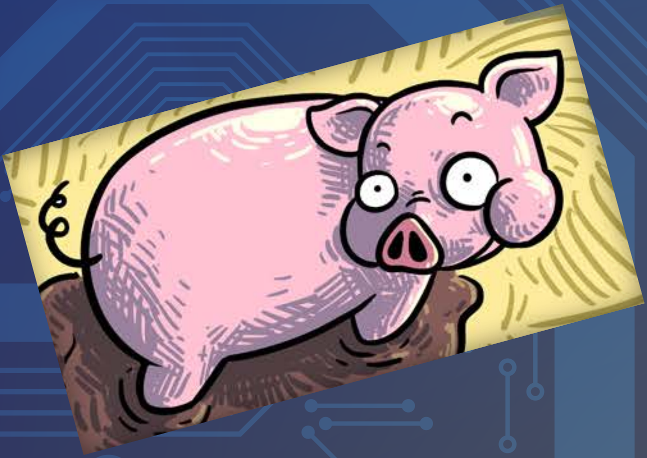
PIGS BEHIND THE TECHNOLOGY

Individual activity monitoring to assess resilience in pigs



Lisette van der Zande

Propositions

1. Technology enabling continuous, activity monitoring of individual animals identifies new phenotypes to quantify resilience.
   (this thesis)

2. Activity pattern changes in response to challenges are promising indicators of (loss of) resilience in pigs.
   (this thesis)

3. Collaboration between academia and industry is essential for implementing academic research.

4. When reviewers of a scientific paper are anonymous to the authors, the authors should be anonymous to the reviewers as well.

5. Red coloured feedback has a negative effect on the affective state of a PhD candidate.

6. Productivity increases when working from home is not restricted to home.

Propositions belonging to the thesis, entitled

Pigs behind the technology – Individual activity monitoring to assess resilience in pigs

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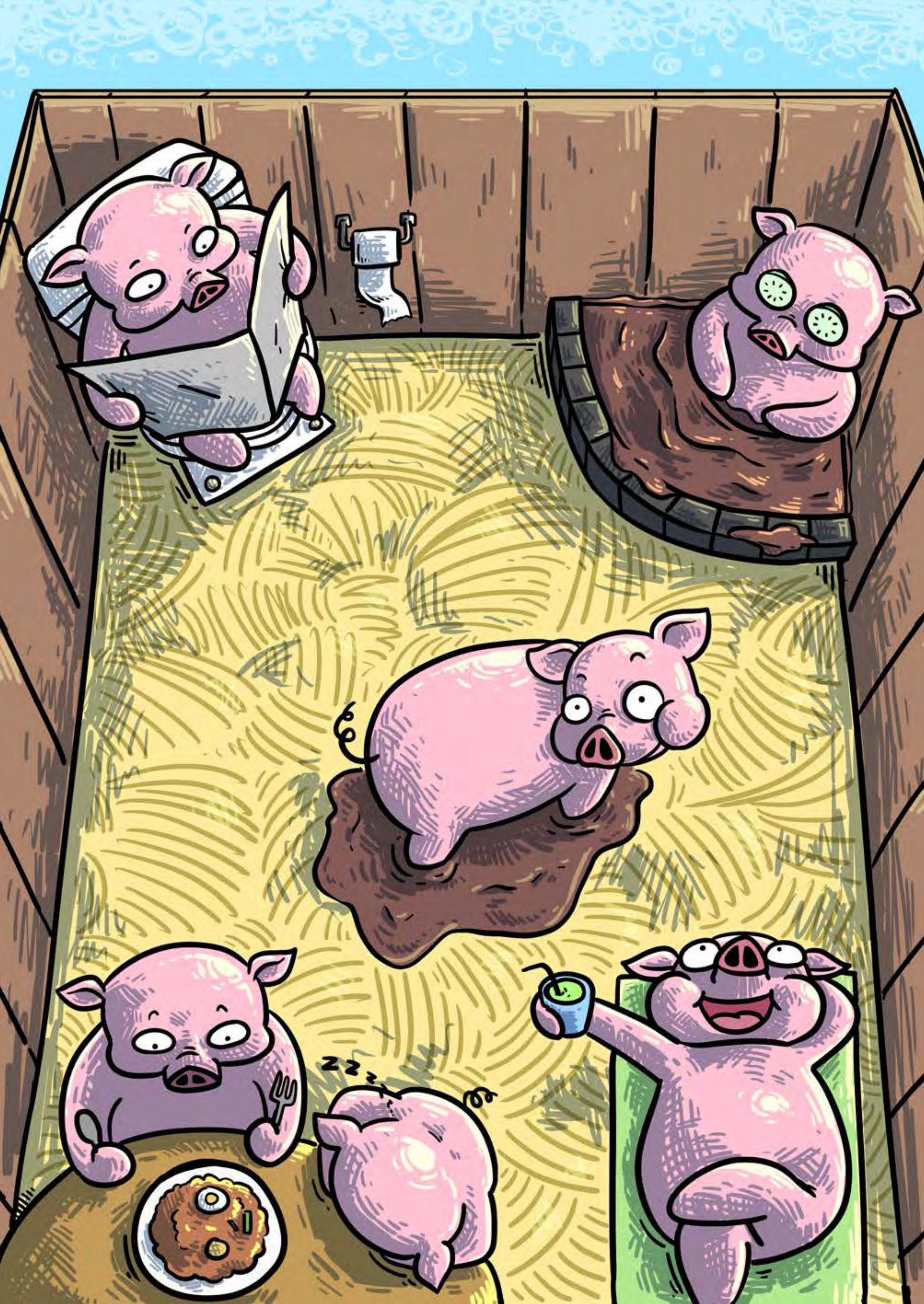
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Pigs behind the technology

Individual activity monitoring to assess resilience in pigs

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Pigs behind the technology
Individual activity monitoring to assess resilience in pigs

Lisette Elise van der Zande

Thesis

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Pigs behind the technology: Individual activity monitoring to assess resilience in pigs

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Abstract

Resilience is a highly relevant trait in pigs, as it may influence their welfare. Resilience is defined as the capacity to be minimally affected by and recover swiftly from a challenge. Resilience is, however, difficult to measure directly. A potentially relevant proxy measure for resilience is activity, as activity patterns deviate under challenging circumstances. The aim of this thesis was to explore novel technologies to measure activity changes as a proxy for resilience. Accelerometers and computer vision were implemented to measure activity following a sickness challenge. Increased root mean square error (RMSE), reflecting variation, and skewness in accelerometer activity three days after infection with porcine reproductive and respiratory syndrome virus (PRRSV), and changes therein compared with pre-infection levels, were associated with higher probability of morbidity and mortality, respectively. Pigs housed in an alternative system comprising group farrowing, delayed weaning, more space and enrichment materials (AHS) appeared to be more resilient and experienced less chronic stress than conventionally housed pigs (CONV), as they were less affected by and/or recovered faster from a transport and a lipopolysaccharide (LPS)-induced sickness challenge, showed less cortisol accumulation in hairs and a lower variance in weight gain. Limited housing effects were found on characteristics of the LPS-induced activity dip, but AHS pigs showed higher baseline activity than CONV pigs. Relationships between the physiological changes and the activity dip in response to the LPS challenge were limited. Hence, sickness-induced changes in activity patterns cannot simply be used as a substitute of physiological data. This does not imply that the activity decrease in itself is not an important aspect of the sickness response which may have consequences for animal health. Taken together, activity patterns and deviations therein are, supplementary to physiological variables, promising indicators of (loss of) resilience in pigs. Overall, the results of this thesis contribute to the biological understanding of resilience and future enhancement of animal welfare.

Contents

Chapter 1

General introduction

1.1 Introduction

Pork is the most consumed meat in the EU (OECD/FAO, 2021). In Europe, the production of pork has increased from 28 million tonnes in 1990 to over 30 million tonnes in 2020 (FAOSTAT, 2022). Although at a slower rate, it is expected that global meat consumption will continue to grow in the future (OECD/FAO, 2021). Large numbers of animals are needed to supply the current and upcoming demand for animal protein. In the Netherlands, over 11 million pigs were housed in 2020 (CBS, 2021). These animals need to be managed in the most efficient way, which is referred to as intensive livestock farming, while addressing societal concerns regarding animal welfare and environmental impact. Intensification of production increases the amount of output generated per unit of input (Place, 2017). In other words, if animals are more productive, fewer animals are needed to produce the same amount of protein. This results in lower emissions of greenhouse gasses and lower land and water usage for the production of animal feed (Steinfeld et al., 2006). Besides affecting the environment, intensive livestock farming also has considerable impact on the animals used for intensive production. It often leads to animals being housed indoors. Indoor housing allows for a controllable environment, however this often coincides with poorer air quality compared to systems with outdoor access (Pedersen et al., 2000). The housing conditions indoors are often barren with (partly) slatted floors and limited possibilities for pigs to express important natural behaviours. Pigs kept in intensive systems have also changed through breeding. Pigs have been bred to use nutrients more efficiently and to grow faster. This high production in intensive systems is demanding for the animals which may, in combination with a suboptimal environment, impair their welfare. Since the 1990s, animal welfare has become an important pillar in the sustainable production of animal protein. The Five Freedoms vision on animal welfare addresses both physical fitness and mental suffering of animals (Farm Animal Welfare Council, 1979). The Five Freedoms vision aims to prevent suffering, and includes the freedom of animals from thirst, hunger and malnutrition, freedom from discomfort, freedom from pain, injury and disease, freedom to express normal behaviour and freedom from fear and distress (Webster, 2001). Fraser et al. (1997) captured animal welfare in three different pillars: biological functioning, emotional state and the ability to express natural behaviour. The more recent Quality of Life model (QoL), unlike the Five

Freedoms vision on welfare, incorporates positive welfare states into a "life worth living" (Mellor, 2016), and includes positive experiences in monitoring welfare.

Negative experiences could occur in different scenarios in pig husbandry, with different causes and effects on the animal. Causes of negative experiences in pigs are, for instance: mixing, heat stress or disease pressure. To reduce the impact of a negative experience to a tolerable minimum, it is important that an individual is able to quickly recover from this negative experience, and this could be referred to as resilience. Resilience is defined as "the capacity of the animal to be minimally affected by a disturbance or to rapidly return to the physiological, behavioural, cognitive, health, affective and production states that pertained before exposure to a disturbance" (Colditz and Hine, 2016). When a pig is resilient to multiple negative experiences, this could be referred to as general resilience. In attempting to cope with and recover from a disturbance or challenge, a range of physiological and behavioural responses is generated. The ability to swiftly recover from a challenge could be partially determined by internal factors, such as the genetic background of animals, but will also depend on the opportunities that are offered in the environment to show adaptive behaviour. Enhanced resilience may benefit animal welfare, given that negative experiences are, because of a quick recovery, brought to a minimum.

Measuring resilience is challenging, since one needs to identify the onset of the negative experience, and also the corresponding recovery curve. Moreover, measuring resilience is often invasive to the animal and/or labour intensive. For instance, often physiological parameters are used to characterize the response to and recovery from a challenge, for which blood sampling, saliva collection or body weight and backfat measurements are needed.

This thesis focusses on the use of technology to measure resilience as reflected in activity changes. The use of technology could make the measurement of resilience a less negative experience for the animal, as it should be less invasive than physical monitoring, and is potentially less labour intensive. In the remainder of this introduction, an overview is provided of resilience in pig husbandry, how resilience could be measured, and which technology could aid in measuring resilience.

