that after feed intake, the body surface temperature of pigs will increase, and HRV will decrease. With feed arrival, the heart rate in pigs increased and did not decrease much after feed intake (Robert *et al.*, 1997). Therefore, the HRV will probably decrease compared to before feed intake. When pigs are housed individually, the hypothesis is that the body surface temperature increases and the HRV decreases compared to paired housing. As individual housing will cause more stress to the pigs, and stress will increase the heart rate and body temperature. Body surface temperature scores and HRV are expected to be used as animal welfare indicators. However, the heart rate is mostly measured with heart rate belts, which need to be placed on the pig to make contact with the skin and can therefore be more stressful for pigs that are not used to being handled that way.

2 Materials and methods

The animals in this study were used for another research study approved by the CCD and IVD. The safety and health department of Carus (Wageningen University and Research, Wageningen, The Netherlands) approved the additional non-invasive handling for this study.

2.1 Animals and housing

A total of 50 male pigs (Tempo x Topig Norsvin TN70) of approximately nine weeks of age and 25 kg body weight were housed at Carus (Animal research facility of Wageningen University and Research) rooms 14 and 15. The rooms consisted of 13 and 12 pens (2.86 x 1.16 m each), each pen housing two pigs. The paired groups and rooms were allocated based on the body weight and litter offspring. The first week was an acclimatisation period to adapt to new housing, diet, and management. The pens were equipped with feeding troughs, drinking nipples, non-edible playing materials (toy on a chain, which was changed every Monday, Wednesday, and Friday), and a rubber mat covered part of the slatted floor to provide the laying area. During the third and fifth week, the pens were split into two pens for five consecutive days for individual housing of the pigs. The room temperature is 25 ºC for the first three days, and 23 ºC thereafter, which is according to the requirements of pigs of this age. From 7.00 to 19.00h the lights are on.

Pigs were fed a basal diet and additional test product twice a day (8.00 and 15.00h). The test product was changed after three weeks. The amount of feed was based on individual body weight. During feeding the fence between the two pens was closed. When all pigs had finished eating, or after a maximum of 2 hours, the fence was removed. Water was always available.

2.2 Experiment and experimental set-up

During the first week, pigs were socialised to humans, so they got used to being touched. From the pigs of which one person could place the belt, five pigs were chosen for this experiment. From the second week onward, three days a week data was collected for approximately three to four hours (Figure 2). A Zephyr BioHarness 3.0 chest strap (Zephyr Technology Corporation) was placed around the chest of the selected five pigs and wrapped with a vetrap bandage to secure the sensor in place. After the chest strap was placed, RBG and IR thermal videos (FLIR T1020) were captured during the different situations. The different situations included before feeding paired (BF_Pair), before feeding separated (BF_Iso), after feeding isolated (AF_Iso), and after feeding paired (AF_Pair). In week 2 the measurements took place after feeding, while in the following weeks, measurements took place before and after feeding. Therefore, each week had different occurring situations. An overview of which situations occurred in which weeks is shown in Table B1 (Appendix B). Thermal videos were captured for about one minute per pig when time allowed for multiple rounds per situation. Two smartphones were mounted to two tripods to collect RGB videos of the behaviour of the pigs during the data collection period, for about five minutes per pig, at 10-15 minutes intervals. Regular video data were not analysed for this study.

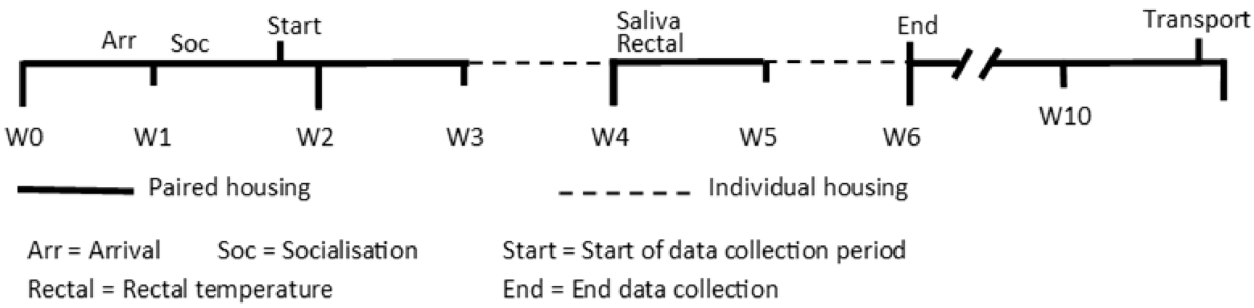

Figure 2: Timeline of the experiment. One day of data collection in week 1, three days of data collection in weeks 2-5. At the end of week 10, heart rate was measured when the pigs were transported to the slaughterhouse. Data collection included heart rate, thermographic imaging and regular videos. In week 4 saliva samples and rectal temperature were also collected.

Data were collected during the afternoon, except on the first three days of the experiment when data were collected in the morning after feeding. In the mornings, pigs were separated and fed. About half an hour after feeding, when the pigs had finished eating, the heart rate belts were placed, and data collection started and continued when the separation fences were removed, and the pigs had paired again. In the afternoon, the heart rate belts were attached before or after separation but before feeding. The collection continued during and after feeding.
In the fourth week, rectal temperature and saliva samples were collected. Rectal temperature was measured in isolation before and after feeding using a microlife vet-temp VT1831 thermometer.
Pigs were encouraged to chew a few seconds to a minute on a Q-tip until the cotton was soaked with saliva. The Q-tip was then placed in a Salivette® (from which the cotton swab was removed), and the tip was cut off.

Saliva samples were stored at 4 ºC for a maximum of two days until the samples were centrifuged at 3000 x g for 10 minutes and pipetted into a 96-Wells plate for storage at -18 ºC. The Cortisol Saliva ELISA (Tecan) analysis was done according to the instructions, and optical density was measured with a photometer at 450 nm. On the first collection day, saliva collection took place during two situations, while on the other two collection days, the saliva collections, were taken during four situations (Figure 3).

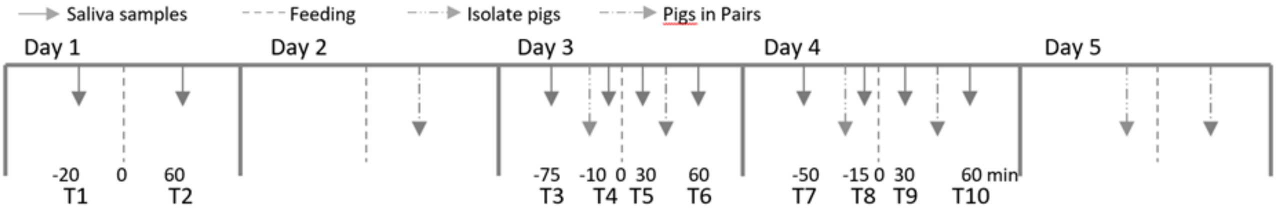

Figure 3: Study design of week 4 with relative time stamps of saliva collection in pigs. Feeding is indicated at minute zero.
For example, '-10' indicates 10 minutes before feeding and '30' indicates 30 minutes after feeding.

