Thermography as a measure of the effects of enrichment and repeated mixing on resilience in pigs.

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Abstract

In current husbandry systems, pigs are frequently exposed to a variety of physiological and psychological challenges that may impact their ability to recover from stress i.e., resilience. Estimating an animals' welfare is a multi-dimensional concept, which requires multiple measurements to obtain a picture of an animals' physical and mental state. In practice, a great deal of animal welfare estimation procedures are highly invasive and in themselves, may induce undue stress. There is a strong demand for alternative and reliable non-invasive methods to gather data, to ensure that the animals do not endure unnecessary suffering.

In this study we investigated temperature responses to the combined effects of differing enrichment conditions and allostatic load, achieved through repeated mixing, on the resilience of pigs. Pigs were either exposed to barren housing (B) post weaning, or provided with enrichment (rooting material, extra toys, access to extra space, and positive human contact (E)). Half of the pigs were exposed to repeated mixing (RM) while the other half were only exposed to one mixing event at weaning (MM) in an attempt to create varying states of allostatic load. To assess their resilience, their temperature response to a physiological challenge in a lipopolysaccharide (LPS) sickness test, and a psychological challenge in a Frustration (isolation) test were measured using infrared thermography (IRT).

Pigs demonstrated a temperature response as a result of the challenges. E pigs had faster recovery rates during the LPS challenge, returning to baseline temperature earlier than B pigs. However, enrichment did not seem to impact the temperature response to the Frustration challenge. Repeated mixing did not have an effect on resilience in both challenges, contrary to the expectations. The accuracy and repeatability of using thermography to measure temperature was convincing for this study, insofar as its intra-observer repeatability which showed strong positive correlations and non-invasive nature.

To conclude, enrichment enhanced the recovery rate in pigs to a LPS challenge, which seems to indicate a better resilience in a sickness challenge. Repeated mixing did not seem to create a state of high allostatic load, and the combination of barren housing and repeated mixing did not seem to exacerbate the effects of lack of enrichment, as measured by temperature. The utilization IRT as a measure of resilience and the use of thermography as a non-invasive measurement tool was promising.

Lay summary

Animal welfare is a continuously growing area of study for scientists. We are consistently looking for ways to make animal farming more ethical and sustainable. Pigs are often subject to poor quality environments that lack enrichment, and can develop illness and stress as a result of barren housing and repeated transportation and social mixing. In this study we looked at using thermal imaging as a non-invasive tool to understand the welfare of pigs. This involved measuring the external temperature of pigs in different enrichment conditions and different amounts of social mixing after two challenges. The challenges included an sickness challenge and a frustration (isolation) challenge. We found that pigs in enriched conditions recover faster from an illness than from barren conditions. We did not get any significant results for response to the frustration challenge, and social mixing did not seem to create excess stress, in this study. The results are promising for the use of thermography as a tool to detect changes in temperature and can be used to indicate possible sickness in pigs. We predict that research in using thermography in animal farming will prove to be a valuable tool in better understanding the welfare of animals.

1. Introduction

Current views on animal welfare have been subject to large social debate in developed countries (Blokhuis, 2004; Glass et al., 2005). Increase in affluence and media perspective on meat has affected the economy of animal farming. Over the past five decades, farming had become increasingly commercial. A large focus on quantity over quality, coupled with public policy in Europe for cheaper and more accessible food, led to an increase in animal production efficiency (Lassen et al., 2006). Effectively, farming was industrialised, which provided meat that was at affordable prices and available to most people. After World War II, farming efficiency was achieved through intensification of farming processes. One of these changes was the shift into more artificial environments, in its attempt to limit/eradicate diseases. However, the idea of welfare and the demand for better animal welfare from consumers only began around the 1960's (Keeling, 2005). Nowadays, there is a strong consumer incentive for farms to maintain high welfare standards in order to be competitive in the economic market.

Currently the Netherlands has a large pig industry, with approximately 12 million pigs (Wageningen Economic Research, 2021). Within large industries, pigs are commonly exposed to conditions that promote cumulative stress, such as sudden weaning, poor environmental conditions, high pathogenic load and repeated regrouping events. These stressors may impact pigs' ability to deal with challenges i.e., resilience. Resilience can be defined as the capacity for an animal to recover from an impact of a physical and/or social stress (Colditz & Hine, 2016). This ultimately influences the health and welfare of the animal. The estimation of welfare itself sometimes leads to stress due to human handling and invasive procedures. Therefore, there is a demand for reliable non-invasive methods to ensure that animals do not endure any undue stress and suffering when invasive welfare or resilience measurement techniques are required.

1.1 Welfare

Defining welfare has largely been of ethical debate, because the 'ideal' is relative. Often times, there is a range of general to specific definitions of the term that can leave grey areas (Keeling, 2005). A widely used definition is "The welfare of an individual is its state as regards to its attempts to cope with its environment" (Broom, 1986). Whereby, to cope with something, can be achieved with little effort and expenditure of resources. But herein lays the small issue of how one measures an animal's ability to 'cope'. Within animal welfare 'science' there are basically 3 animal-centered approaches (Fraser, 1995; Fraser et al., 1997). The first concerns itself with the health and fitness of the animal. Whereby issues such as prevention of disease and/or injury are the most important challenges. Secondly, the focus lies on the animals' preferences and feelings (pleasure/suffering). Lastly, the welfare concerns the ability of animals to express their natural behaviour. In summary these concepts emphasise the importance of animal bodies, minds and natures (Appleby, 1999).

1.2 Societal challenge

Following the World War II, the shift to intensified farming occurred roughly around the same time as an emigration of a majority of the population from rural areas into towns, and perhaps more importantly, away from animal husbandry practices (Keeling, 2005). This led to a mismatch between the public's perception on farming and the true realities of the modern production sector (Blokhuis, 2004). This disparity and/or lack of insight, over time, has made the general public more sensitive to the harsh truths of animal production.

This being said, there was a growing demand from the public for ethical practices and this push led to changes in legislation in Europe. Both the Council of Europe and the European Union (**EU**) had to adjust laws regarding the breeding, transport, slaughter and conservation of farm animals (e.g. European Convention for the Protection of Animals kept for Farming Purposes (ETS87, 1976)) as well as companion animals, wild animals and laboratory animals (Bennett et al., 2002; Blokhuis, 2004; Caporale & Alessandrini, 2005).

There now is a strong incentive for the animal production industry to meet consumers demands for animal-welfare friendly products. Studies have shown that claims such as free-range, low carbon footprint and an animal friendly approach, add value to products, and impact consumers' consumption behaviour (Burnier et al., 2021; Krystallis et al., 2012). A study by Thorslund et al. (2017) ranked the perceived importance for market driven pig welfare. The results strengthened the claim that consumers generally rank animal welfare as important and consider 'good' pig welfare as an important indicator of meat quality. They also found that while consumers had a wide range of pig welfare concerns, the focus was primarily on the 'naturalness' of the pigs' life (e.g., living condition and natural behaviours).

Given the market driven demand for animal welfare and the following legislation. A multitude of changes had to be taken on board, to meet the expectation of the public and governments. Current welfare practices in Europe use the 'Five Freedoms' These consist of: freedom from hunger/thirst, discomfort, pain/injury/disease, fear/distress and expression of normal behaviour (European Commission, 2022). However, more recently in European law, animals are considered ‘sentient’ beings, signifying that they are conscious feeling animals and no longer merely agricultural products (Dawkins, 2017; Korte et al., 2007). This means that as well as investigating improvements in the animals’ housing and care (Five Freedoms), we also must include consciousness, emotions and positive feelings into the investigatory process.

1.3 Homeostasis, allostasis & allostatic load

Broom's (1986) definition of animal welfare “its state as regards its attempts to cope with its environment” implies that an animal is confronted with environmental challenges and it reacts with physiological and behavioural mechanisms to maintain the constant internal characteristics of the body (milieu intérieur) (Korte et al., 2007). This term is coined homeostasis. It implies that the controlled physiological variables are kept at their ‘set point’. This proposes that without environmental challenges good animal welfare can be achieved. However, it disregards the absence of environmental challenges which produces *hypostimulation* and can lead to poor animal welfare (Korte et al., 2007).