1.2 Resilience in pig husbandry

Pigs face all kinds of challenges during their life. The first challenge they encounter is already within hours after birth, when they need to reach the udder and compete with siblings for the best place at the udder. At weaning, piglets are separated from the sow, often mixed with unfamiliar pigs, usually transported, and housed in a new environment. In most cases, they are moved and mixed again at approximately 25 kilos. Social stress might even cause disease in pigs (Proudfoot and Habing, 2015). In addition to the social challenges, housing and management procedures could introduce challenges to pigs. Still most fattening pigs in the Netherlands are housed on barren floors with less than one square meter per pig (van Ooijen, 2021). There is an abundance of literature to prove the beneficial effects of proper environmental enrichment to facilitate explorative behaviour in pigs (Bracke et al., 2006; Buijs and Muns, 2019; Godyń et al., 2019; Mkwanazi et al., 2019; Studnitz et al., 2007; van de Weerd and Day, 2009). Although the provision of suitable enrichment materials is a legal requirement in the EU, the materials provided to the pigs are often suboptimal. For instance, short metal chains are often used as enrichment, however, Bracke et al. (2006) showed that metal objects are not very suitable as enrichment, whereas rubber, rope, wood, roughage and substrates are better. Straw and compound materials were identified as the best possible enrichment for pigs (Bracke et al., 2006). As metal chains are not considered proper enrichment, but are still widely used as part of the enrichment for pigs, it may seem that the scientifically proven benefits of proper enrichment provision are not incorporated in pig husbandry (van de Weerd and Ison, 2019). Most often the lack of incorporation of suitable enrichment is not due to ignorance, but related to costs, labour, and to the difficulty to implement organic (bedding) materials in existing housing and slurry systems. As a result, limitations in housing conditions that hamper the expression of important behaviours are nowadays still a challenge for pigs. Inevitable challenges, such as extreme weather conditions or disease outbreaks, which can only be affected by management to a certain extent, might also cause challenges for pigs. Heat stress or diseases present a significant economic burden to the farmer, besides the obvious pig welfare impairments. For instance, a severe outbreak of porcine reproductive and respiratory syndrome virus (PRRSV) might cost the farmer up to 650 euros per sow per year (Nathues et al., 2017). To treat bacterial infections, antibiotics are used. However, bacteria can mutate and become resistant to the antibiotics and therefore be a direct threat for human health (Lekagul et al., 2019). The best-known example of such a threat are MRSA bacteria (methicillin-resistant *Staphylococcus aureus*), that can seriously threaten hospital patients (Voss et al., 2005). This is why pig farmers, who often carry MRSA, are isolated when admitted to hospital.

In the EU it is prohibited to use antibiotics as a growth promotor to prevent antibiotic resistance since 2006. Since 2009, the Netherlands already realised almost a 70% reduction in the usage of antibiotics in the livestock sector (de Greeff et al., 2020). The achieved reduction of antibiotics usage was mainly accomplished by stopping preventive administration of antibiotics (de Greeff and Mouton, 2017). Antibiotics usage needs to be reduced even further as the ultimate goals is to preserve the effectiveness of antibiotics. The current and upcoming reduction of antibiotic treatments require a different approach of pig health management.

Several terms are used in literature to describe the response to pathogenic health challenges in pigs. We can distinguish between resistant, tolerant and resilient pigs. These terms all refer to the capacity of pigs to adapt to environmental conditions, implying stable performance. However, there are small differences between the three. Resistant animals are immune to a pathogen. Resistance is the ability of a pig to actively decrease the infection pressure (Best et al., 2008). Tolerance implies infection with the disease, but the impact of the infection is limited. However, tolerant animals are not able to actively lower the infection pressure (Best et al., 2008; Schneider and Ayres, 2008). Resistance and tolerance often refer to coping with infectious disease, whereas resilience goes beyond disease. With resilience we refer to challenges rather than diseases only. A resilient pig is a pig that is able to return swiftly to its original state after a challenge (Colditz and Hine, 2016; Pimm, 1984). Resistant animals (i.e., animals who are immune to a pathogen) will require no or fewer antibiotic treatments, however this places selective pressure on the pathogens and will eventually evolve to subvert the resistance (Schneider and Ayres, 2008). Tolerant animals will not experience side effects from a pathogen, but do not actively lower the pathogen load. Therefore, tolerant animals may still need treatment to reduce further spread of the pathogen. On the other hand, resilient animals are able to swiftly recover, without or with lower use of medication. For resilient animals, not only may the negative experience of disease be less intense or shorter, i.e., animal welfare is less impaired following infection, but also less antibiotics are needed for those animals. Therefore, a fast recovering, resilient pig is preferred over a resistant or a tolerant pig. As mentioned earlier, the concept of resilience not only covers coping with and recovery from disease disturbances, but can also be applied to responses to social and management disturbances. Poor resilience may lead to an increased risk to develop behavioural and health problems following a disturbance from which resilient animals swiftly recover and may culminate in severe welfare problems, making resilient animals preferred in pig husbandry.

Not only the farmer will benefit from resilient animals by keeping up with production, the animals themselves will also benefit. It is not a realistic goal to eradicate all challenges livestock are exposed to. Thus, when pigs are exposed to challenges, it is best that they are minimally affected by and quickly recover from these challenges. The suffering and impairment of welfare is brought to a minimum by a quick recovery. Resilient animals might have therefore enhanced levels of animal welfare. Two clear pathways to improve resilience are described in literature. The first pathway is through breeding for enhanced resilience (Harlizius et al., 2020). Putz et al. (2019) used feed intake duration of pigs, as it is sensitive to disease, to estimate resilience indicators (see below). The heritability of these indicators ranged from 15% up to 26%. Homma et al. (2021) estimated heritability of resilience ranging from 31% to 40% for resilience traits based on feed intake. Although the estimated heritability of resilience ranged from 15 up to 40%, genetic variation is found and resilience could therefore be introduced in a breeding program. Berghof et al. (2019) simulated a pig breeding program including resilience. They demonstrated the possibility to successfully introduce a simulated resilience trait into a breeding program, with enhanced resilience in the next generations. The other pathway to enhance resilience of pigs is through improving the early life conditions and living environment of animals. For instance, improving social experience at a young age by allowing piglets of multiple litters to mingle resulted in less diarrhoea, less aggression, fewer skin lesions and improved performance later in life compared to conventional farrowing conditions (Camerlink et al., 2018; van Nieuwamerongen et al., 2015). In addition, proper environmental enrichment might have a positive effect on disease resilience. Van Dixhoorn et al. (2016) showed a faster viral clearance in pigs enriched with straw, branches, extra space and exposure to another litter during lactation, compared to barren housed pigs after co-infection with PRRSV and *Actinobacillus pleuropneumoniae*. The probability of lung damage and the pathological lung tissue damage score was also higher for barren housed pigs than for enriched housed pigs (van Dixhoorn et al., 2016). In addition, pigs exposed to early life enrichment (more space and deep straw, peat and sawdust bedding) were less sensitive to a reward loss (Luo et al., 2020b). Furthermore, pigs reared in an enriched environment experience less stress from common preslaughter handling and transport than pigs reared in a barren environment (de Jong et al., 2000a). Enriched housed pigs showed less manipulation of pen mates and lower cortisol response to mixing at transport and being in lairage compared to barren housed animals. Thus, rearing or housing in enriched conditions might enhance general resilience in pigs. This suggests that resilience in pigs can be influenced by environmental conditions.

1.3 Measuring resilience

As the definition of resilience implies, the animal being minimally affected by and able to quickly recover from a challenge, both these aspects need to be included when measuring resilience. Therefore, it is important to record the starting point when an animal is affected, how severely the animal is affected, and how soon it recovers. Defining the exact onset of response and recovery is challenging as it requires frequent measurements. Ideally, the complete response is monitored continuously (Scheffer et al., 2018). Traits that reflect the response to a disturbance or outcome of poor resilience and could be frequently measured are production traits, physiological traits and behavioural traits. These are often used as proxies to assess resilience. In the remainder of this section, I will explain how these traits can be measured and how they might represent resilience.

1.3.1 Production traits

In multiple species, production traits can be used to estimate (loss of) resilience. Dairy cows that experience mastitis for example have a low milk yield and elevated somatic cell count in milk (Hortet and Seegers, 1998; Sharma et al., 2011). Milk production in dairy cows fluctuates over days, and it has been suggested that not only daily milk production per se, but also the fluctuation therein could be used as indicator of resilience. Poppe et al., (2020) hypothesised that cows with more fluctuations in their milk yield over time would reflect low resilience to disturbances than cows with a more stable milk yield. They calculated parameters that reflect such fluctuations, i.e., the variance, autocorrelation and skewness of daily deviations in lactation curves. Variance in milk yield was genetically correlated to udder health and longevity, indicating a better health status of cows with a low variance in daily milk production over a lactation period. Therefore, it was concluded that the variance in milk yield is a promising resilience indicator. Frequently measured production traits are less commonly available in fattening pigs. Fluctuations in growth and weight could be possible indicators of resilience, however in pig husbandry practice, weight is only measured a couple of times during a pig's life. Putz et al. (2019) used feeding data originating from automatic feeding stations which record pig identity, visits to the station and disappearance of feed. Based on these data, the duration of feeding and 'off-feed' days (i.e., high negative deviation in feed intake) could be calculated. It was found that the proportion of 'off-feed' days was genetically correlated with mortality and the number of (drug) treatments, which reflects the ability to recover from challenges, with correlation coefficients ranging from 0.37 to 0.85. Feed intake data generated from automatic feeding stations might be a potential trait to use as a proxy for resilience. However, feeding stations are costly and therefore not practical to implement on a larger scale.

1.3.2 Physiological traits

Physiological traits, which include heart rate, body temperature, stress hormones (e.g., cortisol) and immunological parameters, might also be potential proxies for resilience. When a pig is confronted with a challenge, it shows a physiological response to restore homeostasis (Etim et al., 2013). Mostly, the response is characterised by a temporary increase or decrease in levels of the physiological parameter, after which – in case of successful coping with the challenge – a return to pre-challenge levels is seen. The strength of the response and recovery can be used as resilience indicators. For example, changes in cortisol levels following transport could be used to characterize the resilience to that challenge. Transport is one of the most common stressors that almost every pig faces at least once in its life. Transport involves handling, loading, and mixing with unknown individuals and results in an increase in cortisol (Sutherland et al., 2014). Pigs that underwent transportation had 41% higher plasma cortisol concentrations four hours after transport compared to controls (McGglone et al., 1993). Also sickness causes elevated levels of cortisol (Nordgreen et al., 2018) as concentrations were elevated for up to four hours in pigs injected with lipopolysaccharide (LPS) to induce sickness compared to saline injected pigs. Often in response to infectious challenges pigs develop a fever, i.e., temporary increase in body temperature. These physiological traits are very suitable to measure the response curve to a challenge. However, the main disadvantage of these traits is the invasive manner to collect the data, e.g., via blood sampling and assessment of rectal temperature which involve handling or even restraining and cause discomfort to the animals. Besides the stress this induces in the animal, the sampling procedures may exert an undesirable effect on the physiological parameters measured. Moreover, it is labour intensive to collect the data frequently enough to measure recovery.

An alternative to measuring the strength of physiological responses and recovery, i.e., return to baseline, as a reflection of resilience, is to assess chronic stress. If pigs are less resilient, they may not be able to cope with or swiftly recover from the challenges they are exposed to. This may cause "wear and tear" on the animals, as reflected in signs of chronic stress, which may further reduce resilience to future challenges. Beattie et al. (2000) measured the weight of adrenal glands at slaughter of barren housed and enriched housed pigs. The enriched housed pigs were housed in a larger pen with straw bedding from three days of age onwards, whereas the barren pens were smaller with slatted floor. They found an increased weight of the adrenal glands of barren housed pigs compared to enriched housed pigs (Beattie et al., 2000). This might be an indication of chronic stress, being the result of chronic increased activation of the hypothalamic-pituitary-adrenal (HPA) axis. Chronic stress is also reflected in cortisol accumulation in the hairs of pigs. Heimbürge et al. (2020) administered adrenocorticotropic hormone (ACTH), which induces the release of cortisol, to pigs. They showed that cortisol in hairs was elevated after four weeks of administrating ACTH compared to control animals. These measurements of chronic stress might be less frequent than those done to assess recovery curves, but the animal handling (except for post-mortem measurements) and lab work involved should not be underestimated.