On the day of transportation (Figure 2), the heart rate belts were attached around the chests of the same five pigs and secured with a vetrap bandage. The five pigs were released from their cage and put together in the corridor in front of all the cages. This resulted in regrouping with pigs that they only had heard, and a new environment to explore. The five pigs were loaded onto the truck together and put in the same compartment before the other pigs were loaded. After reaching the slaughterhouse, the pigs were unloaded last, and put in a separate holding pen just for the five of them, for about half an hour before the heart rate belts were removed.

2.3 Sensors and measurements

2.3.1 Heart rate and accelerometer sensors

The Zephyr BioHarness 3.0 is a belt that can be placed around the chest of humans or animals that are large enough (e.g., pigs, cows, and horses) to measure heart rate, movement, and breathing. The device itself has memory space where collected data is saved. Via Bluetooth, it is possible to connect a laptop to see the live recording.
Table B2 (Appendix B) shows the form and specifics of data that is collected with the Zephyr BioHarness 3.0 sensor.

2.3.2 Thermography

The FLIR T1020 is a handheld infrared thermal camera with an LCD viewfinder. Table B3 (Appendix B) shows the specifications of the FLIR T1020 thermal camera.

2.4 Data and statistical analysis

2.4.1 Heart rate variability

Heart rate variability was analysed in MATLAB R2021a using an HRV tool created by Ali Youssef for the analysis in the time domain. ECG data are acquired at a sampling rate of 250 Hz. To detect the R-peak and acquire the R-R intervals, wavelet was used. Then the cumulative R-R, RMSSD (root mean square of successive differences), HRV (heart rate variability based on relative R-R intervals), and SDSD (standard deviation of successive differences) were computed and plotted over time. Heart rate (bpm) was calculated based on the R-peaks and plotted over time in a graph to be able to compare the heart rate with the HRV. An example of such a graph can be found in Figure 5.

2.4.2 Accelerometer data

Three-dimensional (3D) acceleration (vertical, lateral, and sagittal axes) data are acquired at a sampling rate of 100 Hz. Pre-processing of the acceleration signals was done by applying a Butterworth bandpass filter with cut-off frequencies of 0.05 and 10 Hz. Then the total horizontal displacement of each pig was calculated based on the resultant of the lateral and sagittal displacement according to the following formulas:

$$v_i = \int \frac{da_i}{dt},$$

$$d_i = \int \frac{dv_i}{dt},$$
$$D_t = \sqrt{d_l^2 + d_s^2}\ ,$$
where D_t is the total (resultant) displacement, v_i is the velocity, and a_i is the acceleration of i^{th} axis corresponding to lateral (l) or sagittal (s) axes.

Normalized acceleration, velocity, resultant displacement, and heart rate were plotted in graphs for visual analysis (Figure 6).

2.4.3 Thermography

Thermal video data were analysed in FLIR ResearchIR (64bit) software. Frames were chosen based on the visibility of the number of ROIs and the focus of the frame. Regions of Interest were drawn on one or two frames per video. One frame from the beginning of the video, and one frame near the end of the video. Later it was decided to only take one frame from the beginning of the video for analysis due to time constraints. Regions of interest included the ear bases, inner ears, eyes, base of the head, forehead, nose, and nose disk. In Figure 4 an overview of the location of the ROIs can be found.

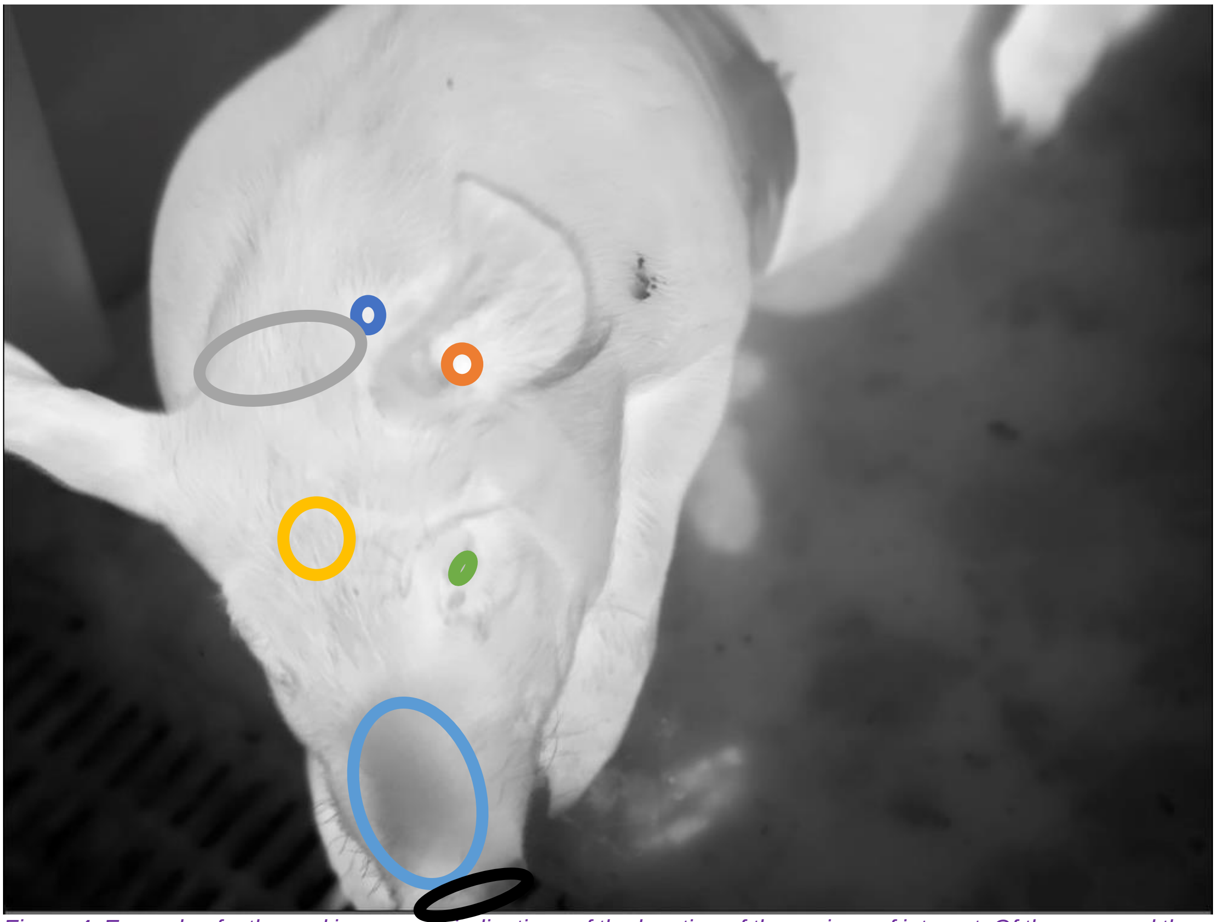

Figure 4: Example of a thermal image with indications of the location of the regions of interest. Of the ears and the eyes only the left side is indicated. Dark blue = ear base, orange = inner ear, grey = base of the head, yellow = fore head, green = left eye, light blue = nose and black = nose disk.