Allostasis is an improved mechanism to explicate the disturbance of animal welfare through hypostimulation or hyperstimulation. The term is defined as the adaptive process for actively maintaining stability through change (Sterling & Eyer, 1988). The definition reflects the cumulative effects of daily life and also includes the consequences of major life challenges that result in health damaging behaviours (Guidi et al., 2021). This mechanism is important during unpredictable events, such as social hierarchy conflicts, resource competition, natural disasters, and predictable events such as seasonal changes (migration and hibernation triggers) (Korte et al., 2009). The emotional brain controls all the mechanisms at the same time using hormones to incorporate influential life factors such as feelings, memories and re-evaluation of needs in anticipation of physiological requirements (Koob & LeMoal, 2001). Some of which are allostatic mediators (adrenal hormones, monoamines like serotonin and dopamine) that act as receptors in certain organs and tissues which can produce an allostatic state (Korte et al., 2009). In essence, a prolonged effect of certain mediators or inefficient management by the organism, means that there’s is a cost the to the body. This is often referred to as ‘allostatic load’ and is described as the cumulative wear and tear to the brain and periphery (McEwen, 1998). This allostatic load may weaken the resilience of these animals when undertaking future challenges (Scheffer et al., 2018).

1.4 Enrichment

Pigs are often housed in stimulus-poor and barren housing conditions in commercial farms. Some of the welfare problems often focussed on, are the monotonous and impoverished housing conditions that are extreme contrasts to the 'free environment' ideal. These conditions don't allow for a natural range of behaviours and often impair health and performance (Zebunke et al., 2013). Therefore, legislation was implemented in the EU outlining the minimum standards for pigs, the details of which are found in Directive 2008/120/EC (Council of the European Union, 2008). The legal document states, among other things, that pigs kept in groups should have access to litter or other materials that provide the possibility for exploration and occupation (Godyń et al., 2019).

Having stated that one of the factors of good animal welfare is the ability to express natural behaviours, the next step is to identify what these behaviours consist of. Feral pigs naturally spend approximately 75% of their active time in foraging related behaviour, such as, rooting grazing and exploring with their snout (Kittawornrat & Zimmerman, 2011). Consequently, the absence of rooting materials alters the attention of pigs to other pen-mates (Beattie et al., 2000). Studies have shown that pigs kept in enriched environments were less involved in harmful social behaviour while pigs kept in barren housing conditions (e.g. common intensive pig farms) showed more behavioural and physiological signs of chronic stress (Beattie et al., 2000; Bolhuis et al., 2006; Fraser, 1995). More specifically, pigs reared in intense systems in Europe demonstrated an increase in incidents of aggression, cannibalism, and tail biting as a result of a lack of environmental enrichment (Guy et al., 2013).

Enrichment can be defined as "an increase of the biological relevance of captive environments by appropriate modifications resulting in an improvement of the biological functioning of captive animals" (Newberry, 1995). The key factors should stimulate an animals' visual, somatosensory and olfactory systems and they should provide an aspect of novelty (Nithianantharajah & Hannan, 2006). Pigs tend to lose attention and/or become habituated to an object within a few days (Ernst et al., 2018). Therefore, constant rotation of different enrichment devices is required to efficiently keep the pigs occupied. As mentioned earlier, rooting material is an important aspect to maintain pigs' natural behaviours. Straw and roughage of some sort are key materials, that when provided enough of, elicit exploratory behaviour. Olsen (2001) demonstrated that pigs that had a combination of straw and roughage had reduced redirected oral activities and skin lesions towards pen mates.

Based on the various studies, the results concerning the effect of enrichment on the welfare of pigs are quite conclusive. Enriching a pigs' environment is a good method of improving the overall welfare of the animal.

1.5 Arousal

Having deduced that animals are considered sentient, there is an implication that having emotional capacities means that an animal will attempt to minimise negative emotions such as fear and frustration, and at the same time seek positive emotions (e.g. pleasure, joy) (Dawkins, 2017). However, identifying emotions on their own is a difficult task, and often we infer their emotional state with a combination of behavioural (valence) and physiological (arousal) measurements (Boissy et al., 2007[a]; Boissy et al., 2007[b]; Smulders et al., 2006). Through combining tests within these two indicators, we are able to create a clearer picture concerning an animal's emotional state.

One of the emotional indicators in animals is arousal. In this context the definition can be summarised as the internal and/or external physiological changes of an animal in response to stimuli (Bliss-moreau et al., 2020; Paul et al., 2005). However, the overarching term of arousal is broad, and is likely that some situations induce different affective states but have the same physiological responses (Paul et al., 2005). For example, Marchant et al., (1995) demonstrated that cows' heart rate increased with activity as well as anticipation of punishing or rewarding stimuli. Therefore, it is necessary to measure multiple physiological indicators to attempt to interpret an animals affective state such as temperature changes, hormone (cortisol) analysis etc. (Broom, Johnson, 1993).

Pigs have a similar cardiovascular pathway to humans. This pathway is controlled by the autonomic nervous system (**ANS**) and is commonly used to study physiological changes and emotional indicators such as heart rate, blood pressure, respiratory rate and skin temperature. The ANS has two divisions: the sympathetic nervous system (**SNS**) and the parasympathetic nervous system (**PSNS**). These two systems act together to regulate the involuntary and reflexive roles in the body (Krause et al., 2017). The PSNS controls the 'rest and digest' functions and maintains the body's internal environment, while the SNS controls the 'fight or flight response' and prepares the animals' body for possible life threatening dangers (Kano et al., 2016; Krause et al., 2017). These changes in an animals internal state consequently lead to the emission (or lack thereof) of heat from the skin (Turner, 2001). In pigs, the effect of an emotional challenging situation and sickness responses both generate short-term metabolism variations which lead to varying bloodstream patterns (Parois et al., in review). Which ultimately, lead to changes in body temperature.

1.6 Thermography

Infrared thermography (**IRT**) is being used to make inferences concerning an animals' physical and emotional state, in particular the response to conditions that may cause stress or cause the animal to be in an aroused state (Boileau et al., 2019; Boissy et al., 2007). All objects on Earth emit electromagnetic radiation (Speakman & Ward, 1998). These radiation emissions can be measured using IRT cameras that allow for real-time visualisation of temperature. This relatively new technology has been a useful tool in animal welfare practices. Initially, IRT was used as a method to measure temperature changes in response to injury, handling, disease, and transportation (Boileau et al., 2019; Schaefer et al., 2004). More recently however, recent studies have demonstrated assuring results about changes in surface temperatures during stressful situations in different animal species cattle - (Church et al., 2014; Scoley et al., 2019; Stewart et al., 2008), chimpanzees, (Kano et al., 2016) and swine - (Boileau et al., 2019; Parois et al., n.d.; Zhang et al., 2016).

Traditional welfare measurement techniques often rely on handling and restraining an animal. Such invasive methods may skew results due to anxiogenic responses from the methods themselves, rather than the stress causing factor being focussed on (McManus et al., 2016; Soerensen & Pedersen, 2015). Other advantages to IRT include the potentially easier and faster diagnostic measures that would otherwise take up time with laboratory analyses. Not to mention the likelihood of being less costly in the long run. Theoretically speaking, the non-invasive nature of IRT makes for a useful tool to assess an animal's state and the possible implications of using IRT are worth investigating.

1.7 Aim

In this study, IRT was used to measure the effect of combining enrichment and social mixing on the resilience of pigs. The study aimed to investigate the effectiveness of using thermal imaging as a non-invasive indicator of welfare, by monitoring the natural process of vasodilation and vasoconstriction in response to psychological and physiological challenges.

Pigs were either exposed to environmentally enriched pens with rooting material and extra enrichment and positive human contact (**E**), or barren (**B)** pens with limited enrichment to mimic common intensive husbandry practices. Half of the pigs were subject to minimal mixing with one social mixing event at weaning (**MM**), while the other half had repeated social mixing (**RM**) events in an attempt create a state of high allostatic load, because of stressors of shifting dominance hierarchies (Ewbank et al., 1974).