1.3.3 Activity as a potential proxy

Pigs show a distinct diurnal activity pattern. Under challenge free circumstances, pigs are most active in the morning and in the late afternoon (Simonsen, 1990). During a challenge, pigs may deviate from their standard behaviour pattern (Matthews et al., 2016). Active behaviour can deviate in both directions, either higher activity or lower activity. For instance, regrouping with unfamiliar pigs results in more aggressive acts between the pen mates, which in turn causes higher activity (Marchant-Forde & Marchant-Forde, 2005; Peden et al., 2018). However, infectious diseases which cause fever and lethargic behaviour as part of the sickness response may result in lower activity (Escobar et al., 2007; Nordgreen et al., 2018; van Dixhoorn et al., 2016). van Dixhoorn et al. (2018) studied the inclusion of behaviour to estimate the resilience of cows, expressed in a total deficit score, during the transition period (from three weeks prepartum to three weeks postpartum). The optimum combination of predictors of resilience consisted of average eating time, variation of ear temperature and daily behaviour patterns. Activity and patterns in activity could be a potential proxy for resilience.

Measuring activity is traditionally done by human observations, often by means of instantaneous scan sampling. Scan sampling consists of observing a group of animals with regular time intervals. This results in an estimation of time expenditure, both at the individual and the group level. To use activity pattern changes as a potential measure resilience, regular and longitudinal observations are needed to assess the exact time of onset of disturbance and recovery. Ideally, 24/7 observations are used to measure resilience. However, with only human observations this is not feasible. The use of technology could aid humans in measuring the activity by tracking individual animals and estimate activity based on sensor data.

1.4 Sensor technology for pigs

The use of technology in livestock is often referred to as precision livestock farming (PLF). Since 2003, the term PLF is increasingly found in literature, following several international research projects on this topic. Technology is already an integrated part of livestock production, for example automatic milking robots and automatic feeding stations have been introduced. These technologies are focussed on labour reduction for the farmer. The use of sensors to collect data on animals, however, is a new emerging field within PLF. Sensors like radio frequency identification (RFID), ultrawide band tracking (UWB), accelerometers and video technology could be applied to the livestock industry (Ellen et al., 2019). The latter two will be discussed in this introduction, since these technologies are used in this thesis.

Accelerometers measure acceleration along three-dimensional axes (x,y,z). With the use of machine learning, accelerations could be translated to behaviours and potentially replace human observations. Oczak et al. (2016) classified accelerations into different postures of sows around farrowing. The overall accuracy of detection of sow postures by accelerometers was 70% with human observations as gold standard. Other studies used accelerometer data to classify number of steps (Grégoire et al., 2013) and feeding behaviour of pigs (Cornou & Lundbye-Christensen, 2008). The main advantages of using accelerometers are that accelerations could be transferred wirelessly to a computer and that the identity of the animal is captured within the hardware, so as long as the animal does not lose the accelerometer, the identity of the animal is always known. However, the attachment of an accelerometer to pigs is difficult. For the posture analysis in sows as performed by Oczak et al. (2016), accelerometers were attached to the legs of the sows, and the sows were housed in individual pens or individual gestation stalls. Data were recorded for a short period of time (approximately 24 hours). For long term recording, the accelerometer could be attached to the ear tag, as attachment to other body parts might be attractive to other pigs when housed in groups. When worn in the ear, the weight of the accelerometers is important, i.e., it cannot be too heavy. This makes it challenging to use accelerometers in younger animals. If placed in the ear, the measurement directly reflects the ear movement. Movements of the head cause noise in the data compared to accelerometers mounted on other parts of the body. This makes it more difficult for a machine learning algorithm to filter out behaviours of interest like locomotion. In addition, behaviours might be very similar in movements but might be very different when observing the animals: this holds, for instance for tail biting or feeding. During both behaviours the pig is chewing, but the behaviours are very different from each other with different consequences and thus making it troublesome if they are mixed up. Additional location information might help to overcome this problem but location data are not collected using accelerometers.

Computer vision is a field of artificial intelligence that enables computers to gain high level understanding from digital images or videos. Multiple studies show the possibility to detect pig behaviour using computer vision. The increase in research towards the implementation of computer vision for pigs represents the potential it may possess (**Table 1.1**). Ott et al. (2014) showed that computer vision could be used to estimate activity of pigs, in high agreement with human observers (R_s=0.92). Video recordings are non-invasive for the pigs as they do not require hardware attached to the animal. In contrast to using accelerometers, the location of the animal can be derived from the video. As exemplified in **Table 1.1**, it is already possible to derive location and some types of behaviour using computer vision using different methods. Computer vision could be applied at group level and individual level. Automated measurement of pig behaviour at group level can have clear benefits for the farmer and the production chain: e.g., providing tools for health and welfare monitoring. Ideally, pig behaviour is monitored at an individual level. Individual data could aid in video assisted treatments or care for specific animals rather than treating the whole pen. Individual data is also necessary to allow for breeding for enhanced resilience. In addition, with complex damaging behaviours like tail biting, the identification of the biter and the victim would be an added value. The main challenge of using computer vision to measure behaviour and activity at the individual level is to establish and maintain the identity of the animal. Pigs have a similar appearance and lie close to each other. These occlusions make it difficult for computer vision algorithms to recognize and keep track of separate pigs. So far, no computer vision algorithm is available for the tracking of activity of individual pigs in a commercial setting.

Table 1.1 – Example studies using computer vision in pigs.

Computer vision method	Feature of interest	Individual or group level	References
Subtraction of reference frame	Locomotion	Group level; all movement within the frame	(Lind et al., 2005)
Ellipse fitting	Location	Individual level; with the use of markings	(Kashiha et al., 2013b)
Region growing (using 3D cameras)	Location	Individual level	(Matthews et al., 2016)
Deep neural networks	Body part detection	Individual level using ear tags	(Psota et al., 2020b)
Anchor based convolutional neural network	Location and postures	Individual and pen-wise	(Alameer et al., 2020a)
Point cloud (using 3D cameras)	Body weight, tail posture	Group level	(D'Eath et al., 2021, 2018)
Threshold segmentation	Location	Group level	(Guo et al., 2015)

Motion detection	Circadian rhythm in activity	Group level	(Chung et al., 2014)
Optical flow patterns	Identification of stationary pigs in slaughterhouse	Group level	(Gronskyte et al., 2016)
Region growing	Location, standing	Group level	(Matthews et al., 2017)
Ellipse fitting	Location, lying	Group level	(Nasirahmadi et al., 2015)
Ellipse fitting	Mounting behaviour	Group level	(Nasirahmadi et al., 2016)
Ellipse fitting and body contour	Water usage	Group level	(Kashiha et al., 2013a)
Neural network	Activity index	Group level	(Oczak et al., 2014)
Gabor and Local Binary Pattern features	Location	Individual level; based on skin colour	(Huang et al., 2018)
Neural network	Tail biting interactions	Individual level; but not linked to identity	(Liu et al., 2020)

1.5 Aim and outline of this thesis

To summarize, there are indications that changes in activity could be used to characterize resilience in pigs. To establish activity patterns of pigs and deviations therein, behaviour should ideally be monitored continuously at the individual pig level, which is not feasible using human observations. Technology to measure behaviour is developing fast and has potential to track activity continuously. Therefore, technology might assist in large scale resilience monitoring. The main aim of this thesis was to explore smart technologies to measure activity and deviations in activity patterns as a proxy for resilience. A schematic overview of this thesis is presented in **Figure 1.1**.

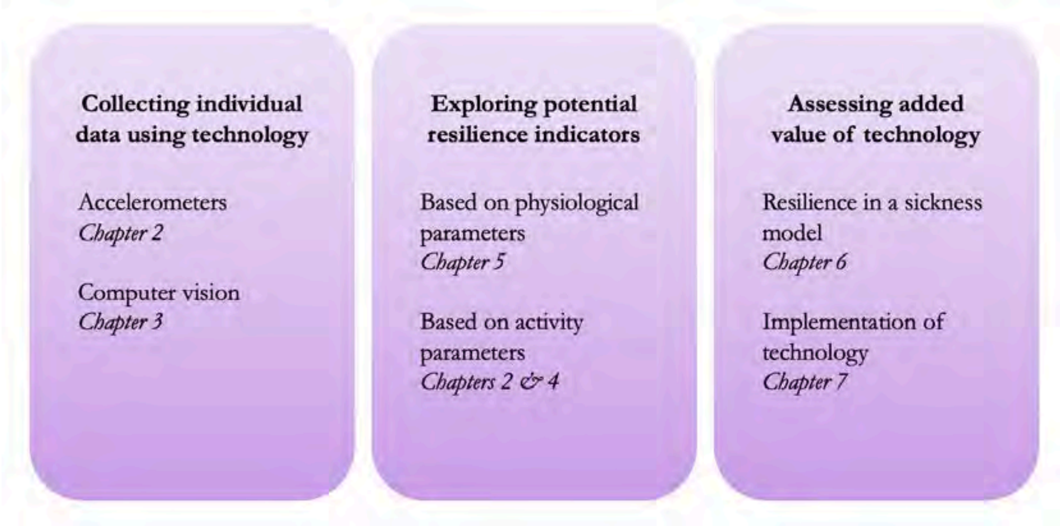

Figure 1.1 – Schematic overview of the contents of this thesis.

Chapter 2

Quantifying individual response to PRRSV using dynamic indicators of resilience based on activity

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Abstract

Pigs are faced with various perturbations throughout their lives, some of which are induced by management practices, others by natural causes. Resilience is described as the ability to rapidly recover from or cope with a perturbation. Using these data, activity patterns of an individual, as well as deviations from these patterns, can potentially be used to quantify resilience. Dynamic indicators of resilience (DIORs) may measure resilience on a different dimension by calculating variation, autocorrelation and skewness of activity from the absolute activity data. The aim of this study was to investigate the potential of using DIORs of activity, such as average, root mean square error (RMSE), autocorrelation or skewness as indicators of resilience to infection with the Porcine Reproductive and Respiratory Syndrome Virus (PRRSV). For this study, individual activity was obtained from 232 pigs equipped with ear tag accelerometers and inoculated with PRRSV between seven and nine weeks of age. Clinical scores were assigned to each individual at 13 days post-challenge and used to distinguish between a resilient and non-resilient group. Mortality post-challenge was also recorded. Average, RMSE, autocorrelation and skewness of activity were calculated for the pre- and post-challenge phases, as well as the change in activity level pre- vs. post-challenge (i.e., delta). DIORs pre-challenge were expected to predict resilience to PRRSV in the absence of PRRSV infection, whereas DIORs post-challenge and delta were expected to reflect the effect of the PRRSV challenge. None of the pre-challenge DIORs predicted morbidity or mortality post-challenge. However, a higher RMSE in the 3 days post-challenge and larger change in level and RMSE of activity from pre- to post-challenge tended to increase the probability of clinical signs at day 13 post-infection (poor resilience). A higher skewness post-challenge (tendency) and a larger change in skewness from pre- to post-challenge increased the probability of mortality. A decrease in skewness post-challenge lowered the risk of mortality. The post-challenge DIOR autocorrelation was neither linked to morbidity nor to mortality. In conclusion, results from this study showed that post-challenge DIORs of activity can be used to quantify resilience to PRRSV challenge.