2.4.4 Statistical analyses

Statistical analysis was executed in SAS On Demand for Academics. The normality of all data was checked. Also, the correlation between thermal data and rectal temperature was determined with the Pearson Correlation Coefficient. The correlation between cortisol and thermal data was determined with the Spearman correlation coefficient, as the cortisol data were not normally distributed.

To quantitatively describe the strength of a relationship between two variables, the effect size can be used. Therefore, Cohen's d was also calculated. Outcome d indicates the number of standard deviations that the groups differ from each other. d = 0.2 indicates a small effect, d = 0.5 indicates a medium effect and d = 0.8

indicates a large effect. Thereby suggesting that an effect smaller than 0.2 implies that the difference is negligible, even when the ANOVA results show a statistically significant difference (McLeod, 2019).

3 Results and discussion

3.1 Heart rate and heart rate variability

3.1.1 Time domain analysis

In this section the effects of the events on the heart rate and HRV of pigs is described. Examples of the graphs can be found in Figure 5. Values (Table 1 and Table 2) in text are given as mean. Individual differences in heart rate were present, but the general trends can be seen in all pigs.

- *Belt attachment*

As can be seen in Table 1, right after the placement of the belt on the pigs in isolation (Figure 5, at approximately 14.25h), an increase in heart rate to an average of 143 bpm is visible and within five minutes the heart rate decreased to 124 bpm. The HRV was 48 ms and within six minutes the HRV decreased to 39 ms.

To place the belts around the pigs' chests, handling was necessary. Therefore, the pigs moved and may have experienced some stress for a few minutes. The increase in heart rate during and shortly after handling was expected. Other studies let the animals get accustomed to the belts before actually starting the experiment, to be sure that placing the belts did not interfere with the experiment. This waiting time ranged from one hour to 24 hours (Brandt & Aaslyng, 2015; Brandt *et al.*, 2017; Correa *et al.*, 2014; Correa *et al.*, 2013; Correa *et al.*, 2010; Goumon *et al.*, 2013). As the resting heart rate of the pigs in this experiment was fluctuating between 100 and 150 bpm, it might be that the stress experienced during the placement of the belt was low and the pigs recovered fast after this event. This indicates that the procedure itself was not very stressful for these pigs that were used to being handled.

- *Separation*

Recording the heart rate during separation of the pigs occurred only on one day. These few recordings did show that the heart rate of the pigs increased immediately when the fence was closed from 130 bpm to 144 bpm. After approximately five minutes the heart rate decreased to 117 bpm. The HRV did show an increase from 31 ms to 37 ms right after separation for about two minutes (Table 1). The increases in both HR and HRV differed between the pigs, some showed a very slight increase while other pigs did have a larger increase.

Table 1: Mean heart rate and heart rate variability of pigs during different events. Before or after an event indicates the period where the HR or HRV was stabilized before or after the event occurred.

	Mean HR in bpm (± std)	**Mean HRV in ms (± std)**
Belt attachment	143 (± 19.6)	48 (± 14.1)
After belt attachment	124 (± 17.3)	39 (± 14.5)
Before separation	130 (± 18.4)	31 (± 8.4)
During separation	144 (± 30.9)	37 (± 6.5)
After separation	117 (± 7.0)	35 (± 0.0)
Before feeding	134 (± 18.5)	55 (± 16.9)
During feeding	167 (± 16.7)	59 (± 15.6)
After feeding	133 (± 15.4)	40 (± 9.1)
Before pairing	144 (± 14.5)	38 (± 15.1)
During pairing	175 (± 37.6)	54 (± 19.1)
After pairing	140 (± 18.5)	37 (± 12.3)

HR = heart rate; HRV = heart rate variability; std = standard deviation

Physical isolation, as occurred in this study, was expected to cause some stress for the pigs. Complete physical isolation of pigs is very stressful based on salivary cortisol levels and a decrease in body temperature (Ruis *et al.*, 2001). Therefore, a large increase in heart rate and a decrease in HRV was expected. However, in this study the separation of the pigs always occurred before feeding and during the first two weeks the pigs were paired again after feeding. The pigs might have been habituated to the repetition of separation, feeding, and pairing, and might not have found the separation as stressful as was anticipated. Similarly, other studies have found that the cortisol levels of isolated pigs had decreased compared to grouped housing (Van der Staay *et al*., 2016). It is thought that the decreased activity due to smaller housing might have contributed to the decrease in cortisol levels (Geverink *et al.*, 2003). During the separation in this experiment, the pigs were still able to hear and smell each other, and even touch neighbouring pigs through the bars. It is possible that the social support received from other pigs has helped the pigs to cope with the stress of separation (Rault, 2012).