The four combinations of experimental groups were then subject to two challenges. The first challenge involved a physiological challenge, whereby the pigs received an injection of lipopolysaccharide (**LPS**) which induces a state of temporary sickness without causing disease (Nordgreen et al., 2018). This imitates possible pathogenic illness that may occur in common husbandry practices. The second challenge was of a psychological nature, whereby, a pig was isolated in a restricted pen facing four other unfamiliar pigs, playing in a play arena for 10-minutes, which may induce frustration. The focal pig was filmed with a thermal imaging camera for the entirety of the 10-minutes and the changes in ear temperature was recorded for each pig in all the test groups.

It was hypothesized that pigs under more enriched conditions would demonstrate to be more resilient, measured by temperature changes as a response to the two challenges. Specifically, the rate at which the peripheral temperature returns to baseline. For the LPS challenge and Frustration challenge the rate would be quicker in enriched pigs. Pigs with greater allostatic load as a result of repeated social mixing events were expected to have lower resilience when facing the challenges, which means that they would recover to baseline slower. Moreover, it was expected that the effects of a greater allostatic load would exacerbate the negative impact of barren living conditions on their resilience. Furthermore, the validity of utilizing IRT as a measurement tool for welfare was investigated as well.

2. Materials and methods

Established principles of laboratory animal care and use mandated by Dutch law on animal experiments were followed. Approved by the Animal Care and Use Committee of Wageningen University & Research.

2.1 Animals

The current study was part of a larger experiment where a total of 384 female piglets (*Sus scrofa domesticus*, TN70 × Tempo crossbred) from 65 sows were used and divided over 6 batches (n=16 litters/batch) (n= 64 pigs/batch) (Luo et al., 2022). However, only 2½ batches (half of batch 4, batch 5 & 6) (n=147 animals) were used for the current study. In short, the piglets were raised in a conventional farm and kept with the sows in individual farrowing pens until weaning at approximately 4 weeks of age. Post weaning (around an average of 4 weeks of age) the pigs were transported to Carus, at Wageningen University & Research, Wageningen, the Netherlands. The pigs were housed in pens of $0.85 m^2$ per pig ($1.2 \times 2.85\ m^2$). Pigs received a standard commercial feed diet for growing pigs *ad libitum* from one feeder and each pen had one drinking nipple. The environment temperature was set at 28°C for the first 12 days, 26°C for the subsequent 12 days and 24°C until the end of the experiment at approximately 7 weeks of age. Pigs were given a 12-hour day-night routine with 115 Lux from 7AM until 7PM and 30 Lux during the night. A gradual day-night transition was done over a 10-minute period. No natural light was available. See Luo et al., (2022) for more information.

2.2 Treatments

After weaning and being transported to Carus, the pigs were placed into one of four treatments in a 2 × 2 experimental design with enrichment (E & B) and two differing social mixing amounts to try and create varying states of allostatic load (MM and RM) see figure 1. The four experimental groups were:

1. Pigs with minimal mixing + enrichment (**E – MM**)
2. Pigs with minimal mixing + no enrichment (conventional), besides minimum legal requirements (**B – MM**)
3. Pigs with four repeated mixings + enrichment (**E – RM**)
4. Pigs with four repeated mixings + no enrichment (conventional), besides minimum legal requirements (**B – RM**)

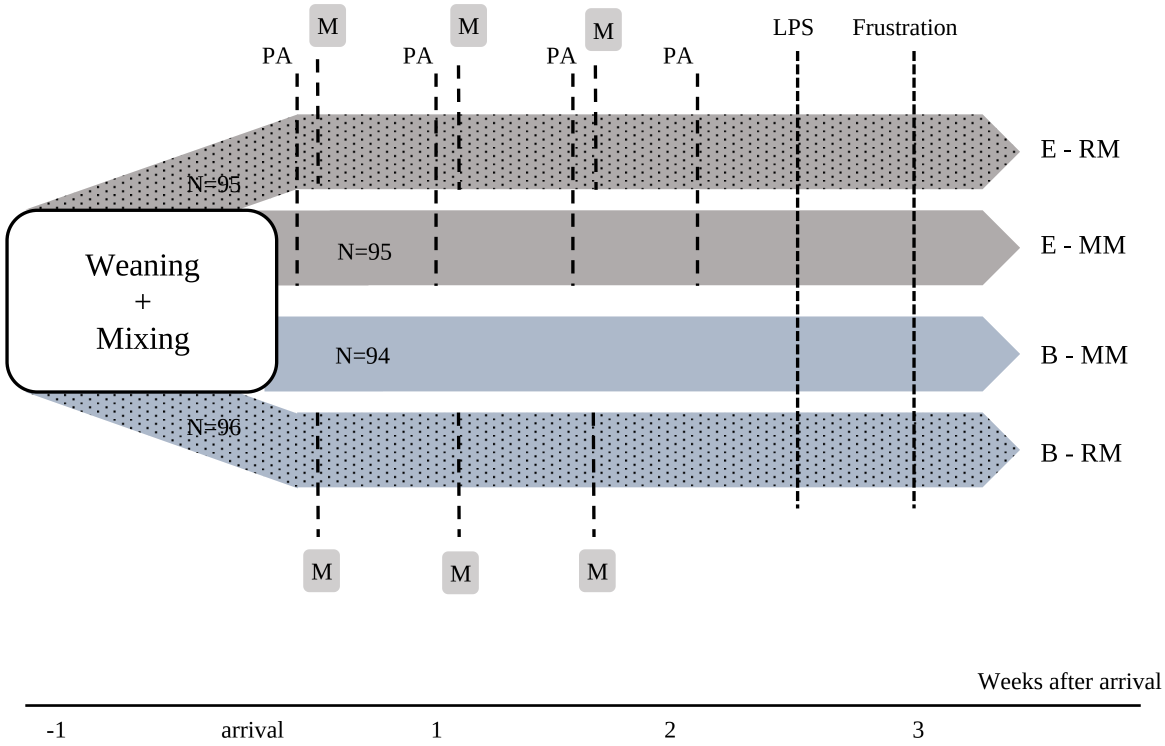

Fig. 1. Experimental design illustrating the four experimental groups and timeline of procedures and challenges. PA indicates the 20-minute access to the 'play arena', and M indicates the mixing events.

2.2.1 Enrichment treatment

The enriched groups (E – MM & E – RM) were provided with environmental enrichment, extra enrichment (toys and regular access to a 'play arena') and positive human contact. Pens for the two enriched groups contained 1cm layer of sawdust on a solid floor, with any soiled spots being cleaned and replaced daily. The pens were provided a mix of permanent toys (jute sack, brush, jute rope) and toys on rotation (composed of a mix of pig and dog toys found on the market. See Luo et al., (2022) for toy list. The toys were rotated every four days. The pigs also received positive human interaction daily, in the form of gentle brushing for 1 minute with a soft broom. The enriched groups were provided access to the 'play arena' with their pen mates for 20 minutes every 4 days, for a total of 4 occasions. The 'play arena' contained extra rooting material (20-22 L of peat), a dog agility tunnel (200cm × 40cm, Trixie) and three microfibre mops suspended 40cm above the ground. On the first and third day the 'play arena' also included a large yellow ball, a plastic snake, a rope and two dog toy plastic bones. On the second and fourth days, a large white bucket, a rubber ring, a KONG Wubba™ and two Pilates balls were included.

Pigs in the conventional barren treatment (B - MM & B - RM) were housed in conventional pens, with partly solid and partly slatted flooring without floor substrate. They were provided one chain with bolts and one chain with a Porcichew® toy, which remained constant throughout the experiment.

2.2.2 Mixing - allostatic load treatment

Half of the pigs (E – RM & B – RM) in each of the enrichment treatments were regrouped in a new pen with the same housing treatment with three new unacquainted pen mates every four days. On day: 0, 4, 8, 12 after weaning. For a total of four mixing events (including mixing at weaning), which was expected to create a state of high allostatic load.

The other half (E – MM & B – MM) experienced minimal mixing (only one mixing event at weaning), which was expected to create a state of low allostatic load.

2.3 Challenges

The resilience of the pigs in all four treatments (E – MM, B – MM, E – RM, B – RM) was measured by analysing the temperature response to an LPS injection to provoke an illness response (physiological) and an isolation (frustration) challenge to induce a psychological stress response.

2.3.1 LPS challenge

At approximately 6 weeks of age the pigs were intravenously injected with 2µg of LPS per kg of body weight in the ear vein (LPS sigma L4391 *Escherichia coli* O111:B4) to stimulate an immune response, without generating disease. Thermal videos of both left and right ears were taken at 0h (baseline) and at 0.75h, 1.32h, 3h, 4.28h and 6h after inoculation.