2.1 Introduction

Resilience is defined as the ability to rapidly recover from or cope with a perturbation (Colditz and Hine, 2016). Perturbations can be of any natural cause (e.g., heat stress) or can, in the case of farm animals, be induced by management practices (e.g., transportation). Pigs face multiple perturbations during their lives. When exposed to a perturbation, pigs may show individual differences in resilience. Improving resilience in pigs may contribute to sustainable pig production for a number of reasons. Resilient pigs are better able to recover from perturbations, including infectious challenges, and require fewer treatments and management interventions. The improved overall health status of resilient animals also results in improved animal welfare. In addition, because resilient pigs are less disturbed by a perturbation, they require less feed than non-resilient pigs for the same amount of growth, and therefore have a better feed efficiency (Hermesch et al., 2015). For these reasons, promoting resilience in pigs by optimizing (early life) conditions or by genetic selection, is desirable for future pig production.

Resilience may be measured in various ways, for instance by using physiological parameters. Blood parameters, such as white blood cell count and haemoglobin level, are examples of physiological parameters used as indicators of resilience (Hermesch and Luxford, 2018). Other physiological variables used are production parameters like body weight and milk yield, which are commonly used to predict health related traits (Berghof et al., 2019a; Poppe et al., 2020). However, despite the number of parameters used, the lack of a golden standard for quantifying resilience remains a challenge. Assessment of physiological parameters can be invasive to animals, and is often labour intensive. Moreover, it is often not feasible to collect physiological data repeatedly, whereas for assessment of recovery time following a perturbation, frequent, or continuous measurements are required. Behaviour is one example of a non-invasive parameter with the potential for easy, repeatable observations. Weary et al. (2009) stated that behaviour is the most commonly used indicator for illness, as reduced activity is a main characteristic of the sickness response that is induced after infection (van Dixhoorn et al., 2016), and may also occur after other stressors (Costa et al., 2014). Locomotor behaviour is therefore often included in the ethogram of studies investigating illness. Traditional behavioural observation methods are labour intensive, especially when animals need to be studied frequently. Precision phenotyping tools such as wearable accelerometers, which are capable of quantifying activity automatically, are therefore an attractive alternative. Accelerometers measure acceleration along the x, y, and z-axis. Using machine learning models, acceleration can be translated to activity which can, in turn, possibly be used to quantify resilience.

Apart from changes in the level of activity per se, dynamic changes in activity patterns may be related to resilience (van Dixhoorn et al., 2018). Dynamic indicators of resilience (DIORs), which are capable of quantifying deviations in functioning of biological systems, are proposed by Scheffer et al. (2018) and have been adopted for farm animals as resilience indicators (Berghof et al., 2019a). Such DIORs are, for instance, variance, and autocorrelation in repeatedly measured variables, which may include activity. It is expected that resilient pigs will show less variation in activity following a perturbation. In general, the activity level of pigs following a health challenge will be reduced. Pigs that recover more quickly from such a challenge (i.e., resilient pigs) will return to their initial level of activity faster than non-resilient pigs. This should result in a lower Root Mean Square Error (RMSE) of activity. Putz et al. (2019) found a positive genetic correlation between RMSE of feed intake and mortality, suggesting that RMSE of feed intake can be used as an indicator of resilience. Autocorrelation represents the degree of similarity between two given time periods and ranges from −1 to 1. It is hypothesized that resilient pigs will have a (lag-1) autocorrelation of activity around zero (Berghof et al., 2019b), as their fast recovery results in less resemblance to previous days. Less resilient pigs recover more slowly from a perturbation, resulting in more similarity in activity of previous days for a longer period of time, i.e., a high autocorrelation. Skewness indicates the direction of the response to perturbation, i.e., a positive or negative response. It is expected that resilient pigs will have a skewness around zero as they recover more quickly from a perturbation than non-resilient pigs. All DIORs are expected to be most informative immediately following a perturbation. It can be observed directly whether a decrease in activity occurs, how steep the slope of the decrease is, and how long it persists. However, it has been suggested that dynamic patterns in repeatedly measured biological systems before a major perturbation might also be predictive of resilience. Systems losing resilience, approaching a tipping point to an alternative state (e.g., disease) may also show slower recovery from small, natural perturbations in the environment, resulting in, for instance, higher autocorrelation, and variance [see Scheffer et al. (2018), for review].

In this study, DIORs based on activity were used to measure and potentially predict resilience following a Porcine Reproductive and Respiratory Syndrome Virus (PRRSV) infection. PRRSV is a common infection among pig populations (Almeida et al., 2018). As its name implies, PRRSV results in two main pathologies: reproductive failure and respiratory disease. Reproductive failure occurs in pregnant sows and results in abortions, mummified piglets, and weak live born piglets. Growing pigs infected with PRRSV may suffer from high fever, have loss of appetite and become lethargic or less active, leading to reduced growth and feeding efficiency, and increased mortality. The course of the clinical signs is on average two weeks. Despite the availability of vaccines, PRRSV remains a difficult disease to control and regular outbreaks occur. Besides the impairment of pig welfare, PRRSV causes severe economic losses for the farmer.

The aim of this study was to investigate whether activity levels, or DIORs such as RMSE, autocorrelation or skewness of activity patterns, can be used as dynamic indicators of resilience following PRRSV infection in pigs.

2.2 Material and Methods

Data for this paper were obtained from a subset of pigs in an experiment executed by Pipestone Veterinary Research and Topigs Norsvin USA. Prior to the start of that experiment, Pipestone Applied Research (PAR) institutional animal care and use committees (PAR IACUC 1-18) reviewed and approved the trial.

2.2.1 Animals and Housing

A total of 2,186 commercial crossbred pigs from a commercial sow farm were used for the study we obtained data from. Upon weaning at approximately three weeks of age, pigs were shipped to a commercial research facility in the US. Each pen had fully slatted floors, with two cup waterers and a 4-hole dry feeder which provided 35 cm of feeder space per pig. Feed and water were provided *ad libitum*. Pigs originated from three genetic groups. Two groups were sired by boars from the same genetic line, but these boars were selected based on a different breeding goal. The third group was sired by a different genetic line. Upon arrival at the research facility, pigs were penned by genetic group and balanced by sex with 27 pigs housed per pen (0.65 m^2/pig) in 81 pens in total and all pigs were vaccinated per the label instructions using a PRRS modified live virus vaccine (IngelVac ATP, Boehringer Ingelheim). Pens had fully slatted concrete floors. Lights were on in the facility from 8:00 to 20:00 with a night light turned on outside of these hours. Four weeks later, pigs were experimentally inoculated with PRRS virus variant 1-7-4 at a total dose of 1×10^5 TCID50 via the IM route [SD15-174 (lineage 1)-TB3-P8, SDSU, Brookings, USA] (Dee et al., 2018). At 0, 13, and 42 days post-infection, corresponding with expected peak PRRS viremia and viral clearance at 13 and 42 days post-infection, pigs were weighted and clinical scores were assigned using a 6-point scoring system (Hess et al., 2016; Lopez and Osorio, 2004). Scores were assigned as follows where: “1”, healthy; “2”, mild signs of disease; “3”, moderate signs of disease; “4”, advanced signs of disease; “5”, extreme signs of disease; and “6”, deceased (including day) (Pantoja et al., 2013). We could not define the recovery period using activity, because clinical scores were not assessed daily. Therefore, clinical scores at 13 days post-infection were used to distinguish pigs with a favourable or unfavourable outcome of the infection, where pigs with a clinical score of “1” were classified as “resilient”, and pigs with a clinical score “>1” were classified as “non-resilient”.

2.2.2 Collection of Accelerometer Data

A subset of 232 pigs, originating from nine pens (three pens per genetic group), were equipped with individual accelerometer ear tags at five weeks of age (Remote Insights, Minneapolis, USA). Accelerometer data were recorded from 23 days prior to infection with PRRSV to 42 days post-infection. Videos of the pigs were annotated for activity by Remote Insights. The annotations were used as training and validation data for a machine learning model to classify their activity (Remote Insights, Minneapolis, USA). A 5-s window was classified as active or inactive, based on the output of the machine learning model, which resulted in 720 windows per hour. Data were transformed to minutes per hour. Forty-seven animals were removed from the final dataset, due to missing data for more than 20 consecutive hours, resulting in a total of 185 animals used for analyses. Missing values influence the calculation of DIORs. To avoid this, a rolling average was used for the analysis with a window of 12 h.

2.2.3 DIORs Calculation

Dynamic indicators of resilience (DIORs) were calculated per individual for the pre-challenge (from 23 days pre-challenge until challenge) and post-challenge (from challenge until three days post-challenge) phases, as well as the change in activity level from three days pre-challenge vs. three days post-challenge (i.e., delta). Pre-challenge data were used to potentially predict resilience, based on clinical scores on day 13 post-challenge, without the influence of the PRRSV inoculation. DIORs post-challenge, based on data from the first data days post-challenge, were also used to potentially predict resilience and mortality. The first three days post-challenge were chosen, because on the fourth day post-challenge the first pig died, so all animals have data collection up to three days post-challenge. The delta of DIORs following inoculation was calculated by subtracting DIORs of three days pre-challenge from DIORs of three days post-challenge.

Root Mean Square Error (RMSE) of activity of the j^{th} individual was calculated as:

$$RMSE_j = \sqrt{\frac{\sum_{i=1}^{n_j} (x_{f_{ij}} - x_{o_{ij}})^2}{n_j}},$$

where $x_{f_{ij}}$ is the forecasted observation i of the j^{th} individual, $x_{o_{ij}}$ is the observed observation i of the j^{th} individual, and n_j is the number of observations of the j^{th} individual.

Autocorrelation of activity of the j^{th} individual was calculated as:

$$Autocorrelation = \frac{\sum_{i=1}^{n_j - k} (x_{ij} - \bar{x}_j)(x_{(i+k)j} - \bar{x}_j)}{\sum_{i=1}^{n_j} (x_{ij} - \bar{x}_j)^2},$$

Where n_j is the number of observations of the j^{th} individual, x_{ij} the i^{th} observation of the j^{th} individual, and $\bar{x}_j$ the sample mean of the j^{th} individual.

Skewness of activity of the j^{th} individual was calculated as:

$$Skewness = \frac{\sqrt{n_j(n_j-1)}}{n_j-2} * \frac{m_3}{m_2^{3/2}},$$

where n_j is the number of observations of the j^{th} individual, $m_k = \frac{1}{n_j}\sum_{i=1}^{n_j}(x_{ij} - \bar{x}_j)^k$, where x_{ij} is the i^{th} observation of the j^{th} individual, and $\bar{x}_j$ the sample mean of the j^{th} individual.

2.2.4 Statistical Analysis

All models were fitted using R (R Core team, 2013). A generalized linear mixed model using a binomial distribution with logit link function was used to test whether DIORs were different for resilient and non-resilient pigs (based on assigned clinical scores). DIORs were tested independent of each other. Fixed effects in the generalized linear mixed model were DIOR and clinical score at the day of inoculation as some pigs already had early or moderate signs of clinical disease. Pen was included as a random effect. Mortality was tested using Cox regression survival analysis. Fixed effects in the Cox regression model were DIOR and clinical score at the day of inoculation. Pen was included as a random effect.

2.3 Results

Two pigs had died prior to inoculation. At day 13 post-challenge, 92 pigs had a clinical score of “1” (i.e., resilient group), where 93 pigs had a clinical score of “2” or greater (i.e., non-resilient group). The resilient group had significantly ($P < 0.001$) higher average daily gain between inoculation and day 13 post-challenge compared to the non-resilient group (0.47 ± 0.02 vs. 0.23 ± 0.02 kg). At day 13 post-challenge, seven pigs had died between one day pre-challenge and 12 days post-challenge. By the end of the study (at 42 days post-challenge), 13 pigs had died between one day pre-challenge, and 27 days post-challenge.