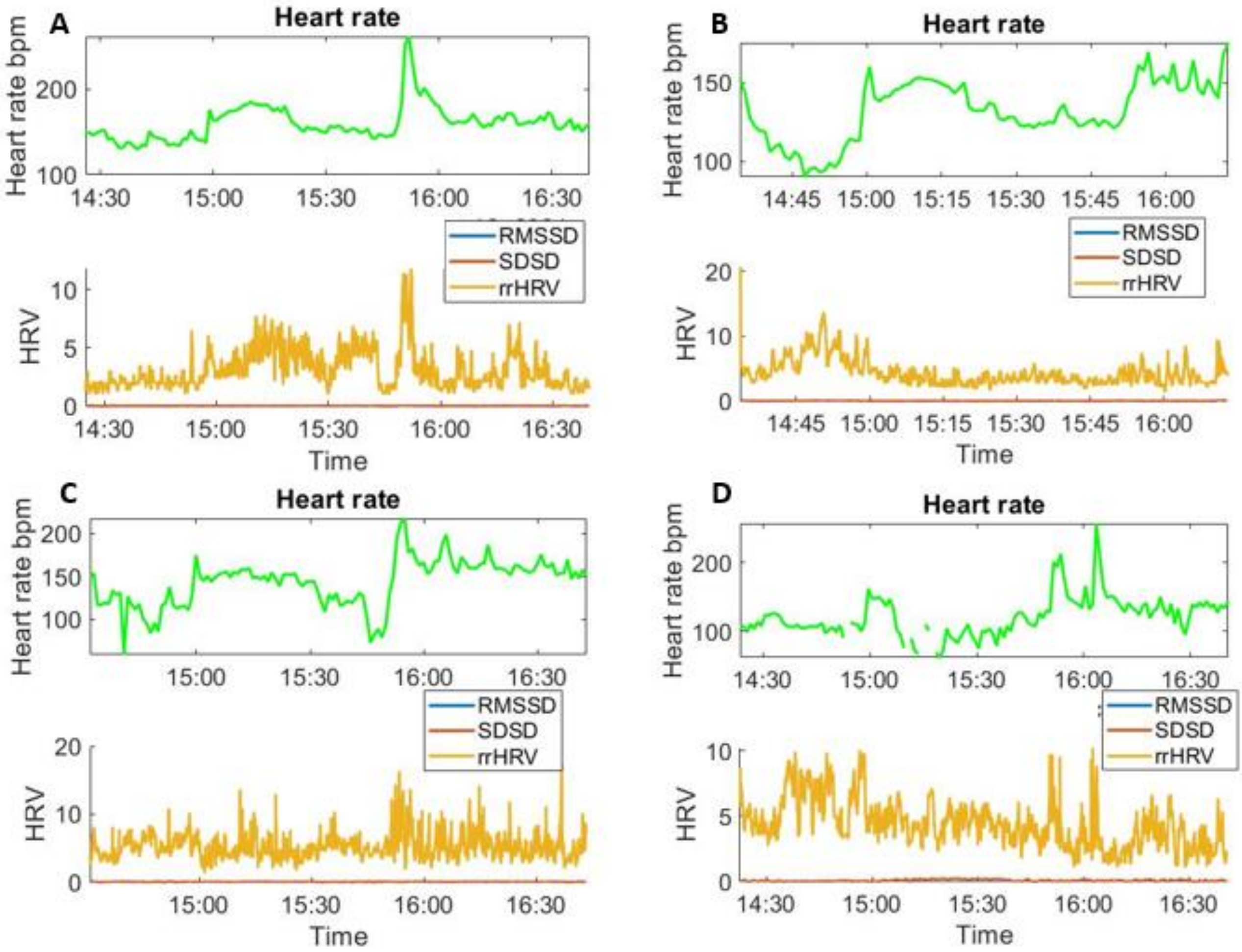

*Figure 5: Four graphs (A-D) of the heart rate and heart rate variability of four individual pigs on the same day. The unit of the HRV is cs (*10 ms). At 15.00h the pigs were fed and at 15.45h the pigs were paired.*

- *Feeding*

As can be seen in Table 1, about 10 minutes before feeding (Figure 5, at approximately 14.50h), the heart rate increased from 121 bpm to 134 bpm and increased further after feeding to 167 bpm for about 15 minutes. Then the heart rate decreased slowly to 133 bpm. The HRV also increased approximately 10 minutes before feeding from 51 ms to 55 ms. After feeding the HRV increased to 59 ms. After 15 minutes the HRV decreased to 40 ms.

Noticeably, most pigs showed an increase for about 15 minutes right after feeding, only one pig showed this increase for about 30 minutes. 15 minutes after feeding, most pigs had finished eating and started resting or playing with their toys. The pig that had an increased heart rate for 30 minutes, was the pig that was taking more time to finish eating. This suggests that eating might be accompanied by an increased heart rate.

Pigs were always fed in the same order. First the pigs in room 14, then the pigs in room 15. The sounds from the other room were noticeable, which already alerted the pigs in room 15, who became more restless and started screaming. This can be interpreted as arousal or excitement for the arrival of food. This excitement, approximately 10 minutes before actual feeding, can explain the increased heart rates and HRV 10 minutes before feeding. This is according with Robert *et al.* (1997), who found that the heart rate of pigs increased when the food arrived.

- *Pairing*

Right before removing the fence between the cage mates, the heart rate of the pigs is steady at 144 bpm. At removal of the fence (Figure 5, at approximately 15.45h), the heart rate increased immediately and stayed at 175 bpm for about 15 minutes before decreasing to 140 bpm.
Recordings after pairing took around 30-60 minutes. Some recordings were ended early because the cage mates did not stop playing with the belts or were able to remove the belts. In those cases it was decided to remove the belt before it was seriously damaged. This resulted in less data after pairing, so there was not always a clear stabilization of the heart rate and HRV visible after the fence removal.
Some recordings showed much variation in the heart rate after pairing, this might be (partly) due to noise or artefacts from the chewing of the other pig on the belt, which was observed during the experiment.

In general, the placement of the belts on the pigs resulted in a slightly increased heart rate. After a few minutes, the heart rate decreased to a resting heart rate. Before feeding the heart rate increased more and reached its peak after feeding, while the pigs were still eating. About 15 minutes after feeding the heart rate restored to resting rates. Removal of the fences resulted in immediately increased heart rates for a few minutes, after which the heart rate decreased rapidly to a slightly higher than resting heart rate. The relatively fast decrease to a lower heart rate after an event suggests that the pigs did not stay stressed and adapted to the changes in the situation.

The HRV did not show the results as expected. It was expected that during a more stressful situation, like separation or pairing, that the HRV would be lower, and that during non-stressful situations (when paired and resting) the HRV would be higher (McCraty & Shaffer, 2015). However, in these results, during the events, the HRV showed peaks instead of drops. Possible explanations for this are an error in the MATLAB-code or the events and situations were not as stressful as was anticipated for the pigs.

3.1.2 Frequency domain analysis

All data looks normally distributed, which is also confirmed by the skewness and kurtosis values of all variables. When looking at the ANOVA analysis (Table C1, Appendix C), treatment (BF_Pair, BF_Iso, AF_Iso and AF_Pair) does not seem to affect the average R-R interval and LF/HF ratio of the heart rate of these pigs. There seems to be an effect of treatment on the RMSSD. However, with further comparison, only AF_Iso differs from BF_Iso.

This is in contradiction to the results of Cohen's d (Table C1Table , Appendix C). Treatments seem to have only small effects on the average R-R interval (<0.37). Except for the comparison between AF_Pair and BF_Pair where there seems to be no effect on the average R-R interval (0.10). The effect of AF_Iso and BF_Iso can even be considered medium (-0.53). The effect of treatment on the RMSSD can be considered medium (>0.67). AF_Iso has a large difference from all other treatments (>1.32), while AF_Pair does not differ from BF_Pair (0.04). The effect of treatment on the LF/HF ratio can cautiously be considered large (>0.99), as only AF_Iso compared to BF_Pair (0.20) and AF_Pair compared to BF_Iso (-0.33) experience small effects from treatments.

3.2 Acceleration and heart rate during transport

The graphs (Figure 6) and Table 2 show that when the pigs were placed in a larger area with other pigs (regrouping), the displacement and heart rate increased to 200 bpm and 3000 m. In this place the pigs were running back and forth, using all the newly available space. However, about 11 minutes after the heart rate had increased, it decreased to 146 bpm, while the displacement of the pigs decreased after 25 minutes to 800 m.
The increase in heart rate might be partly explained by the increased activity of the pigs, as they were running around in a place that was much larger than the cages they were used to. Another part that probably influenced the increased heart rate, was the regrouping of the animals. They were suddenly put with other unfamiliar pigs that they could only hear for three months. The peaks in heart rate decreased almost as rapidly as they increased, the peaks were only present for a few minutes, and the heart rate restored quickly after. This might indicate that these pigs did not experience much stress or stay stressed by moving to a new environment with unfamiliar pigs and thus increased social stress.
At the time of loading, the heart rate of the pigs immediately increased to 203 bpm. The displacement increased to 2350 m. Probably ten minutes after loading, when the pigs were put in their compartment and the handlers were handling the other pigs, the heart rate of the pigs decreased to 143 bpm and the displacement decreased to 1188 m.