2.3.2 Frustration challenge

At an average of 7 weeks of age, individual pigs were separated from their pen and moved into a new pen (1.2 × 0.6m) with direct view of the 'play arena' containing unfamiliar pigs, which the tested pig could not join. The pig was isolated for a 10min period. Each pig was recorded with a thermal imaging camera placed directly above (1m) for the entire 10 minutes.

2.4 Thermal imaging analyses

Thermal videos for both challenges were taken with the FLIR T1020 camera (lens: FOL 36mm, Emissivity: 0.98, Reflected temp: 26°C, Relative Humidity: 60%) for all pigs. Pictures were extracted from the footage using the graphical tools from FLIR Tools and FLIR Studio by a single observer (for this study).

For the LPS challenge, thermal pictures of both ears (when visible) were taken from the footage. The pictures were then used for temperature extraction (figure 2a and 2b). The measurements taken were the maximum and average temperature of ¼ of the total distance between the hottest spot inside the ear to the tip of the ear. The hottest spot was defined using a circle tool drawn around the ear canal (software creates a maximum temperature point within selected area). The hottest point was used as an anatomical reference to counteract the effect of morphology differences between pigs' ears (Parois et al., in review)

For the Frustration challenge, pictures from the footage were extracted at 30 second intervals from when a pig entered the pen until the end of the 10-minute period. Maximum temperature was measured of both ears using the same circle selection tool from the software (figure 2a and 2b).

Fig. 2a. Temperature extraction for LPS challenge of right ear. El1 was the circle identifying hottest point in ear. Li1 was the line from hottest point to ear tip. Li2 was $^{1}/_{4}$ of Li1 and the line where max and average temperatures were measured.

Fig. 2b. Temperature extraction for Frustration challenge. El1 was the circle indicating the max. temperature of left ear and El2 was the circle indicating the max. temperature of the right ear.

2.5 Statistical analyses

Statistical analyses were performed using SAS (SAS OnDemand for academics, v. 3.1.0). One pig from this study's section was omitted from the LPS challenge analysis as it had mistakenly received an incorrect dose of LPS.

A correlation test (PROC CORR of SAS) was used to test for similarities in left and right ear temperatures. Temperature (maximum and average) did not differ between left and right ear. Therefore, average values of maximum ear temperature and average of left and right ear temperature were used. However, for Frustration only the average maximum temperature was used of both ears because the average maximum temperature was less prone to external temperature variation factors. PROC CORR was also utilised to assess intra-observer reliability, by extracting temperatures on a group of 30 images on two separate occasions, without reference to previous extractions.

For all data, distribution of residuals of response variables were tested for normality. Normality of distribution was assessed by kurtosis and skewness (between -1 and 1). The maximum and average ear temperature in the LPS study (model 1) were analysed separately from the maximum temperature from the Frustration study (model 2). A backwards stepwise elimination process was used for all biologically significant interactions in both models.
A mixed model (PROC MIXED of SAS) was used for both models 1, with enrichment, mixing and time, as well as their interactions, as fixed effects.

Pen was nested with each batch, enrichment, mixing, and time, and together with batch and litter, included as random effects. Values in both models were given as least square means and their standard errors of the mean. P-values were regarded as significant if $P \leq 0.05$ and considered a tendency if $P < 0.1$ and presented after a Tukey adjustment.

3. Results

All results in this study were from 2½ batches (half of 4, entire batches 5 & 6). One pig was omitted from the LPS analysis as it had received the wrong dose. Average temperature did not significantly differ to maximum ear temperature; therefore, maximum ear temperature was used to convey results.

3.1 Thermal imaging measurement's reliability

The temperature extractions in this study were measured by a single trained experimenter. The intra-observer reliability of the measurement was tested per challenge with replicate pictures (Table 1).

Table 1. Intra-observer correlation values of two repeated groups of pictures (P < 0.0001)

Pearson Correlation Coefficients		r^2	r^2
		Maximum 2	Average 2
LPS	Max 1	**0.99945**	.
	Average 1	.	**0.88682**
Frustration	Max 1	**1.0000**	.
	Average 1	.	**0.89758**

3.2 LPS challenge

The interaction between enrichment and mixing in the 4 treatment groups had no significant effect on temperature over time *(F* = 0.63, ns). Temperature means over 6 time points are given in Table 2 with fixed effects and interactions. Enrichment resulted in a quicker increase in temperature (P ≤ 0.05) while mixing did not (*F = 2.9, ns)*.

Pigs in the B – MM group had lower mean temperatures at all the 6 time points (figure 3). The E – RM, B – RM and E – MM experimental groups had similar mean temperature reductions from time points 1-2 (P ≤ 0.05), however, E – MM temperatures started to increase at a faster rate after time point 2 compared to E – RM and B – RM. Similarly, E – RM temperature increased rapidly after time point 3. While B – RM only increased back to baseline temperature after time point 4. Pigs in the enriched housing treatments (E – RM and E – MM) started recovery earlier than pigs in the non-enriched housing treatments (figure 4).

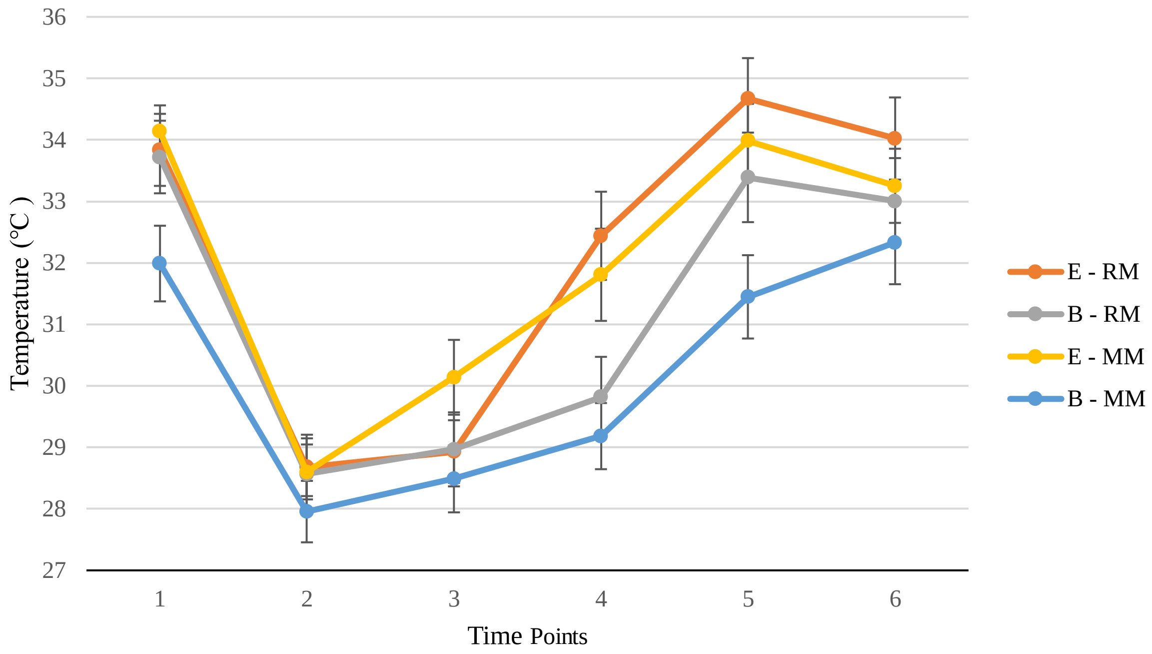

Fig. 3. The maximum temperature means (both ears) of 4 experimental groups over time after receiving LPS dose.