Table 2.1 shows the means and standard deviations of DIORs pre- and post-challenge, illustrating that the average activity levels decreased following challenge, whereas the impact on other DIORs was minimal.

Table 2.1 – Means and corresponding standard deviation in parentheses for DIORs of activity (min/hour) pre-challenge and post-challenge.

DIOR	Pre-challenge[a]	Post-challenge[b]
Average activity	12.17 (1.63)	8.41 (2.00)
RMSE of activity	3.75 (0.60)	3.60 (0.97)
Autocorrelation of activity	0.94 (0.01)	0.91 (0.03)
Skewness of activity	0.24 (0.34)	0.31 (0.38)

[a] Pre-challenge is from 23 days pre-challenge until challenge.
[b] Post-challenge is from challenge until three days post-challenge.
[c] In minutes per hour.

2.3.1 Association between DIORs pre-challenge and morbidity and mortality

Odds ratios given in **Tables 2.2, 2.4** reflect the probability of being non-resilient, i.e., showing clinical signs at day 13 post infection, over the probability of being resilient. The hazard ratios presented in **Tables 2.3, 2.5** give the probability of mortality in respect of time.

Table 2.2 – Odds ratios with 95% confidence intervals (CI) for DIORs of activity pre-challenge (based on 23 days) using generalized linear mixed models for resilience (i.e., morbidity) following PRRSV inoculation.

DIOR[a]	Odds ratio (95% CI)	P-value
Average activity	1.14 (0.92 – 1.40)	0.32
RMSE of activity	1.14 (0.66 – 1.97)	0.61
Skewness of activity	0.99 (0.36 – 2.77)	0.71

[a] Odds ratio of autocorrelation could not be estimated. The variation in autocorrelation was minimal, resulting in very high confidence intervals.

Table 2.3 – Hazard ratios with 95% confidence intervals (CI) for DIORs of activity pre-challenge (based on 23 days) using Cox regression models for mortality following PRRSV inoculation.

DIOR[a]	Hazard ratio (95% CI)	P-value
Average activity	1.10 (0.77 – 1.60)	0.60
RMSE of activity	1.24 (0.49 – 3.20)	0.65
Skewness of activity	0.27 (0.04 – 1.40)	0.11

[a] Hazard ratio of autocorrelation could not be estimated. The variation in autocorrelation was minimal, resulting in very high confidence intervals.

Table 2.4 – Odds ratios with 95% confidence intervals (CI) of DIORs of activity three days post-challenge using generalized linear mixed model for resilience (i.e., morbidity) following PRRSV inoculation.

DIOR[a]	Odds ratio (95% CI)	P-value
Average activity	1.04 (0.88 – 1.24)	0.65
RMSE of activity	1.42 (1.01 – 2.05)	0.05
Skewness of activity	1.30 (0.56 – 3.04)	0.54

[a] Odds ratio of autocorrelation could not be estimated. The variation in autocorrelation was minimal, resulting in very high confidence intervals.

Table 2.5 – Hazard ratios with 95% confidence intervals (CI) of DIORs of activity three days post-challenge using Cox regression models for mortality following PRRSV inoculation.

DIOR[a]	Hazard ratio (95% CI)	P-value
Average activity	0.80 (0.58 – 1.10)	0.18
RMSE of activity	1.09 (0.59 – 2.00)	0.78
Skewness of activity	3.02 (0.92 – 10.00)	0.07

[a] Hazard ratio of autocorrelation could not be estimated. The variation in autocorrelation was minimal, resulting in very high confidence intervals.

DIORs pre-challenge did not relate to the probability of being non-resilient (**Table 2.2**). In addition, probability of mortality post-challenge could not be predicted by DIORs pre-challenge (**Table 2.3**).

2.3.2 Association between DIORs of activity post-challenge and morbidity and mortality

RMSE of activity 3 days post-challenge tended to be different between resilient and non-resilient groups (**Table 2.4**). The odds ratio of RMSE indicates that for every one-unit increase in RMSE, the odds of being non-resilient increases by 1.42 times. Skewness of activity tended to relate to mortality (**Table 2.5**). Every one-unit increase in skewness, the relative risk of mortality tended to increase 3.02 times.

2.3.3 Association between change in DIORs from pre- to post-challenge and morbidity and mortality

The change in DIORs was calculated by subtracting the DIOR for three days pre-challenge from the DIOR for three days post-challenge. **Table 2.6** shows that changes in average activity and RMSE from pre-challenge to post-challenge tended to affect the probability of a non-resilient outcome of the infection. When the average activity decreased post-challenge by one-unit, the probability of being non-resilient was 22% higher (1 divided by 0.82). The effect of changes in RMSE was in the opposite direction. One-unit increase in RMSE tended to increase the odds of being non-resilient by 1.34. The change in skewness significantly affected the probability of mortality (**Table 2.7**). For every one-unit increase in skewness, the relative risk of mortality increased by 3.70.

2.4 Discussion

This study investigated the use of DIORs, including average, RMSE, autocorrelation, and skewness of activity to quantify resilience following PRRSV infection. It was expected that DIORs pre-challenge could be predictive of morbidity or mortality post-challenge. However, no DIOR pre-challenge was identified as predictive for morbidity or mortality in this study. Previous studies that investigated DIORs in livestock calculated DIORs using the entire study period, including the challenge period. This study identified associations between DIORs based on activity and resilience after the PRRSV challenge only, indicating that these DIORs are only associated with resilience when the animal is challenged.

Table 2.6 – Odds ratios with 95% confidence intervals (CI) of the difference in DIORs of activity pre-challenge and post-challenge using generalized linear mixed models (n = 185) for resilience (i.e., morbidity) groups following PRRSV inoculation.

DIOR[a]	Odds ratio (95% CI)	P-value
Average activity	0.82 (0.66 – 1.01)	0.06
RMSE of activity	1.34 (0.98 – 1.87)	0.07
Skewness of activity	1.18 (0.56 – 2.22)	0.75

[a] Odds ratio of autocorrelation could not be estimated. The variation in autocorrelation was minimal, resulting in very high confidence intervals.

Table 2.7 – Hazard ratios with 95% confidence intervals (CI) of the difference in DIORs of activity pre-challenge and post-challenge using Cox regression models for mortality following PRRSV inoculation.

DIOR[a]	Hazard ratio (95% CI)	P-value
Average activity	0.79 (0.52 – 1.20)	0.23
RMSE of activity	1.21 (0.66 – 2.20)	0.54
Skewness of activity	3.70 (1.5 – 9.0)	0.004

[a] Hazard ratio of autocorrelation could not be estimated. The variation in autocorrelation was minimal, resulting in very high confidence intervals.

To our knowledge, this is the first study to investigate pre-challenge DIORs as potential indicators of resilience in livestock. Gijzel et al. (2019) explored the association between DIORs and frailty levels of elderly people. Results showed greater variation in the physical, mental, and social domain, for frail elderly individuals than non-frail elderly individuals. It should be noted, though, that in this between-subject study within-subject changes in resilience were not investigated. Thus, although DIORs pre-challenge may be associated with resilience, results from this study did not support predictive value of DIORs related to activity for the recovery of pigs from a PRRVS infection.

It was expected that activity would decrease following PRRSV inoculation, given that sickness behaviour is typically characterized by a decrease in locomotor activity (Hart, 1988). The results from this study support this by showing that a decrease in activity post-challenge as compared with pre-challenge levels, increased the risk of being classified as non-resilient, i.e., showing clinical signs on day 13 post challenge. This suggests that changes in activity levels in the early stage of infection may be a useful DIOR following PRRSV infection. Several studies have reported a decrease in activity following PRRSV infection (Escobar et al., 2007; van Dixhoorn et al., 2016) or other diseases (Reiner et al., 2009). However, occasionally, an increase in activity may be observed post-infection. For example, pigs infected with Salmonella were more active (Rostagno et al., 2011). Another perturbation, such as regrouping, is also associated with an increase in activity. After regrouping, pigs show an increase in activity (Camerlink et al., 2013). Therefore, the desired direction of activity changes for identifying resilient pigs may differ depending on the specific perturbation.

RMSE post-challenge and the change in RMSE following PRRSV inoculation were linked to morbidity. A higher increase in RMSE following and a higher RMSE post-challenge tended to increase the risk of a non-resilient outcome, i.e., morbidity or mortality. No associations were identified between RMSE and mortality alone, whereas Putz et al. (2019) found that a higher RMSE of feed intake following natural disease challenge was associated with higher mortality. One possible explanation for this finding could be that a much lower mortality rate was observed for this study (7%) compared to the mortality rate observed by Putz et al. (2019) (26%). The perturbation used by Putz et al. (2019) included various viral and bacterial diseases, whereas this study used only one experimentally induced viral disease as a perturbation. Furthermore, deviations in feed intake may be more informative for mortality than deviations in activity. Another explanation could be the smaller sample size in this study.

Autocorrelation was expected to be around zero for resilient animals. However, autocorrelation had little to no variation between animals. The confidence interval of odds and hazard ratio had a range of more than one thousand (data not shown). Multiplying autocorrelation by 100 lowered the confidence interval. However, autocorrelation in activity remained uninformative regarding morbidity or mortality. Apart from the possibility that the time series resolution and length may not have been optimal for calculation of this DIOR, not all variables are characterized by critical slowing down, of which autocorrelation is a typical indicator. It has been argued that only time series of physiological variables that are maintained close to a pre-determined setpoint and fluctuate around an equilibrium, "regulated variables" exhibit critical slowing down when resilience is reduced (Gijzel, 2020). In line with this, Berghof et al. (2019a) and Poppe et al. (2020) concluded that autocorrelation in body weight of layer chickens and milk yield of dairy cattle seem to be less informative for quantifying resilience.

In contrast with RMSE of activity, which tended to be related to morbidity, skewness in activity post-challenge, and particularly the change in skewness from pre- to post-challenge, was associated with mortality rather than morbidity. Skewness was expected to be around zero for resilient animals. Lower skewness post-challenge indeed increased the odds of being resilient. Skewness post-challenge had a mean of 0.31 (**Table 2.1**), so a decrease in skewness indicates a movement towards zero. However, skewness has a range of −1 to 1, so a one-unit shift in skewness is very unlikely. Berghof et al. (2019a) and Poppe et al. (2020) concluded that skewness in body weight of layer chickens and milk yield was less informative for health and longevity traits than other DIORs. This is also in line with the findings from this study, which indicate that skewness is not related to morbidity. Skewness could be sensitive to outliers, which could be the case for individual recordings of milk yield and activity (Poppe et al., 2020). Results from this study did, however, identify an association between reduced skewness (movement towards zero) with decreased risk of mortality.

For young animals, activity decreases over time irrespective of a perturbation (Bolhuis et al., 2005). This study did not correct for this decrease in activity. DIORs post-challenge and their deviations from pre-challenge values were calculated based on three days, and it is therefore assumed that the changes in these three days are due to the perturbation. To use activity of the whole period, control animals should be added to be able to correct for the decrease in activity due to aging.

The results obtained from this study demonstrated the value of DIORs based on activity to quantify resilience to disease challenge in pigs, although studies with larger sample sizes are needed to confirm this. The accelerometers used in this study measured acceleration using three axes and machine learning models to calculate activity, which is a black box approach. Based on accelerations, activity could be assessed, but spatial distribution, specific behaviours (e.g., whether a pig was shaking its head or running around) or social interactions could not be measured. Conversely, computer vision, allowing for immediate identification of a pig in a video and registering of its coordinates, could be used to extract the location and specific behaviour of the animal. Additional information captured using computer vision might include distance moved, velocity, spatial distribution, and social interactions. Taken together, these parameters would allow for the analysis of more complex activity and behavioural traits. Therefore, data generated via computer vision technology may improve estimation of DIORs, compared to using accelerometer data. However, accelerometers are currently commercially available, while camera technology is not yet ready for implementation at the commercial level. In the future, the cost/benefit of accelerometers vs. cameras will need to be evaluated on a case-by-case basis.