When the truck started driving there was a slight peak of an increased heart rate of 150 bpm (pig 21 did not show this increase), which decreased within 5 minutes to 145 bpm. At the start of driving the displacement was 1188 m, which decreased to 650 m after about five minutes. After the highway, there were more curves and stops on the road, which might have caused the increase in displacement (1833 m). The mean heart rate shows a decrease (138 bpm), however, in two out of three pigs the heart rate increased the third pig showed a larger decrease. The temperature on the day of transport was 5-9 ºC (Homan, 2022). It was cloudy, dry and there was not much direct sunlight. The transport took about an hour.

When the truck arrived at the slaughterhouse and stopped driving, the pigs were still waiting inside the truck. There was a slight drop in heart rate (133 bpm) and a decrease in displacement (900 m).
When the pigs were unloaded from the truck, and thus being handled and moved into a new space, the heart rate increased to 185 bpm for a few minutes and the displacement increased to 2850 m. When the pigs were put in the holding pen a rapid decrease in heart rate to 145 bpm was visible and the displacement decreased to 600 m. Unloading means people in the truck, guiding the pigs to a holding pen. The people and the equipment they used (large plastic bags) might be a factor for slight stress, which can be indicated by the higher heart rate, but this increase can also be due to walking or running to the new environment. The rapid return to the normal resting heart rate indicates that these pigs did not experience unloading as very stressful.

Table 2: Mean heart rate and mean displacement of pigs on the day of transport. After an event indicates the period where the HR or displacement was stabilized after the event. End of the drive indicates the last 5-10 minutes of the drive, after leaving the highway.

	Mean HR in bpm (± std)	**Mean displacement in m (± std)**
Regrouping	200 (± 8.2)	3000 (±1620)
After regrouping	146 (± 12.9)	800 (± 495)
Loading	203 (± 25.1)	2350 (± 770)
After loading	143 (± 13.0)	1188 (± 1081)
Driving	150 (± 7.9)	1188 (± 817)
During drive	145 (± 9.4)	650 (± 802)
End of drive	138 (± 20.1)	1833 (± 624)
Arrival	133 (± 20.1)	900 (± 294)
Unloading	185 (± 5.0)	2850 (± 650)
Holding pen	145 (± 5.0)	600 (±400)

HR = heart rate; std = standard deviation

- *Heart rate sensor*

With no other instructions than “place the belts around the chest”, the belts were placed around the chest of the pigs in a way and at a place that seemed most logical and provided data to the software via Bluetooth. No shaving of hairs or cleaning of the skin occurred before placing the belts. This might have influenced the connectivity of the sensors on the skin, especially when the pigs grew more hair. Another thing that was noticed during the experiment, is that when the pigs laid down, and thereby lie on the belt, the belt did not always attach properly to the skin while the pigs breathed out. Depending on the side and way the pigs lied down, this could result in loss of data or on-off connection related to the breathing of the pigs. The vetwrap did keep the belts clean and secured during the measurement period.
Placing the belt on separated pigs was doable, and other than the loss of contact while the pigs were lying down, the measurements were fine most of the time. When the pigs were paired, most other cage mates did play with the heart rate belts by biting the belt, the ends of the vetwrap and as far as observed during the sampling period, mostly the places where there was a bump (excess of the belt or the recording device) under the vetwrap. The other pigs could grasp these parts and that could have encouraged them to continue showing this behaviour. After a few minutes, most cage mates did leave the belts alone and lay down together with the pig to rest. Some cage mates did not rest and kept on biting in the belt. A future option to discourage this behaviour, might be to make sure that the belts are as flat as possible and there are no bumps that the other pigs can grasp with their mouths. Or adding something that does not taste well, but is not harmful to the pigs when ingested.