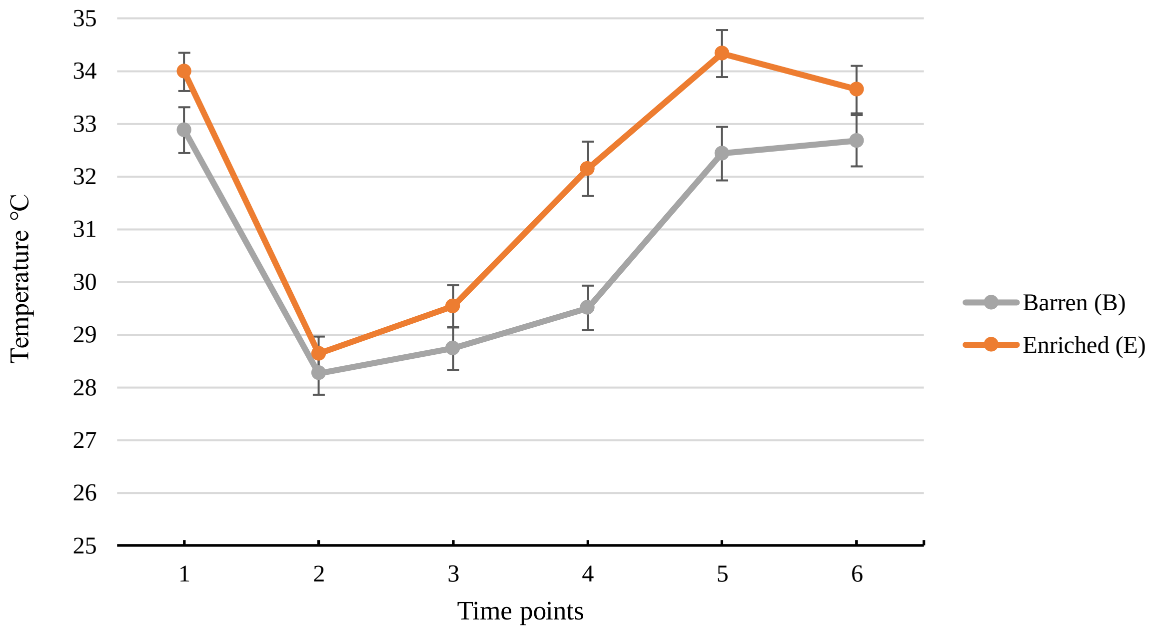

Fig. 4. Interaction between enrichment treatments on mean temperature over time after LPS dose.

Table 2. Mean maximum ear temperature of pigs in each of the 4 experimental groups in the LPS challenge over 6 time points post inoculation.

		Group				*P*-values fixed effects and interactions						
	Time points	E – RM	B – RM	E – MM	B – MM	time	Enrichment	Mixing	E*time	time*Mxng	tmt*Mxng	E*Mxng*time
Max temp ℃ ± SE	1	33.84 ±0.59	33.72 ±0.59	34.14 ±0.42	31.99 ±0.61	<.0001	**0.0089**	0.0967	**0.0475**	0.4541	0.3673	0.6801
	2	28.68 ±0.47	28.57 ±0.64	28.6 ±0.45	27.96 ±0.50							
	3	28.93 ±0.51	28.97 ±0.60	30.14 ±0.61	28.49 ±0.54							
	4	32.44 ±0.72	29.82 ±0.65	31.81 ±0.75	29.18 ±0.54							
	5	34.67 ±0.66	33.39 ±0.73	33.98 ±0.60	31.45 ±0.68							
	6	34.02 ±0.67	33.00 ±0.70	33.25 ±0.60	32.33 ±0.68							

Pigs in the enriched group demonstrated a return to baseline temperature earlier and at a faster rate than pigs in the barren treatment ($P < 0.05$, Fig.4). Pigs in both the enriched and barren housing treatments showed an initial mean maximum temperature decrease after LPS treatment from 34°C and 33°C to ≈ 28.5°C between time point 1 (baseline temperature) and time point 2 (1h30). After which, pigs in both groups started a gradual increase towards time point 3. After time point 3 enriched pigs showed a rapid increase toward baseline temperature while pigs in the barren treatment increased but at a slower rate. At time point 4 enriched pigs had a mean temperature of ≈ 32°C while barren pigs were only at ≈ 29.5°C. After time point 4 enriched pigs maintained a high recovery rate, and barren pigs started to increase more rapidly. By time points 5 and 6 pigs in both experimental groups returned to baseline temperature.

3.3 Frustration challenge

Frustration inducing isolation challenge did not differ between the four treatments ($P > 0.05$). All interactions were likewise insignificant. Figure 5 illustrates the mean temperatures over time. The temperature of pigs in all experimental groups showed an increase in mean maximum temperature during the 10-minute Frustration challenge. The increase was approximately 1°C from ≈ 38°C to ≈ 39°C.

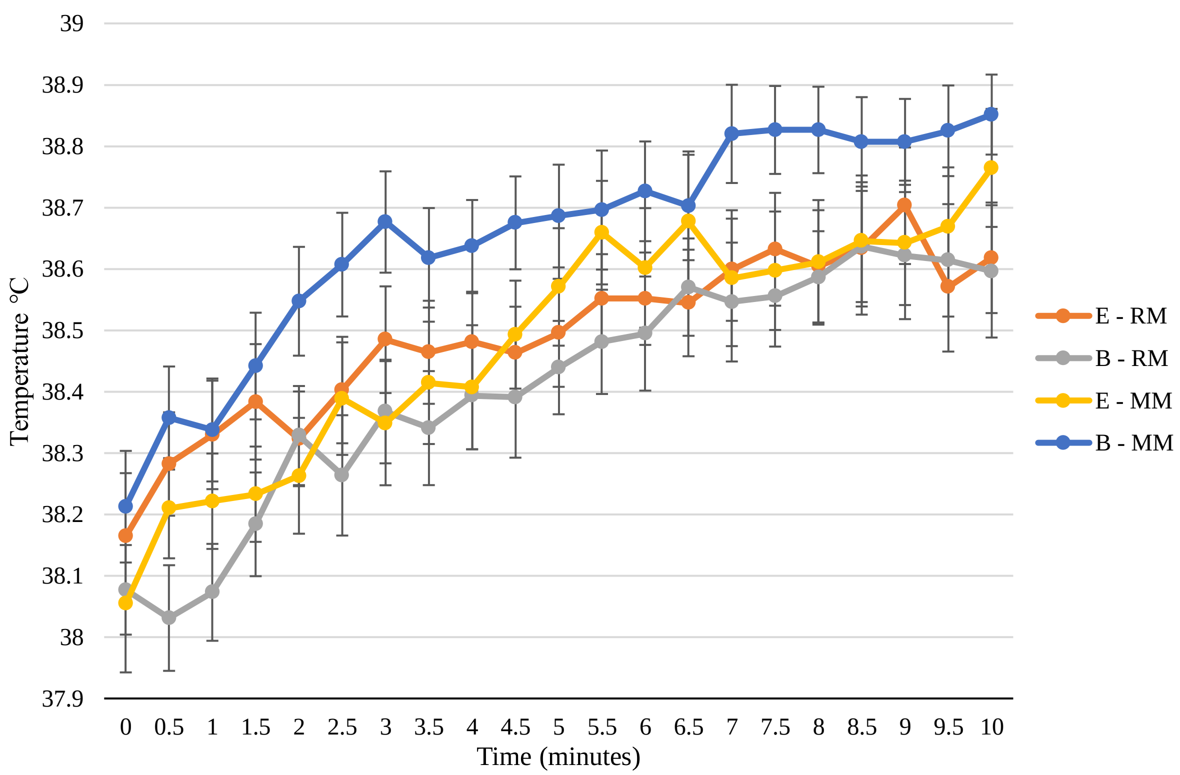

Fig. 5. Mean maximum temperature of 4 experimental groups over 10-minute Frustration challenge.

4. Discussion

The objective of the current study was to evaluate the effects of enrichment and mixing on the capacity of pigs to recover from two challenges, measured by infrared thermography and evaluate the ability by which it can be used as a potential reliable non-invasive measurement. Pigs in the enriched treatment were expected to demonstrate enhanced resilience compared to pigs kept in the barren treatment, which is inspired by the conditions pigs are exposed to in common husbandry practices. Pigs in the repeated mixing group (which was expected to create a state of high allostatic load) were expected to show reduced resilience to challenges. The combinations of treatments were expected to exacerbate the effects of the challenges. In this study, enrichment or lack thereof, impacted pigs' temperature response to the LPS challenge. While social mixing did not demonstrate a temperature response.