2.5 Conclusion

Results from this study showed that DIORs based on activity pre-challenge could not predict morbidity and mortality following a PRRSV infection. However, RMSE in the three days post-challenge and the change in RMSE and average activity from pre- to post-challenge tended to be associated with morbidity 13 days after infection. Skewness post-challenge tended to be associated with mortality, and the change in skewness was significantly related to mortality. Thus, DIORs based on activity showed their value to quantify resilience to a disease challenge. To explore the full potential of DIORs more in depth, more elaborate measurements of behaviour are desirable. Computer vision may allow for these in-depth measurements which cannot be assessed using accelerometers.

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Chapter 3

Individual detection and tracking of group housed pigs in their home pen using computer vision

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Abstract

Modern welfare definitions not only require that the Five Freedoms are met, but animals should also be able to adapt to changes (i.e., resilience) and reach a state that the animals experience as positive. Measuring resilience is challenging since relatively subtle changes in animal behaviour need to be observed 24/7. Changes in individual activity showed potential in previous studies to reflect resilience. A computer vision (CV) based tracking algorithm for pigs could potentially measure individual activity, which will be more objective and less time consuming than human observations. The aim of this study was to investigate the potential of state-of-the-art CV algorithms for pig detection and tracking for individual activity monitoring in pigs. This study used a tracking-by-detection method, where pigs were first detected using You Only Look Once v3 (YOLOv3) and in the next step detections were connected using the Simple Online Real-time Tracking (SORT) algorithm. Two videos, of 7 h each, recorded in barren and enriched environments were used to test the tracking. Three detection models were proposed using different annotation datasets: a young model where annotated pigs were younger than in the test video, an older model where annotated pigs were older than the test video, and a combined model where annotations from younger and older pigs were combined. The combined detection model performed best with a mean average precision (mAP) of over 99.9% in the enriched environment and 99.7% in the barren environment. Intersection over Union (IOU) exceeded 85% in both environments, indicating a good accuracy of the detection algorithm. The tracking algorithm performed better in the enriched environment compared to the barren environment. When false positive tracks where removed (i.e., tracks not associated with a pig), individual pigs were tracked on average for 22.3 min in the barren environment and 57.8 min in the enriched environment. Thus, based on proposed tracking-by-detection algorithm, pigs can be tracked automatically in different environments, but manual corrections may be needed to keep track of the individual throughout the video and estimate activity. The individual activity measured with proposed algorithm could be used as an estimate to measure resilience.

3.1 Introduction

Successful adaptation to changes is besides the Five Freedoms a critical pillar in modern animal welfare definitions (Mellor, 2016). Animals should be able to cope with challenges in their environment and reach a state that the animals experience as positive. In other words, to enhance pig welfare, pigs should be not only free from any kind of discomfort but also be resilient to perturbations. Resilient pigs are able to cope or rapidly recover from a perturbation (Colditz and Hine, 2016). Perturbations in pig production could be management related (e.g., mixing, transport) or environment related (e.g., disease, climate). Non-resilient pigs have more difficulty recovering or cannot recover at all from perturbations and therefore experience impaired welfare. The lack of ability to cope with perturbations causes a risk for these non-resilient animals to develop intrinsic problems like tail biting or weight loss (Bracke et al., 2018; Rauw et al., 2017).

To prevent welfare problems in pigs, a management system that provides information on resilience will most likely be needed in the future. With such a management system, the farmer will know when resilience is impaired and which animals are concerned. These animals labelled by a management system as non-resilient could be assisted when required. However, such a system is difficult to develop since resilience is difficult to measure. Resilience consists of many parameters that could be monitored. Currently, mainly physiological parameters are used to measure resilience in pigs. Blood parameters such as white blood cell count or haemoglobin levels, but also production parameters such as body weight are used to measure resilience (Berghof et al., 2019b; Hermesch and Luxford, 2018). However, measuring these physiological parameters requires invasive handling of the animal. In addition, these parameters represent a delayed value due to the nature of the measurements, and therefore they are less suitable for immediate decision support.

Recent studies investigated activity and group dynamics as traits to measure resilience. Several studies show a reduction in activity as a response to sickness (Nordgreen et al., 2018; Trevisan et al., 2017; van der Zande et al., 2020; van Dixhoorn et al., 2016). Pigs are lethargic during sickness; they spend more time lying down and less time standing and feeding. Not only sickness affects activity, but also climate has an influence on the activity of pigs, with pigs showing lower activity levels when temperature increases. Costa et al. (2014) showed that relative humidity affected pig activity as well and that pigs had a preference to lay close to the corridor when relative humidity was high. To conclude, activity could be a suitable indicator of resilience to perturbations of different nature.

It is extremely time-consuming to measure activity and location of individuals in multiple pens continuously by human observations. The use of sensors could facilitate automatic activity monitoring and minimize the need for human observers. The activity of an individual could be measured by using accelerometers, which could be placed in the ear of the pig, just like an ear tag. Accelerometers measure accelerations along three axes. With the use of machine learning, accelerations can be transformed into individual activity levels (van der Zande et al., 2020). The main advantage of using accelerometers is that the devices usually have a static ID incorporated in their hardware. In other words, identities of animals are known all the time unless they lose the accelerometer. On the other hand, accelerometer placement could affect readings and therefore introduce extra noise in raw acceleration data. With placement in the pig's ear, ear movements can cause confounding of true levels of physical activity. Noise in acceleration data could lead to false positive activity. In addition, the location of the animal is not known when using accelerometers, which further limits more precise resilience measurements since proximity and location preference could be included when the location is known.

As an alternative to sensors placed on animals, computer vision allows for non-invasive analysis of images or videos containing relevant individual activity and location data. Several studies investigated computer vision algorithms to recognize a pig and track it in a video to estimate activity (Larsen et al., 2021). The main advantage of using computer vision to measure activity is that activity is calculated from the pig's location in each frame, allowing for the calculation of proximity to pen mates and location preferences. Ott et al. (2014) measured activity on a pen level by looking at changes in pixel value between consecutive frames of a video. They compared the automated measured activity with human observations and found a strong correlation of 0.92. This indicated that the use of algorithms for automated activity monitoring could minimize the need for human observations. The limitation of the approach of Ott et al. (2014) is that in their method, the activity is expressed at pen level, where individual information is preferred for a management system. Pigs observed from videos are difficult to distinguish individually, so Kashiha et al. (2013) painted patterns on the back of pigs to recognize individuals. An ellipse was fitted to the body of each pig, and the manually applied recognition pattern was used to identify the pig. On average, 85.4% of the pigs were correctly identified by this algorithm. Inspired by patterns, Yang et al. (2018) painted letters on the back of the pigs and trained a Faster R-CNN to recognize the individual pigs and their corresponding letters. Tested on 100 frames, 95% of the individual pigs was identified correctly. These studies mainly concentrated on detecting the manually applied markings/patterns for pig identification and while the approach showed relatively good performance, the manually applied marking is labour intensive. Markings must be consistent and at least be refreshed every day to be able to see the markings properly.

Huang et al. (2018) used an unspecified pig breed with variation in natural coloration and made use of this natural variation to identify pigs from a video. A Gabor feature extractor extracted the different patterns of each individual and a trained Support Vector Machine located the pigs within the pen. An average recognition of 91.86% was achieved. However, most pigs in pig husbandry do not have natural coloration. Another possibility is to recognize individuals by their unique ear tag (Psota et al., 2020a). Pigs and ear tags were detected by a fully-convolutional detector and a forward-backward algorithm assigned ID-numbers, corresponding to the detected ear tags, to the detected pigs. This method resulted in an average precision >95%. Methods using manual markings are successful but could still be invasive to the animal and labour intensive.

The studies that do not rely on manual marking of animals, have difficulties in consistent identification of individuals during the tracking. Ahrendt et al. (2011) detected pigs using support maps and tracked them with a 5D-Gaussian model. This algorithm was able to track three pigs for a maximum of 8 min. However, this method was also computationally demanding. Cowton et al. (2019) used a Faster R-CNN to detect pigs at a 90% precision. To connect the detections between frames (DEEP) Simple Online Realtime Tracking (SORT) was used. The average duration before losing the identity of the pig was 49.5 s and the maximum duration was 4 min. Another method used 3D RGB videos rather than 2D RGB videos to track pigs (Matthews et al., 2017). Pigs were detected with the use of depth data combined with RGB channels, and a Hungarian filter connected the detected pigs between frames. The average duration of a pig being tracked was 21.9 s. Zhang et al. (2019) developed a CNN-based detector and a correlation filter-based tracker. This algorithm was able to identify an average of 66.2 unique trajectories in a sequence of 1,500 frames containing nine pigs. Despite the variety of methods, none could track a pig while maintaining the identity for longer than one min on average. In practice, this would result in a human observer correcting IDs more than 360 times for an hour-long video with six pigs being monitored. A computer vision algorithm used to measure activity should be able to maintain identity for a longer period of time to lower human input.

All the previous studies based on different convolutional neural network (CNN) architectures showed a robust performance when it comes to single pig detection. However, continuous detection across several frames and under varying conditions remains challenging. You Only Look Once v3 (YOLOv3) is a CNN with outstanding performance (Benjdira et al., 2018). SORT could be used for tracking across several frames. SORT is an online tracker which only processed frames from the past (and not from the future). The main advantage of an online tracking algorithm is improved speed, but this algorithm is fully dependent of the quality of the detections. The fast and accurate detections of YOLOv3 and the connection of the detections across frames by SORT might allow for longer tracking of individual pigs. Therefore, the aim of this study was to investigate the potential of state-of-the-art CV algorithms using YOLOv3 and SORT for pig detection and tracking for individual activity monitoring in pigs.

3.2 Material and Methods

3.2.1 Ethical Statement

The protocol of the experiment was approved by the Dutch Central Authority for Scientific Procedures on Animals (AVD1040020186245) and was conducted in accordance with the Dutch law on animal experimentation, which complies with the European Directive 2010/63/EU on the protection of animals used for scientific purposes.

3.2.2 Animals and Housing

A total of 144 crossbred pigs was used in this study. The pigs originated from the same farm but were born and raised in two different environments: a barren and an enriched environment. Piglets from the barren environment were born in farrowing crates, and the sow was constrained until weaning at four weeks of age. Upon weaning, eight pigs per litter were selected based on body weight and penned per litter in pens with partly slatted floors until nine weeks of age. A chain and a jute bag were provided as enrichment. Feed and water were provided ad-libitum. The second environment was an enriched environment, where piglets were born from sows in farrowing crates. After three days post-farrowing, the crate was removed, and the sow was able to leave the farrowing pen into a communal area consisting of a lying area, feeding area, and a dunging area together with four other sows. Seven days post-farrowing, the piglets were also allowed to leave the farrowing pen into the communal area and were able to interact with the four other sows and their litters. The piglets were weaned at nine weeks of age in this system.

All pigs entered the research facility in Wageningen at nine weeks of age. The pigs originating from the barren environment remained in a barren environment. Each barren pen (0.93 m2/pig) had a partly slatted floor and a chain and a ball were provided as enrichment. The pigs originating from the enriched environment were housed in enriched pens (1.86 m2/pig) which had sawdust and straw as bedding material. A jute bag and a rope were alternated every week. Once a week, fresh peat was provided, as were cardboard egg boxes, hay or alfalfa according to an alternating schedule. Additionally, six toys were alternated every two days. Each pen, independent of environment, consisted of six pigs, balanced by gender, and feed and water were available ad-libitum. Lights were on between 7:00 and 19:00 h and a night light was turned on between 19:00 and 7:00 h. The experiment was terminated at 21 weeks of age.