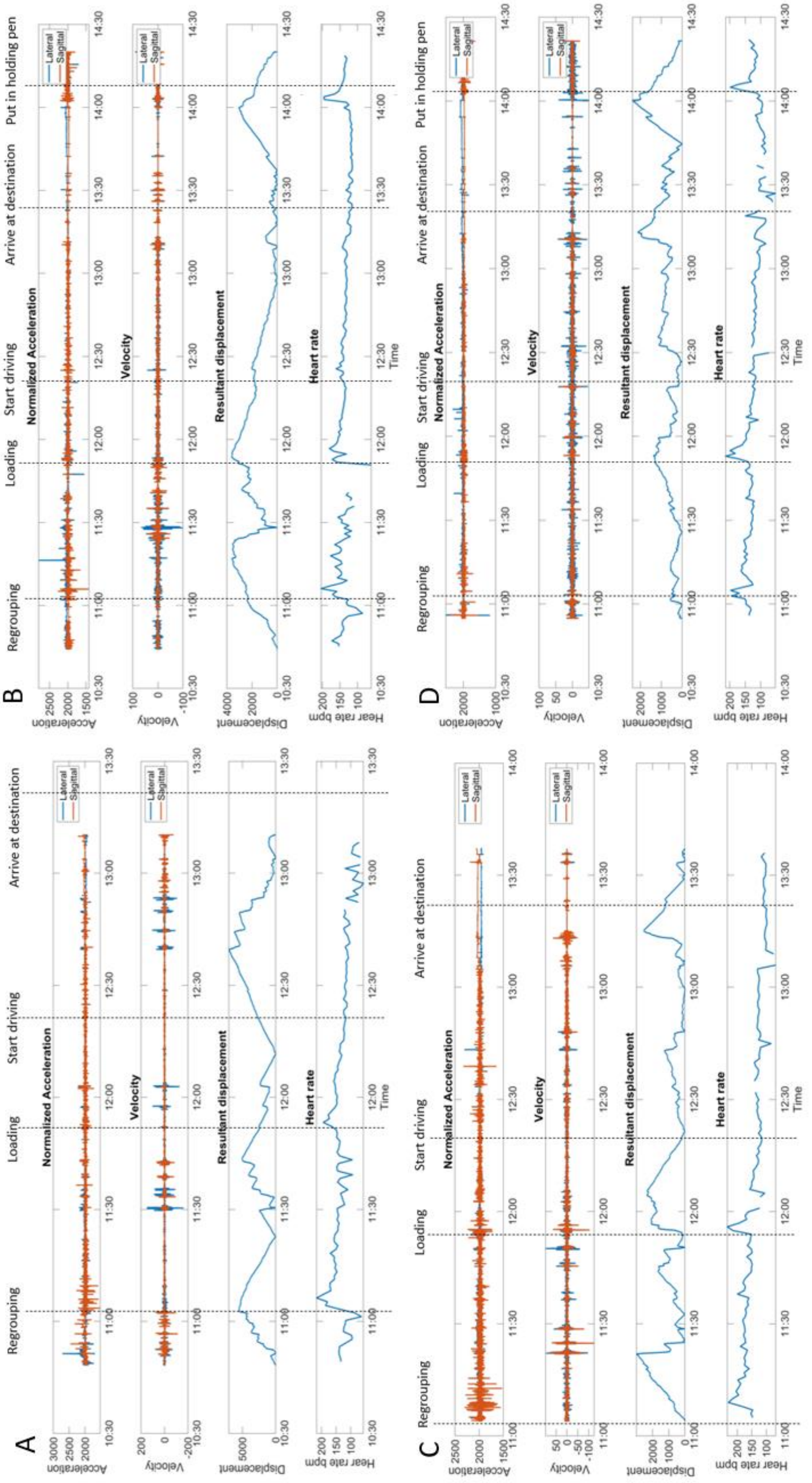

Figure 6: Four graphs (A-D) of the acceleration, activity and heart rate of pigs on the day of transport. The dotted lines indicate when the events took place.

3.3 Infrared thermography

Pig seems to affect all thermographic variables ($P < 0.0001$) and there seems to be a difference between the left and right inner ear ($P = 0.013$). Treatment does not seem to affect the skin temperature of pigs measured by a thermographic camera (Table C2, Appendix C). However, in this experiment, the data were very unbalanced. Not all treatments occurred on all test days, and not all treatments occurred in the same amounts on the test days. This makes it difficult to draw any conclusions. Another thing that needs to be considered when looking at this data, is that the data were not corrected for the incorrect camera settings on several, but not all, days.

Although this study did not look at the ambient temperatures and climates, that still might affect the surface body temperature (Arduini *et al.*, 2017) and thereby possibly also the heart rate data and thus results during transport. In Carus the ambient temperatures and humidity were controlled. During transport that was not possible.

In this study, a FLIR thermal camera was used to measure the body surface temperature of pigs. However, the disadvantages of this camera are the accuracy of the measurements, the non-tracking of the moving pigs and the difference in distance between the lens and captured animal. The accuracy is not enough to register small temperature differences in the pigs, as the differences measured can also be due to variations of the camera. The pigs movements made it harder to keep them within the set range of the camera. It's not possible to adjust the distance settings during recording. And while filming, the pigs were able to turn and have their bodies or other objects blocking the view of their heads. Therefore using more than just the head for body surface temperature analysis might be useful in the future.
Even though the FLIR camera has a high resolution, which is needed to be able to predict the body temperature of the pigs (Stukelj *et al.*, 2022), the accuracy at which the camera can predict, and not even measure, the pig's body temperature, is low.

An ROI that is found to be useful for measuring skin temperature to measure the pig's body temperature was the inner ear (Stukelj *et al.*, 2022), as that one was most comparable to the rectal temperature measured.
In calves, the eye temperature decreases very rapidly after a stressful situation and increases later again (Stewart *et al.*, 2008). This is not found in the internal temperature, which does decrease at a much slower rate after a stressful situation. This might be explained by the thermoregulation of homeothermic animals. The blood flow to the skin, gut and other organs can be regulated, depending on the situation (e.g., heat, cold, stress) (Jorquera-Chavez *et al.*, 2019; Taylor *et al.*, 2014). The eye or inner eye canthus are found to be less accurate to measure or predict the body temperature of pigs. With the thermal camera, the region in the frame is often too small. A very high resolution would be needed, and a short distance between the camera and the pig to get a good view of the region and have enough pixels available (Stukelj *et al.*, 2022). Even when that is possible, eyelashes can be in the eye region in a 2D image, and thereby influencing the measured temperatures of that region (Stukelj *et al.*, 2022).

Visible light might have a great impact on the quality of the thermal images. When there is low illumination, the images have less noise, when the illumination increases, the images will have more noise. Another factor that could influence the amount of noise in the images is temperature. Higher temperatures will result in more noise (Zhang *et al.*, 2019).

3.3.1 Rectal temperatures

There does not seem to be a linear relationship between thermographic temperatures and rectal temperatures in treatments BF_Iso and AF_Iso (Table C3, Appendix C).

Due to the lack of determined gold standards for pig body temperature measurements and the fact that measuring rectal temperatures is relatively easy and cheap, it is often used as the gold standard for comparison (Stukelj *et al.*, 2022). A promising measurement that can be used as the gold standard in the future is the temperature of the inner ear of pigs measured via IRT (Stukelj *et al.*, 2022). It is already considered in humans, it is convenient to measure and not as influenced by the environment in contrast to other ROIs.

3.4 Salivary cortisol

Pig has the most effect on salivary cortisol levels (Table C4, Appendix C), meaning that there are variations between individual pigs. Treatment and day ($P = 0.270$ and $P = 0.931$) do not seem to affect the cortisol levels in this experiment.

When assumed that a linear relationship between two variables is considered strong with $r > 0.70$. There does not seem to be a linear relation between salivary cortisol levels and thermographic temperatures ($-0.44481 < r < 0.22235$).

The activity of the HPA-axis caused by stressors can be indicated by salivary cortisol levels (Escribano *et al.*, 2015). Increased salivary cortisol levels indicate that pigs experienced a stressor (Escribano *et al.*, 2019; Escribano *et al.*, 2015). After regrouping, the salivary cortisol was higher, while isolation showed no raised cortisol levels (Escribano *et al.*, 2015). However, salivary cortisol might not be the best option to indicate social stress responses (Escribano *et al.*, 2019). Measuring salivary cortisone might be a better indicator than salivary cortisol (Bae *et al.*, 2019).

Since pig has a large effect on the salivary cortisol levels in this study, there is a need for much larger sample size for being able to research the effect of the treatment on the salivary cortisol levels. Therefore, this experiment cannot draw conclusions about the correlation between the salivary cortisol levels and the thermographic video temperature or HRV.
Next to that, the timing of sampling was not ideal, sometimes just a few minutes had passed in the next situation, which might not have been enough time for the cortisol levels in the saliva to change.

Conclusion

This study aimed to investigate the potential of using wearable and non-contact sensors, namely heart rate monitors and thermal cameras, for real-time monitoring of pig welfare status. Having the ability to continuous monitor the animals' heart rate and body surface temperature seems to be useful for monitoring animal welfare. However, probably not all physiological changes can be monitored as they are too small for the accuracy of the sensor. Separate housing and feeding do not seem to significantly influence the body surface temperature, heart rate, and heart rate variability of pigs in this experiment. Using wearable heart rate sensors in pigs in separated and paired housing and during transport might, with a few adaptations to ensure connection and security, be a good way to monitor the changes in heart rate. Infrared thermography seems a promising tool to monitor temperature changes in animals when the accuracy of the cameras are higher.