4.1 Enrichment effects

Pigs in the enriched treatments received sawdust bedding, toys on rotation, positive human contact and had regular access to extra space in the "play arena". These enrichment factors have been proven to increase pig welfare (Beattie et al., 2000; Bolhuis et al., 2006; Fraser, 1995). Enrichment in the current study did improve the pigs' ability to recover from the LPS challenge, but not in the Frustration challenge. After the initial decrease in ear temperature, as a reaction to LPS inoculation, ear temperature of pigs in the enrichment treatments started to increase to baseline earlier and at a faster rate than pigs in the barren treatment. Similarly, in the study of Van Dixhoorn et al., (2016), pigs in the barren treatment retained a high rectal temperature for 24 hours while pigs in the enriched treatment decreased faster, an hour and a half hours after receiving the LPS dose after co-infection of PRRSV and *A. pleuropneumoniae*. The results where similar in that enriched pigs demonstrated a stronger recovery response, albeit showing an inverse correlation between rectal temperatures and IRT temperatures. The reasoning behind why there was an inverse correlation between rectal temperature and thermographic measurements, is because the LPS sickness inflammatory process is symmetrical and decreases temperature in the peripheral parts of the body. This was probably due to activation of the SNS which causes vasoconstriction in the periphery and blood flow is redistributed to vital organs in attempt to fight against infection (Stewart et al., 2008). Enriched pigs still demonstrate faster recovery in both studies; however, rectal temperatures may be more adept in identifying the effects of the LPS sickness on core body temperature. Thermographic temperature can identify the overall temperature as a result nervous system processes, at the same time indicating the effect of possible disease.

Enrichment had no effect during the Frustration test, contrary to the initial hypothesis. Pigs from both housing condition showed approximately a 1°C increase in mean ear temperature as a result of being isolated for a 10-minute period. This increase in temperature may be due to an increase in heart rate as a result of the challenge. In one study, pigs had undergone similar isolation tests for a 15-minute period and their heart rate was found to only be elevated during the first 5 minutes of the test (Reimert et al., 2014). Contrastingly, the same study found that pigs from barren treatments spent more time demonstrating standing alert and escape behaviour (Luo et al., 2022; Reimert et al., 2014). For example, barren pigs often displayed tucked tails, which was a fear related behaviour while curled tails are a marker of a positive emotional state (Lassen et al., 2006). Curled tails were also observed to be more common in enriched pens compared to barren pens (Czycholl et al., 2020). Based on behavioural observation findings in the Luo et al., (2022) study, pigs from enriched housing conditions seemed less stressed. It may be too, that the effect of isolation alone may overcome the positive effects of enrichment. The temperature after 10-minutes looked as if it continued to increase in this study. Perhaps a longer isolation period may show differences, as the initial effect was shared between pigs from both housing conditions.

4.2 Mixing effects

In this study we expected that repeated mixing would create a state of high allostatic load. Contrary to expectations, repeated mixing did not seem to create enough cumulative stress on the pigs and therefore, did not create the desired state of high allostatic load. Repeated mixing did not seem to have an effect on the pigs' resilience, compared to the group with minimal social mixing. Contrary to the results, repeated social mixing events was expected to increase stress levels due to repeated process of establishing new hierarchies between the pigs in the pen (Camp Montoro et al., 2021). The process often involves aggressive behaviour, and has been demonstrated that repeated mixing can lead to chronic stress measured by an increased levels of salivary cortisol compared to pigs from minimal mixing environments. (Coutellier et al., 2007). Therefore, it was expected that the cumulative stress as a result of mixing i.e., high allostatic load, would negatively affect the resilience of pigs during the two challenges.

There was no significant difference in temperature response over time for both the LPS and the Frustration challenges between repeated mixing and minimal mixing. There could be a few possible reasons for the lack of effect on temperature by repeated mixing. Firstly, the disparity between the repeated mixing group (4 mixing events) and the minimal mixing group (1 mixing event at weaning) may not have been large enough. Perhaps meaning that the number of mixing events has varying effects and that the first mixing may have a greater effect than the subsequent events. Some studies have indicated negative effects of mixing, with pigs that have never been socially mixed (i.e. remained with their litter) (Camp Montoro et al., 2021; Coutellier et al., 2007). That being said, pigs may become habituated to going through mixings, so the effect is minimal at some threshold. Secondly, repeated mixing may not be enough of a factor on its own to create a state of high allostatic load. The effect may have been too slight and therefore easy to cope with and possibly even nullified the effect before the next mixing event took place. This would renounce the idea of cumulative stress. That being said, it could also play into the concept of mild stress possibly helping animals cope with future challenges (McEwen, 1998). However, in this study the groups that were subject to repeated mixing did not show an increased ability to cope with the challenges either. Furthermore, pigs in the mixing groups may have been habituated to novel pens during the mixing procedures, so entering the isolation pen may not have been as stressful as presumed and thus counteract the hypothetical mixing effects. Lastly, age at mixing may have varied effects. Pigs in this study were mixed at a fairly young age, one could expect stronger effects of mixing in older pigs. In contrast, one study found that pigs demonstrated a drop in temperature in several locations on the front of the face in response to isolation (Parois et al., in review), which may indicate that ear temperatures alone were not enough of an indicator of frustration.

4.3 Interaction between enrichment and mixing

It was expected that the effects of repeated mixing would exacerbate the negative effects of barren housing. However, repeated mixing did not seem to create a state of high allostatic load, and therefore, the combinations of treatments likewise did not follow the hypothesised trends for either challenge. There was no indication that mixing had less of an effect on pigs in the barren treatment based on temperature alone. However, in the behavioural analysis barren pigs tended to show more aggressive behaviour than the enriched pigs (Luo et al., 2022). It is also possible that the process of mixing provided behavioural stimuli to barren pigs, which lacked the space and enrichments in their home pens.

4.4 Infrared Thermography

IRT was used as a tool to demonstrate that temperature changes during the two challenges could be considered reliable non-invasive indicators of welfare.

Temperature extractions showed good intra-observer reliability with strong correlations between temperature extractions of the same group of pictures taken on two separate occasions.

This is because the locations of the measurements were almost always perfectly visible, even if the animal's position differed between pictures. However, the average temperatures during the LPS challenge slightly lower correlations (≈ 0.85). The cause of this may be that measurements of lower temperatures were more sensitive to background noise. When measuring the average, the line from the hottest point to the ear tip covers more area. Often the ear tip is not always easy to distinguish, especially during lower temperatures when it occasionally blends with its surroundings. Paired with the fact that the ear is a mobile body part, so image clarity is not always ideal. However, the repeatability of the process within and between observers when measuring maximum temperature of specific spots in calves has been proven to yield good results (Scoley et al., 2019).

In this study, it seems that IRT is sensitive enough to pick up the drop in temperature to the animals' periphery as a response to LPS and perhaps other sickness inducing agents, especially as a measure over time. However, it might not be sensitive enough to pick up on psychological stressors. Attempts to measure a negative state of mind are more commonly studied than a positive state of mind, and the use of IRT might be a useful tool in attempts to understand positive emotionality.

Furthermore, it is important to identify which factors affect thermoregulation in pigs in order to validate the areas on the body that have been identified as markers for physiological changes. Environment also has to be considered when utilising IRT, as certain factors such as wind speed, direct sunlight and humidity (Church et al., 2014; Zhang et al., 2016). It is also evident that coat length may impact heat emissivity of horses (Turner, 2001). It was also a notable observation that in the current study, there was variation of pigs' hair lengths and densities as well as thickness, on the body as well as the ears. Therefore, further investigation may be worthwhile to see if hair impacts IRT measurements in pigs.

5. Conclusion

In conclusion, pigs in an enriched housing environment were more resilient, in terms of their ability to recover from an LPS challenge in temperature. Enrichment did not influence pigs during a frustration challenge, nor did repeated mixing as an attempt to achieve a state of high allostatic load. IRT in this study proved to be a reliable non-invasive tool to measure ear temperature, especially for LPS sickness recovery. IRT seems to be a promising indicator of physiological changes as a result of stress and sickness.

References

Appleby, M. C. (1999). What should we do about animal welfare? *Journal of Agricultural and Environmental Ethics*, *18*(3), 293–303.