3.2.3 Data

An RGB camera was mounted above each pen and recorded 24 h per day during the experiment. The videos were 352 by 288 pixels and recorded in 25 fps. Due to the smaller width of the barren pens, neighbouring pens were visible on the

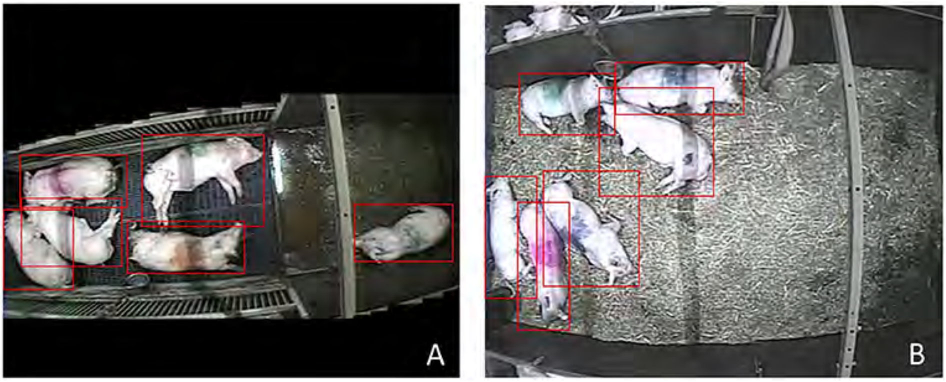

Figure 3.1 - Example frames with annotated bounding boxes (red boxes) in the barren (A) environment with blocked neighbouring pens and the enriched (B) environment.

videos of the barren pens. To avoid that the pigs from neighbouring pens were detected and to allow an equal comparison between the barren and enriched environment, the neighbouring pens were blocked prior to the analysis (**Figure 3.1A**). Frames were annotated using LabelImg (Tzutalin, 2015). The contours of the pig were labelled by a bounding box, where each side of the bounding box touches the pig (**Figure 3.1**). One annotation class (pig) was used, and only pigs in the pen of interest were annotated.

Three different detection models were evaluated to assure the best detection results possible under varying circumstances: using frames where young pigs were annotated (young model), using frames where old pigs were annotated (old model), and a combination. The young model contained annotations of randomly selected frames from pigs around 10 weeks of age. The training dataset consisted of 2,000 annotated frames, where 90% of the frames was used for training, and 10% was used for validation. The old model was trained on 2,000 annotated randomly selected frames of pigs from 17 to 21 weeks of age, where 90% was used for training, and 10% was used for validation. The combined model consisted of young and old animals' annotations, with 4,000 annotated frames split into 90% training data and 10% validation data.

To review a possible difference in the performance of tracking between environments, one video of ~7 h (n frames = 622,570) of each environment without any human activity except for the activity of the caretaker was used for tracking. The pigs were 16 weeks of age in this video, which is an age that was not used for training of the detection models. Every 1780th frame was annotated to obtain 350 equally distributed frames per environment and to evaluate the three different detection models (young, old and combined). All the frames were then used to test the success of the multiple-object tracking.

3.2.4 Detection Method

To assure high computational speed and robust multiple object detection, the You Only Look Once version 3 (YOLOv3) algorithm was used to detect pigs in their home pens (Redmon and Farhadi, 2018). YOLOv3 is an accurate object detection network that features multi-scale detection, a more robust feature extraction backbone compared to other convolutional neural networks (CNN)-based detectors and an improved loss function calculation. The YOLOv3 framework consists of two main multi-scale modules: the Feature Extractor and the Object Detector (**Figure 3.2**). The input for YOLOv3 are frames/images of interest. First, an input frame/image passes through the Darknet-53, which is a deep convolutional neural network consisting of 53 layers and used for initial feature extraction. The output of the feature extraction step consists of three different feature maps, where the original input image is down sampled by 32, 16, and 8 times from its original size, respectively. These feature maps are then passed through another 53 fully convolutional layers of the Object Detector module of the YOLOv3 network to produce actual detection kernels. The final YOLOv3 architecture is a 106 layer deep neural network, which produces detections at three different scales (using previously produced feature maps of different sizes) to allow accurate detection of objects with varying size. The tree detection kernels produced at layers 82, 94, and 106 are then combined in a vector with the coordinates of all three detections and corresponding probabilities of the final combined bounding box being a pig.

3.2.5 Tracking Method

Simple Online and Real-Time (SORT) was used to track pigs in their home pen (Bewley et al., 2016). The detections produced by YOLOv3 network were used as the input for the SORT tracking algorithm. The performance of SORT is highly dependent on the quality of the initial detection model since SORT has

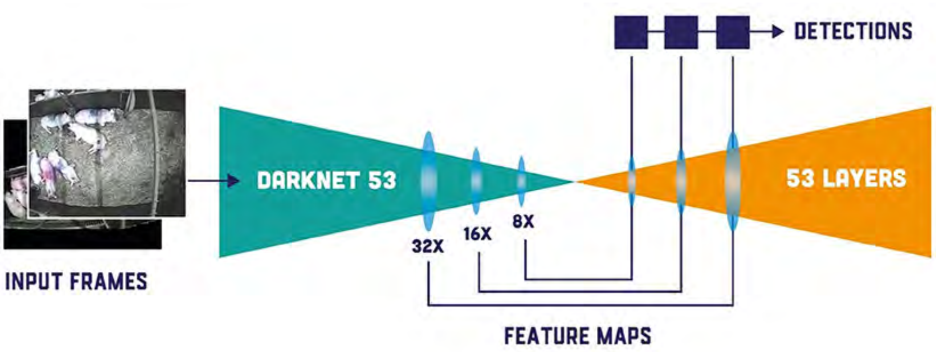

Figure 3.2 – Graphical overview of You Only Look Once v3 (YOLOv3).

no such functionality itself. The SORT algorithm utilizes the combination of common techniques such as the Hungarian algorithm and Kalman filter for object tracking. The Kalman filter is used to predict future positions of the detected bounding boxes. These predictions serve as a basis for continuous object tracking. This filter uses a two-step approach. In the first prediction step, the Kalman filter estimates the future bounding box along with the possible uncertainties. As soon as the bounding box is known, the estimates are updated in the second step and uncertainties are reduced to enhance the future predictions. The Hungarian algorithm predicts whether an object detected in the current frame is the same as the one detected in the previous adjacent frame. This is used for object re-identification and maintenance of the assigned IDs. The robust re-identification is crucial for continuous and efficient multiple object tracking. The Hungarian algorithm uses different measures to evaluate the consistency of the object detection/identification (e.g., Intersection over Union and/or shape score). The Intersection over Union (IoU) score indicates the overlap between bounding boxes produced by the object detector in one frame and another frame. If the bounding box of the current frame overlaps the bounding box of the previous frame, it will probably be the same object. The shape score is based on the change in shape or size. If there is little change in shape or size, the score increases, guaranteeing re-identification. The Hungarian algorithm and the Kalman filter operate together in SORT implementation. For example, if object A was detected in frame t, and object B is detected in frame t+1, and objects A and B are defined as the same object based on the scores from the Hungarian algorithm, then objects A and B are confirmed being the same object. The Kalman filter could use the location of object B in frame t+1 as a new measurement for object A in frame t to minimize uncertainty and improve the overall score.

3.2.6 Evaluation

Detection results were evaluated by using mean average precision (mAP), intersection over union (IOU), number of false positives (FP) and number of false negatives (FN). mAP is the mean area under the precision-recall curve for all object classes. IOU represents the overlap between two bounding boxes. FP are detections of a pig which is not a pig, where FN are missing detections of a pig.

The tracking algorithm generates more tracks (i.e., part of the video with an assigned ID) than individuals, so each track was manually traced back to the individual that was tracked. Not all tracks could be traced back to a pig, and these tracks are referred to as FP tracks (**Figure 3.3A**). Occasionally, individuals take over the track of another pig. This is referred to as ID switches (**Figures 3.3B, 3.3C**).

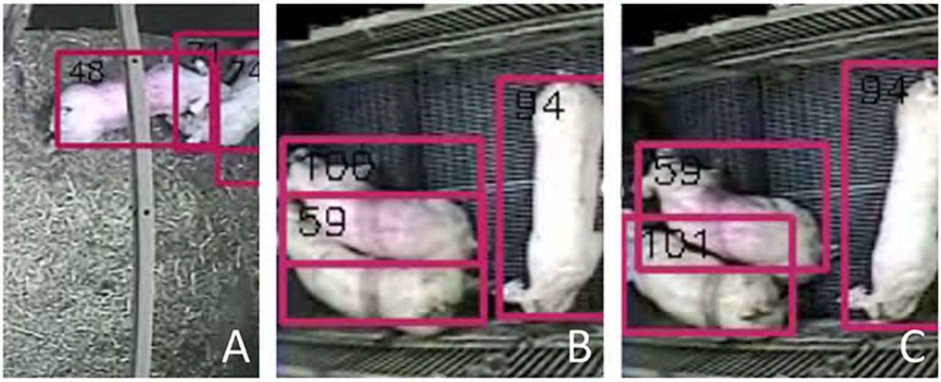

Figure 3.3 – Examples of FP tracks and ID switches: (A) An example of a false positive (FP) track, where two pigs are visible, but three bounding boxes are identified. The bounding box at the far right (nr. 74) is labeled as a FP track since it could not be assigned to a pig; (B) The moment just before the ID switch of pig nr. 59; (C) Just after the ID switch, where bounding box nr. 59 has moved up to another pig compared to (B). The original pig nr. 59 received a new ID.

3.3 Results

3.3.1 Detection

Figure 3.4 shows the mean average precision (mAP) and intersection over union (IOU) for all three detection models. In both environments, the mAP was over 99%. The combined detection model reached a mAP of 99.95% in the enriched environment. In both environments, IOU was the lowest with the young detection model. Adding older animals (i.e., combined detection model) improved the IOU in the enriched environment. The old detection model had the highest IOU in the barren environment.

Figure 3.5 shows the number of FP and FN for all detector models in both environments. The detector trained on young animals found 128 FP in the barren environment where it only found two FP in the enriched environment. FP in the barren environment dropped drastically when older animals were used in or added to the detection model. FN (undetected pigs) decreased in both environments when older animals were used compared to only using younger animals. For both environments, FN dropped even further when young and old animals were combined in the detection model. The combined detection model was used in tracking since it performed best in both environments.

3.3.2 Tracking

In the barren environment, more tracks were identified compared to the enriched environment (**Table 3.1**). In both environments, approximately one third of the tracks were a FP track. In other words, one-third of the IDs found could not be assigned to a pig. More IDs were switched in the barren environment compared to the enriched environment. FP tracks had a short duration in both environments. On average, the length of FP tracks was 9.9 s in the barren environment and 2.2 s in the enriched environment. When these short FP tracks were excluded, on average individual pigs were tracked for 22.3 min in the barren environment and 57.8 min in the enriched environment.