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Appendix A

Introduction

Table A1: Overview of available sensor technology and what it can be used for.

Sensor technology	Indicator	Measure variables	Contact, invasive	References
Accelerometer	Activity, heat, behaviour	Speed, distance	Contact, non-invasive	Cornou *et al.*, 2011; Gomez *et al.*, 2021; Zhang *et al.*, 2022
Infrared thermography	Health, fertility, locomotion problems, behaviour	Body surface temperature	Non-contact, non-invasive	Gomez *et al.*, 2021; Sykes *et al.*, 2012; Zhang *et al.*, 2022
pedometer	Activity, locomotion problems, behaviour		Contact, non-invasive	Zhang *et al.*, 2022
Visual sensors (cameras)	Activity, behaviour, locomotion problems, position		Non-contact, non-invasive	Gomez *et al.*, 2021; Ott *et al.*, 2014; Zhang *et al.*, 2022
Water flowmeters	Drinking behaviour	Water flow	Non-contact, non-invasive	Larsen *et al.*, 2019; Zhang *et al.*, 2022
Cardiovascular sensors	Activity, health, affective state	Heart rate, ECG	Contact, non-/minimally invasive	Gerritzen *et al.*, 2013; Goumon *et al.*, 2013
Microphone	Health, affective state	Sound	Non-contact, non-invasive	da Silva Cordeiro *et al.*, 2013; Gomez *et al.*, 2021; Van Hirtum & Berckmans, 2004

Table A2: Overview of used heart rate sensors on pigs in literature.

Article	Application	Heart rate sensor	Collected data
Bøgh *et al.* (2020)	Under sedation	Cortrium C3	ECG, respiration curves, surface temperature and accelerometer
Yousef *et al.* (2019)	Under sedation; ear, upper tail and the left back leg	Shimmer Optical Pulse sensing probe	Heart beat through changes in blood flow
Gerritzen *et al.* (2013)	Pad electrodes and data logger in a metal box and leather pouch attached to an elastic belt on the pig's back.	Remote telemetric loggers	ECG
Gerritzen *et al.* (2013)	Heart rate belt	Polar + wristwatch	Heart rate
Goumon *et al.* (2013)	Shaved and lubricant gel	Polar	Heart rate
Brandt *et al.* (2015), Brandt *et al.* (2017)	Heart rate belt applied with water and gel	Polar Team2 Pro	Heart rate
Zupan *et al.* (2016)	Heart rate belt	Polar belt and wristwatch computer	HRV
Correa *et al.* (2014;Correa *et al.*, 2013; Correa *et al.*, 2010)	Heart rate belt	Polar	HR

Table A3: Literature table on use of thermal camera in practice. ROI is Region of Interest.

Article	Application	Dataset info	ROI	Thermal camera
Lu *et al.* (2018)	Automatic ear base temperatures extraction from top view	20 Meishan piglets, 7-8 days old	Ear base	Fluke TI31, 320x240
Basak *et al.* (2020)	Pig body temperature prediction based on ambient temperatures	6 Yorkshire piglets, 10 weeks old	Left and right side, forehead and back of piglet	IR sensor MI3
Arduini *et al.* (2017)	Measure of stress after transportation	1400 crossbred (Durock x (Landrace x Large White))	Dorsal and ocular	Avivo thermoGear Nec G120 EX
Teixeira *et al.* (2020)	Assess effect of tail lesions severity on skin temperature of pigs	269 (Large White x Landrace) 100-110 kg of body weight	Base of tail and ear	TESTO 875-2i
Stukelj *et al.* (2022)	Determine suitable ROIs for similarity with rectal temperature, predict pig body temperature	15 breeding sows and 1 boar (Landrace x Yorkshire)	Ear canal, eye canthus, outer ear and perianal area	Flir T650sc and Fluke TiS45

Appendix B

Materials and methods

Table B1: Overview of measurement situations during the collection period.

Week	Situations
2	Isolated after feeding Paired after feeding
3	Isolated before feeding Isolated after feeding
4	Paired before feeding Isolated before feeding Isolated after feeding Paired after feeding
5	Isolated before feeding Isolated after feeding
10	Transportation

Table B2: Specifics of data collected by Zephyr BioHarness 3.0 (Zephyr Technology, 2010).

Summary and Waveform Log

Parameter	Reporting Frequency (Hz)	Range	Units	Description
All summary parameters	1			As Summary Log Format
Breathing Sensor Waveform	25		*	
Vertical Axis Accelerometer	100	0 – 4095	Bits	Centered on 2048 1g = 83
Lateral Axis Accelerometer	100	0 – 4095	Bits	Centered on 2048 1g = 83
Sagittal Axis Accelerometer	100	0 – 4095	Bits	Centered on 2048 1g = 83
ECG	250	0 – 4095	Bits	Indicative
Heart Rate RR intervals	Per R detection	250 – 1500	ms	40 – 240bpm equivalent
Breathing BB intervals	Per B detection	850 - 15000	ms	4 – 70Bpm equivalent
Event	Per event			See Event Descriptions

*Raw breathing sensor output.

Table B3: Specifications of the FLIR T1020 thermal camera. Adapted from Teledyne FLIR (2022).

Built-in digital camera	5 Mpixel with LED light
Detector Pitch	17 µm
Detector type	Focal plane array (FPA), uncooled microbolometer
Field of view (FOV)	12º x 9º
f-number	1.2
Focal length	83.4 mm
Focus	One shot or manual
Frame rate	30 Hz
Image frequency	30 Hz
Image models	Thermal, thermal MSX, picture in picture, digital camera
Infrared image	Full color infrared image
IR resolution	1024 x 768; up to 3.1 MP with UltraMax
Minimum IR focus distance	1.3 m
MSX resolution	1024 x 768 pixels
Multispectral Dynamic Imaging (MSX)	Thermal image with enhanced detail present
Thermal sensitivity	< 20 mK @ 30 ºC
Area	5 + 5 areas (boxes and circles) with max./min./average
Atmospheric transmission correction	Automatic, based on the inputs for distance, atmospheric temperature, and relative humidity
Difference Temperature	Delta temperature between the measurement functions and the reference temperature
Emissivity Correction	Variable from 0.01 to 1.0 or selected from the materials list
External optics & windows correction	Automatic, based on the inputs of the window transmission and temperature
Measurement corrections	Emissivity, reflected temperature, relative humidity, atmospheric temperature, object distance, external infrared window compensation
Object temperature range	-40 ºC to 2000 ºC
Object temperature range Accuracy	± 1 ºC or ±1% at 25 ºC for temperatures between 5 ºC to 150 ºC or ±2% of reading at 25 ºC for temperatures up to 1200 ºC.
Reference temperature	Manually set using the difference temperature
Reflected apparent temperature correction	Automatic, based on the input of the reflected temperature

Appendix C

Results

Table C1: One-way ANOVA analysis and effect size of different situations on the HRV parameters; average R-R interval, RMSSD and LF/HF-ratio. Where Mean = Least Square Means, Stdev = standard deviation.