Beattie, V. E., O'Connell, N. E., Kilpatrick, D. J., & Moss, B. W. (2000). Influence of environmental enrichment on welfare-related behavioural and physiological parameters in growing pigs. *Animal Science*, *70*(3), 443–450. https://doi.org/10.1017/S1357729800051791

Bennett, R. M., Anderson, J., & Blaney, R. J. P. (2002). Moral intensity and willingness to pay concerning farm animal welfare issues and the implications for agricultural policy. *Journal of Agricultural and Environmental Ethics*, *15*(2), 187–202. https://doi.org/10.1023/A:1015036617385

Bliss-moreau, E., Williams, L. A., & Santistevan, A. C. (2020). Supplemental Material for The Immutability of Valence and Arousal in the Foundation of Emotion. *Emotion*, *20*(6), 993–1004. https://doi.org/10.1037/emo0000606.supp

Blokhuis, H. J. (2004). Recent developments in European and international welfare regulations. *World's Poultry Science Journal*, *60*(4), 469–477. https://doi.org/10.1079/WPS200430

Boileau, A., Farish, M., Turner, S. P., & Camerlink, I. (2019). Infrared thermography of agonistic behaviour in pigs. *Physiology and Behavior*, *210*(February), 112637. https://doi.org/10.1016/j.physbeh.2019.112637

Boissy, A., Arnould, C., Chaillou, E., Désire, L., Duvaux-Ponter, C., Greiveldinger, L., Leterrier, C., Richard, S., Roussel, S., Saint-Dizier, H., Meunier-Salaün, M. C., Valance, D., & Veissier, I. (2007). Emotions and cognition: A new approach to animal welfare. *Animal Welfare*, *16*(SUPPL.), 37–43.

Boissy, Alain, Manteuffel, G., Jensen, M. B., Moe, R. O., Spruijt, B., Keeling, L. J., Winckler, C., Forkman, B., Dimitrov, I., Langbein, J., Bakken, M., Veissier, I., & Aubert, A. (2007). Assessment of positive emotions in animals to improve their welfare. *Physiology and Behavior*, *92*(3), 375–397. https://doi.org/10.1016/j.physbeh.2007.02.003

Bolhuis, J. E., Schouten, W. G. P., Schrama, J. W., & Wiegant, V. M. (2006). Effects of rearing and housing environment on behaviour and performance of pigs with different coping characteristics. *Applied Animal Behaviour Science*, *101*(1–2), 68–85. https://doi.org/10.1016/j.applanim.2006.01.001

Broom, Donald M. Johnson, K. G. (1993). *Stress and animal welfare* (1st ed.). Chapman & Hall.

Broom, D. M. (1986). Indicators of poor welfare. *British Veterinary Journal*, *142*(6), 524–526. https://doi.org/10.1016/0007-1935(86)90109-0

Burnier, P. C., Spers, E. E., & Barcellos, M. D. de. (2021). Role of sustainability attributes and occasion matters in determining consumers' beef choice. *Food Quality and Preference*, *88*(August 2020), 104075. https://doi.org/10.1016/j.foodqual.2020.104075

Camp Montoro, J., Boyle, L. A., Solà-Oriol, D., Muns, R., Gasa, J., & Garcia Manzanilla, E. (2021). Effect of space allowance and mixing on growth performance and body lesions of grower-finisher pigs in pens with a single wet-dry feeder. *Porcine Health Management*, *7*(1), 1–8. https://doi.org/10.1186/s40813-020-00187-7

Church, J. S., Hegadoren, P. R., Paetkau, M. J., Miller, C. C., Regev-Shoshani, G., Schaefer, A. L., & Schwartzkopf-Genswein, K. S. (2014). Influence of environmental factors on infrared eye temperature measurements in cattle. *Research in Veterinary Science*, *96*(1), 220–226. https://doi.org/10.1016/j.rvsc.2013.11.006

Colditz, I. G., & Hine, B. C. (2016). Resilience in farm animals: Biology, management, breeding and implications for animal welfare. *Animal Production Science*, *56*(12), 1961–1983. https://doi.org/10.1071/AN15297

Council of the European Union. (2008). Council Directive 2008/120/EC laying down minimum standards for the protection of pigs. *Official Journal of the European Union*, *5*, L47/5-13.

Coutellier, L., Arnould, C., Boissy, A., Orgeur, P., Prunier, A., Veissier, I., & Meunier-Salaün, M. C. (2007). Pig's responses to repeated social regrouping and relocation during the growing-finishing period. *Applied Animal Behaviour Science*, *105*(1–3), 102–114. https://doi.org/10.1016/j.applanim.2006.05.007

Czycholl, I., Hauschild, E., Büttner, K., Krugmann, K., Burfeind, O., & Krieter, J. (2020). Tail and ear postures of growing pigs in two different housing conditions. *Behavioural Processes*, *176*(January), 104138. https://doi.org/10.1016/j.beproc.2020.104138

Dawkins, M. S. (2017). Animal welfare with and without consciousness. *Journal of Zoology*, *301*(1), 1–10. https://doi.org/10.1111/jzo.12434

Ernst, K., Ekkelboom, M., Kerssen, N., Smeets, S., Sun, Y., & Yin, X. (2018). *Play behavior and environmental enrichment in pigs*.

European Commission. (2022). *Animal Welfare*. https://ec.europa.eu/food/animals/animal-welfare_en#related

Ewbank, R., Meese, G. B., & Cox, J. E. (1974). Individual recognition and the dominance hierarchy in the domesticated pig. The role of sight. *Animal Behaviour*, *22*(2), 473–480. https://doi.org/10.1016/S0003-3472(74)80046-1

Fraser, D. (1995). Science, Values and Animal Welfare: Exploring the "Inextricable Connection." *Animal Welfare*, *4*(2), 103–117.

Fraser, D., Weary, D. M., Pajor, E. A., Milligan, B. N., & Milligan, &. (1997). *WellBeing International WellBeing International WBI Studies Repository WBI Studies Repository 1997 A Scientific Conception of Animal Welfare that Reflects Ethical A Scientific Conception of Animal Welfare that Reflects Ethical Concerns Concerns Recommende*. 187–205.

George, F. K., & Michel Le, M. (2001). Drug Addiction, Dysregulation of Reward, and Allostasis. *Neuropsychopharmacology*, *24*(2), 97.

Glass, C. A., Hutchinson, W. G., & Beattie, V. E. (2005). Measuring the value to the public of pig welfare improvements: A contingent valuation approach. *Animal Welfare*, *14*(1), 61–69.

Godyń, D., Nowicki, J., & Herbut, P. (2019). Effect of Environmental Enrichment on Pig Welfare. *Animals*, *9*(383). https://doi.org/10.3390/ani9060383

Guidi, J., Lucente, M., Sonino, N., & Fava, G. A. (2021). Allostatic Load and Its Impact on Health: A Systematic Review. *Psychotherapy and Psychosomatics*, *90*(1), 11–27. https://doi.org/10.1159/000510696

Guy, J. H., Meads, Z. A., Shiel, R. S., & Edwards, S. A. (2013). The effect of combining different environmental enrichment materials on enrichment use by growing pigs. *Applied Animal Behaviour Science*, *144*(3–4), 102–107. https://doi.org/10.1016/j.applanim.2013.01.006

Kano, F., Hirata, S., Deschner, T., Behringer, V., & Call, J. (2016). Nasal temperature drop in response to a playback of conspecific fights in chimpanzees: A thermo-imaging study. *Physiology and Behavior*, *155*, 83–94. https://doi.org/10.1016/j.physbeh.2015.11.029

Keeling, L. J. (2005). Healthy and happy: Animal welfare as an integral part of sustainable agriculture. *Ambio*, *34*(4–5), 316–319. https://doi.org/10.1579/0044-7447-34.4.316

Kittawornrat, A., & Zimmerman, J. J. (2011). Toward a better understanding of pig behavior and pig welfare. *Animal Health Research Reviews / Conference of Research Workers in Animal Diseases*, *12*(1), 25–32. https://doi.org/10.1017/S1466252310000174

Korte, S. M., Olivier, B., & Koolhaas, J. M. (2007). A new animal welfare concept based on allostasis. *Physiology and Behavior*, *92*(3), 422–428.

https://doi.org/10.1016/j.physbeh.2006.10.018

Korte, S. M., Prins, J., Vinkers, C. H., & Olivier, B. (2009). On the origin of allostasis and stress-induced pathology in farm animals: Celebrating Darwin's legacy. *Veterinary Journal*, *182*(3), 378–383. https://doi.org/10.1016/j.tvjl.2009.08.023

Krause, A., Puppe, B., & Langbein, J. (2017). Coping style modifies general and affective autonomic reactions of domestic pigs in different behavioral contexts. *Frontiers in Behavioral Neuroscience*, *11*(May), 1–13. https://doi.org/10.3389/fnbeh.2017.00103

Krystallis, A., Grunert, K. G., de Barcellos, M. D., Perrea, T., & Verbeke, W. (2012). Consumer attitudes towards sustainability aspects of food production: Insights from three continents. *Journal of Marketing Management*, *28*(3–4), 334–372. https://doi.org/10.1080/0267257X.2012.658836

Lassen, J., Sandøe, P., & Forkman, B. (2006). Happy pigs are dirty! - conflicting perspectives on animal welfare. *Livestock Science*, *103*(3), 221–230. https://doi.org/10.1016/j.livsci.2006.05.008

Luo, L., Zande, L. E. Van Der, Marwijk, M. A. Van, Knol, E. F., Rodenburg, T. B., Bolhuis, J. E., & Parois, S. P. (2022). *Impact of Enrichment and Repeated Mixing on Resilience in Pigs*. *9*(March), 1–16. https://doi.org/10.3389/fvets.2022.829060

Marchant, J. N., Mendl, M. T., Rudd, A. R., & Broom, D. M. (1995). The effect of agonistic interactions on the heart rate of group-housed sows. *Applied Animal Behaviour Science*, *46*(1–2), 49–56. https://doi.org/10.1016/0168-1591(95)00636-2

Marie-Laure, A. (2020). The EU pig meat sector. *Eprs*, *September 2020*.

McEwen, B. S. (1998). Protective and Damaging Effects of Stress Mediators. *New England Journal of Medicine*, *338*(3), 171–179. https://doi.org/10.1056/nejm199801153380307

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

Newberry, R. C. (1995). Environmental enrichment: Increasing the biological relevance of captive environments. *Applied Animal Behaviour Science*, *44*(2–4), 229–243. https://doi.org/10.1016/0168-1591(95)00616-Z

Nithianantharajah, J., & Hannan, A. J. (2006). Enriched environments, experience-dependent plasticity and disorders of the nervous system. *Nature Reviews Neuroscience*, *7*(9), 697–709. https://doi.org/10.1038/nrn1970

Nordgreen, J., Munsterhjelm, C., Aae, F., Popova, A., Boysen, P., Ranheim, B., Heinonen, M., Raszplewicz, J., Piepponen, P., Lervik, A., Valros, A., & Janczak, A. M. (2018). The effect of lipopolysaccharide (LPS) on inflammatory markers in blood and brain and on behavior in individually-housed pigs. *Physiology and Behavior*, *195*(March), 98–111. https://doi.org/10.1016/j.physbeh.2018.07.013

Olsen, A. W. (2001). Behaviour of growing pigs kept in pens with outdoor runs I. Effect of access to roughage and shelter on oral activities. *Livestock Production Science*, *69*(3), 255–264. https://doi.org/10.1016/S0301-6226(01)00172-5

Parois, S. P., Rodenburg, T. B., Zande, L. E. Van Der, Kemp, B., Bolhuis, J. E., Group, A. P., & Health, E. (n.d.). *Thermography: a promising non-invasive methodology to study pigs' welfare and recovery*.

Paul, E. S., Harding, E. J., & Mendl, M. (2005). Measuring emotional processes in animals: The utility of a cognitive approach. *Neuroscience and Biobehavioral Reviews*, *29*(3), 469–491. https://doi.org/10.1016/j.neubiorev.2005.01.002

Reimert, I., Bolhuis, J. E., Kemp, B., & Rodenburg, T. B. (2014). Social support in pigs with different coping styles. *Physiology and Behavior*, *129*, 221–229. https://doi.org/10.1016/j.physbeh.2014.02.059

Schaefer, A. L., Cook, N., Tessaro, S. V., Deregt, D., Desroches, G., Dubeski, P. L., Tong, A. K. W., & Godson, D. L. (2004). Early detection and prediction of infection using infrared thermography. *Canadian Journal of Animal Science*, *84*(1), 73–80. https://doi.org/10.4141/A02-104

Scheffer, M., Elizabeth Bolhuis, J., Borsboom, D., Buchman, T. G., Gijzel, S. M. W., Goulson, D., Kammenga, J. E., Kemp, B., van de Leemput, I. A., Levin, S., Martin, C. M., Melis, R. J. F., van Nes, E. H., Michael Romero, L., & Olde Rikkert, M. G. M. (2018). Quantifying resilience of humans and other animals. *Proceedings of the National Academy of Sciences of the United States of America*, *115*(47), 11883–11890. https://doi.org/10.1073/pnas.1810630115

Scoley, G. E., Gordon, A. W., & Morrison, S. J. (2019). Use of thermal imaging in dairy calves: Exploring the repeatability and accuracy of measures taken from different anatomical regions. *Translational Animal Science*, *3*(1), 564–576. https://doi.org/10.1093/tas/txy126

Smulders, D., Verbeke, G., Mormède, P., & Geers, R. (2006). Validation of a behavioral observation tool to assess pig welfare. *Physiology and Behavior*, *89*(3), 438–447. https://doi.org/10.1016/j.physbeh.2006.07.002

Soerensen, D. D., & Pedersen, L. J. (2015). Infrared skin temperature measurements for monitoring health in pigs: A review. *Acta Veterinaria Scandinavica*, *57*(1). https://doi.org/10.1186/s13028-015-0094-2

Speakman, J. R., & Ward, S. (1998). Infrared thermography: Principles and applications. *Zoology*, *101*(3), 224–232.

Sterling, P., & Eyer, J. (1988). Handbook of Life Stress, Cognition and Health Edited by S. Fisher and J. Reason Copyright 1988 John Wiley 8. Sons. *Handbook of Life Stress, Cognition and Health*, *January 1988*.

Stewart, M., Webster, J., & Schaeder, A. (2008). Infrared thermography and heart rate variability for non-invasive assessment of animal welfare. *ANZCCART Humane Science News*, *Figure 1*, 1–6.

Thorslund, C. A. H., Aaslyng, M. D., & Lassen, J. (2017). Perceived importance and responsibility for market-driven pig welfare: Literature review. *Meat Science*, *125*, 37–45. https://doi.org/10.1016/j.meatsci.2016.11.008

Turner, T. A. (2001). Diagnostic thermography. *The Veterinary Clinics of North America. Equine Practice*, *17*(1), 95–114. https://doi.org/10.1016/s0749-0739(17)30077-9

V. Caporale, B. Alessandrini, P. D. V. & S. D. P. (2005). Global perspectives on animal welfare: Europe. *Revue Scientifique et Technique-Office International Des Epizooties*, *24*(2), 567.

Van Dixhoorn, I. D. E., Reimert, I., Middelkoop, J., Bolhuis, J. E., Wisselink, H. J., Koerkamp, P. W. G. G., Kemp, B., & Stockhofe-Zurwieden, N. (2016). Enriched housing reduces disease susceptibility to co-infection with porcine reproductive and respiratory virus (PRRSV) and actinobacillus pleuropneumoniae (A. Pleuropneumoniae) in young pigs. *PLoS ONE*, *11*(9), 1–24. https://doi.org/10.1371/journal.pone.0161832

Wageningen Economic Research. (2020). *Number of pigs in the Netherlands from 2000 to 2019 (in millions) [Graph]*. Statista. https://www.statista.com/statistics/647035/total-number-of-pigs-in-the-netherlands/

Zebunke, M., Puppe, B., & Langbein, J. (2013). Effects of cognitive enrichment on behavioural and physiological reactions of pigs. *Physiology and Behavior, 118,* 70–79. https://doi.org/10.1016/j.physbeh.2013.05.005

Zhang, K., Jiao, L., Zhao, X., & Dong, D. (2016). An instantaneous approach for determining the infrared emissivity of swine surface and the influencing factors. *Journal of Thermal Biology*, *57*, 78–83. https://doi.org/10.1016/j.jtherbio.2016.03.003