Variable	Treatment1	Treatment2	Mean1	Stdev1	Mean2	Stdev2	P-value	Cohen's d	r
AVG_RR	AF_Iso	BF_Iso	0.4221	0.0249	0.4427	0.0494	0.7350	-0.5265	-0.2546
	AF_Iso	AF_Pair	0.4221	0.0249	0.4307	0.0219	0.9725	-0.3664	-0.1802
	AF_Iso	BF_Pair	0.4221	0.0249	0.4285	0.0214	0.9884	-0.2746	-0.1360
	AF_Pair	BF_Iso	0.4307	0.0219	0.4427	0.0494	0.9306	-0.3139	-0.1550
	AF_Pair	BF_Pair	0.4307	0.0219	0.4285	0.0214	0.9995	0.1023	0.0511
	BF_Iso	BF_Pair	0.4427	0.0494	0.4285	0.0214	0.8914	0.3734	0.1835
RMSSD	AF_Iso	BF_Iso	0.0950	0.0025	0.0576	0.0224	0.0285*	2.3531	0.7620
	AF_Iso	AF_Pair	0.0950	0.0025	0.0739	0.0188	0.3183	1.5764	0.6190
	AF_Iso	BF_Pair	0.0950	0.0025	0.0730	0.0235	0.2873	1.3162	0.5497
	AF_Pair	BF_Iso	0.0739	0.0188	0.0576	0.0224	0.5344	0.7882	0.3666
	AF_Pair	BF_Pair	0.0739	0.0188	0.0730	0.0235	0.9999	0.0395	0.0197
	BF_Iso	BF_Pair	0.0576	0.0224	0.0730	0.0235	0.5761	-0.6734	-0.3191
LF/HF_Ratio	AF_Iso	BF_Iso	0.4603	0.0631	0.5739	0.0579	0.1744	-1.8766	-0.6842
	AF_Iso	AF_Pair	0.4603	0.0631	0.5460	0.1037	0.3880	-0.9979	-0.4465
	AF_Iso	BF_Pair	0.4603	0.0631	0.4438	0.0968	0.9889	0.2014	0.1002
	AF_Pair	BF_Iso	0.5460	0.1037	0.5739	0.0579	0.9497	-0.3326	-0.1641
	AF_Pair	BF_Pair	0.5460	0.1037	0.4438	0.0968	0.2476	1.0182	0.4537
	BF_Iso	BF_Pair	0.5739	0.0579	0.4438	0.0968	0.1012	1.6315	0.6321

Table C2: Analysis of body surface temperature, N = number of samples, LSM = Least Square Mean, SEM = Standard Error Mean, P = P-value.

		Ears				Eyes				Inner ears				Base head			
R-Square		0.453				0.547				0.395				0.653			
Intercept			34.8	0.2			32.2	0.2			33.7	0.6			33.9	0.2	
		N	LSM	SEM	P	N	LSM	SEM	P	N	LSM	SEM	P	N	LSM	SEM	P
Treatment	AF_Iso	112	35.7	0.1	0.802	112	33.3	0.1	0.964	92	35.0	0.2	0.986	56	34.5	0.1	0.713
	AF_Pair	60	35.5	0.1		60	33.3	0.1		48	34.9	0.3		29	34.4	0.1	
	BF_Iso	90	35.6	0.1		90	33.3	0.1		74	35.0	0.2		45	34.5	0.1	
	BF_Pair	20	35.6	0.2		20	33.3	0.1		15	35.0	0.4		10	34.4	0.2	
Side	Left	141	35.6	0.1	0.928	141	33.3	0.1	0.450	123	35.2	0.2	0.013	–	–	–	–
	Right	141	35.6	0.1		141	33.3	0.1		106	34.7	0.2		–	–	–	–
Pig	13	58	36.1	0.1	<.0001	58	33.0	0.1	<.0001	56	35.6	0.2	<.0001	29	34.8	0.1	<.0001
	15	56	35.5	0.1		56	33.3	0.1		52	35.2	0.2		28	33.6	0.1	
	17	58	35.8	0.1		58	33.6	0.1		24	35.7	0.3		29	35.0	0.1	
	21	54	35.4	0.1		54	33.4	0.1		50	34.0	0.2		27	34.3	0.1	
	22	54	35.2	0.1		54	33.1	0.1		46	34.3	0.2		27	34.4	0.1	

Table C2 continued

		Forehead				**Nose**				**Nose disk**			
R-Square		0.734				0.788				0.758			
Intercept			34.0	0.2			26.0	0.6			23.0	0.8	
		N	**LSM**	**SEM**	**P**	**N**	**LSM**	**SEM**	**P**	**N**	**LSM**	**SEM**	**P**
Treatment	**AF_Iso**	56	34.6	0.1	0.768	56	30.7	0.2	1.000	55	29.0	0.2	0.999
	AF_Pair	29	34.5	0.1		29	30.7	0.3		28	29.0	0.3	
	BF_Iso	45	34.6	0.1		45	30.7	0.2		45	29.0	0.3	
	BF_Pair	10	34.6	0.2		10	30.7	0.4		10	29.0	0.6	
Side	**Left**	–	–	–	–	–	–	–	–	–	–	–	–
	Right	–	–	–	–	–	–	–	–	–	–	–	–
Pig	**13**	29	35.1	0.1	<.0001	29	30.4	0.2	<.0001	29	27.8	0.3	<.0001
	15	28	33.8	0.1		28	30.3	0.3		26	29.0	0.3	
	17	29	35.3	0.1		29	33.2	0.2		29	32.0	0.3	
	21	27	34.0	0.1		27	30.6	0.3		27	30.3	0.3	
	22	27	34.8	0.1		27	29.0	0.3		27	25.9	0.3	

Table C3: Correlation between rectal temperature and the body surface temperature measured by the thermal camera.

	N	**Pearson Correlation Coefficient**	**R-Square**	**P-value**
Ears	27	0.488	0.238	0.010
Eyes	27	0.237	0.056	0.234
Inner ears	19	0.584	0.083	0.009
Base Head	27	0.289	0.106	0.144
Forehead	27	0.326	0.023	0.097
Nose	27	-0.024	0.340	0.453
Nose disk	23	0.151	0.001	0.914

Table C4: Cortisol analysis.

R-Square	0.511896				
Intercept			0.105216	0.104695	
		N	**LSMean**	**SEM**	**P-value**
Treatment	**AF_Iso**	15	0.310	0.060	0.270
	AF_Pair	10	0.224	0.079	
	BF_Iso	15	0.408	0.060	
	BF_Pair	10	0.259	0.079	
Pig	**13**	10	0.124	0.075	<.0001
	15	10	0.482	0.075	
	17	10	0.165	0.075	
	21	10	0.597	0.075	
	22	10	0.131	0.075	
Day	**26**	10	0.297	0.082	0.931
	28	20	0.288	0.052	
	29	20	0.315	0.052	