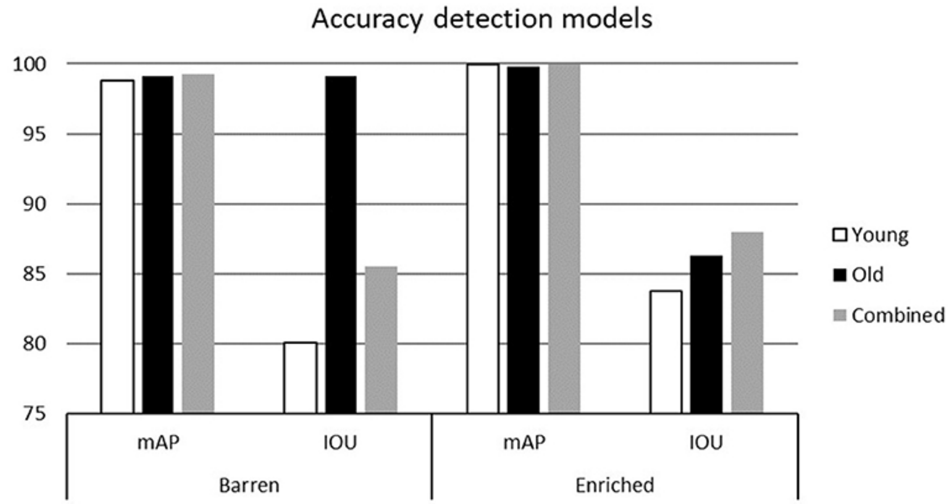

Figure 3.4 – Mean Average Precision (mAP) and Intersection Over Union (IOU) for the barren and enriched environment using the "young," "old," and "combined" detection model.

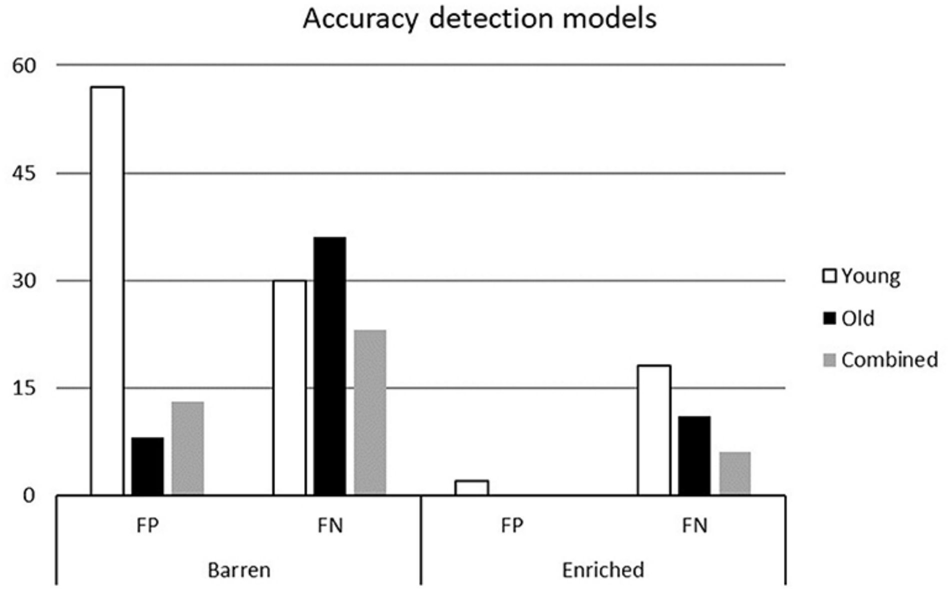

Figure 3.5 – The number of false positives (FP; i.e., tracks not associated with a pig) and false negatives (FN; i.e., undetected pig) for the barren and enriched environment using the "young," "old," and "combined" detection model.

Table 3.1 – Summary of tracking results in barren and enriched environment.

	Barren	Enriched
Number Ids	225	100
False positive track	76	31
Switched Ids	20	4
% tracked of video	99.3	99.9
Average track without FP[a] (min)	22.3	57.8
Average track with FP[a] (min)	11.3	24.8
Longest track (min)	222.9	315.7

[a] FP, false positive.

Table 3.2 - Tracking summary per individual in barren environment.

			Barren		
Individual	# tracks	Switches	Total tracked frames	Percentage tracked	Average length (min)
A	20	2	610,719	98.09%	20.4
B	57	4	609,563	97.91%	7.1
C	10	4	622,566	100.00%	41.5
D	21	4	622,028	99.91%	19.7
E	14	4	622,508	99.99%	29.6
F	27	2	620,416	99.65%	15.3

Table 3.3 – Tracking summary per individual in enriched environment.

Enriched					
Individual	**# tracks**	**Switches**	**Total tracked frames**	**Percentage tracked**	**Average length (min)**
A	7	0	622,281	99.96%	59.3
B	3	0	622,559	100.0%	138.3
C	23	1	620,568	99.68%	18.0
D	20	2	620,948	99.74%	20.7
E	10	0	622,463	99.99%	41.5
F	6	1	622,387	99.97%	69.2

There was variation between individuals in the performance of the tracking algorithm (**Tables 3.2, 3.3**). In the barren environment the highest number of tracks traced back to one individual was 57, whereas in the enriched environment this was only 23 tracks. The lowest individual average track length was therefore 18 min, where the highest was 138.3 min in the enriched environment. The average individual track length in the barren environment varied between 7.1 and 41.5 min.

Figures 3.6 and **3.7** display all tracks per individual including FP tracks. In both environments there was a period between ~11:24 and 13:48 where all IDs were maintained. In this period, the pigs were mostly lying down. Especially before and after this period of resting most new IDs were assigned to individuals. In these periods the pigs were actively moving around and interacting with pen mates.

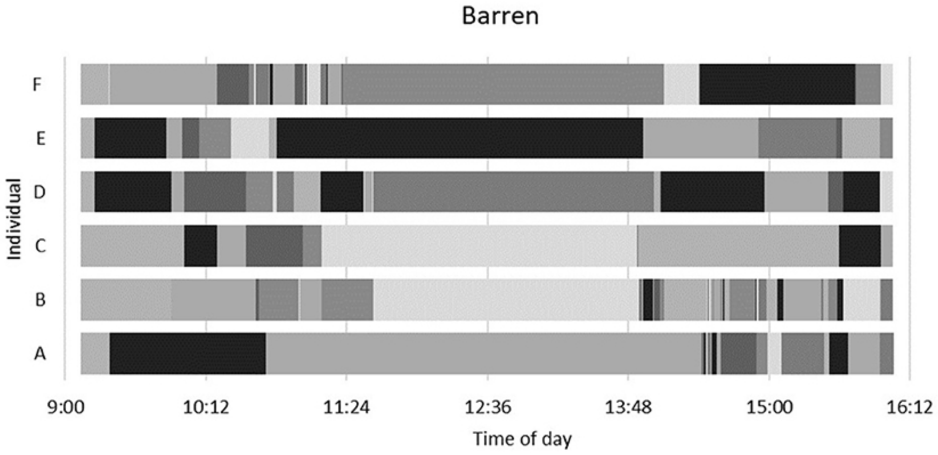

Figure 3.6 - Tracks per individual in the barren environment including false positive tracks corresponding to the time of day.

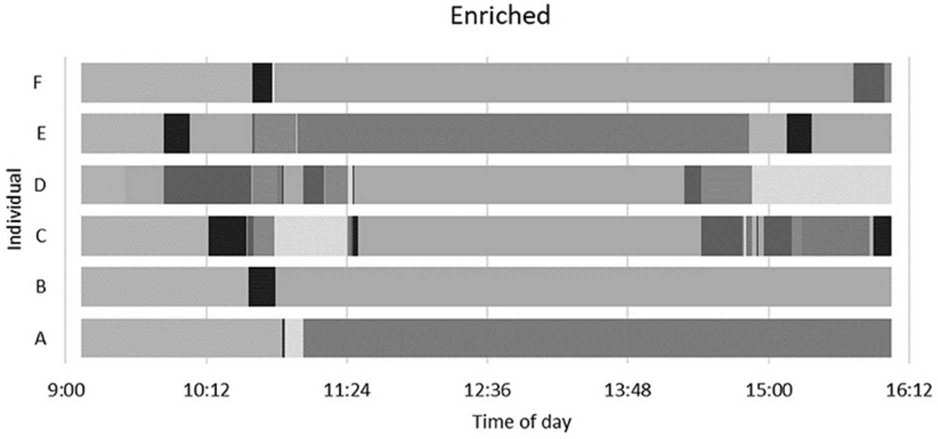

Figure 3.7 - Tracks per individual in the enriched environment including false positive tracks corresponding to the time of day.

3.4 Discussion

The aim of this study was to investigate the potential of state-of-the-art CV algorithms using YOLOv3 and SORT for pig detection and tracking for individual activity monitoring in pigs. This study showed the potential of state-of-the-art CV algorithms for individual object detection and tracking. Results showed that individual pigs could be tracked up to 5.3 h in an enriched environment with maintained identity. On average, identity was maintained up to 24.8 min without manual corrections. In tracking-by-detection methods, as used in this study, tracking results are dependent on the performance of the detection method. No literature was found showing an algorithm maintaining identity for longer than one minute on average without manually applied marking. The highest average tracking time reported until now was 49.5 s (Cowton et al., 2019). This study outperformed existing literature in maintaining identity in tracking pigs with an average tracking duration of 57.8 min. However, this study used a long video sequence of 7 h, while pigs are known to be active during certain periods of time. This might result in a distorted comparison between studies. However, when the average length of tracks is calculated based on trajectories during active time, the average length of the enriched housed pigs is still between 6.4 and 17.2 min. The average track length of barren housed pigs was lower (3.3–24.6 min), but still higher than found in the literature. The main difference between this study and others is the use of YOLOv3 as a detector.

The proposed tracking algorithm was trained and tested on annotated frames from different ages. Yang et al. (2018) tested their algorithm on different batches within the same pig farm and results were “quite good.” They state: “the size of pigs does not matter much.” This study, however, proves otherwise. There is a difference in performance between different ages within the same environment (i.e., different size of pigs). Psota et al. (2020) also had a training set that consisted of different pen compositions, angles and ages. They reported that a dataset containing frames from finisher pigs performed better than a dataset containing frames from nursery pigs. This is in line with results presented in the current study, where IOU of the old detection model was higher than the IOU of the young detection model. Another phenomenon was shown in current results: in the enriched environment, the IOU of the combined detection model exceeded the IOU of the young and the old detection model, while in the barren environment the old detection model performed best. This interaction between environment and age could be explained by unoccupied surface in the pen. Enriched housed pigs had twice as much space available than barren housed pigs. In addition, pigs grow rapidly and especially in the barren environment, pigs are more occluded when growing older. Visually, the frames of the old detection model are more similar to the test frames than the frames of the young detection model (**Figure 3.8**). Thus, the old detection model fits the test frames the best in the barren environment, and therefore has the best performance. When annotations of younger animals are added, some noise is added in the detections, creating a more robust detection model (higher mAP) with a lower IOU.

Besides the difference in age, there was also a difference in the environment in the current study. The tracking algorithm performed better in the enriched environment than in the barren environment. The only difference between the two environments was the use of bedding material and enrichments, and the space allowance per pig. The bedding material was not observed to be detected as a pig, so the space allowance is responsible for the difference in performance. The most difficult situations to detect pigs individually is when pigs are touching each other. When in close proximity, IDs can be lost or switched, which could happen more often when there is less space available per pig.

The appearance of pigs (i.e., spots or colour marking) appeared to be irrelevant in the performance of the tracking algorithm. Some pigs were coloured for identification in the experiment with a saddle-like marking that was prominently visible to the human observer. The tracking algorithm was not affected by the colouring. A pig with a pink marker had the most tracks in the barren environment (**Table 3.2**; individual C) but was among the pigs with the fewest tracks in the enriched environment (**Table 3.3**; individual F). Creating more tracks per individual appears to be more strongly related to unfortunate placement of the pig within the pen rather than disturbance by background colours or shadows.

ID switches are a difficult problem in tracking. Not only do you lose the identity, but identities are switched without any visibility in tracking data except when IDs are checked manually. Psota et al. (2020) also reported ID switches. An example showed that despite all animals were detected, only seven out of 13 had the correct ID. We expected that two tracks would exchange their IDs, however, that only happened twice out of 24 switches identified. The other 22 switches showed one individual receiving a new ID number, and the other individual took over the other animal's original ID.

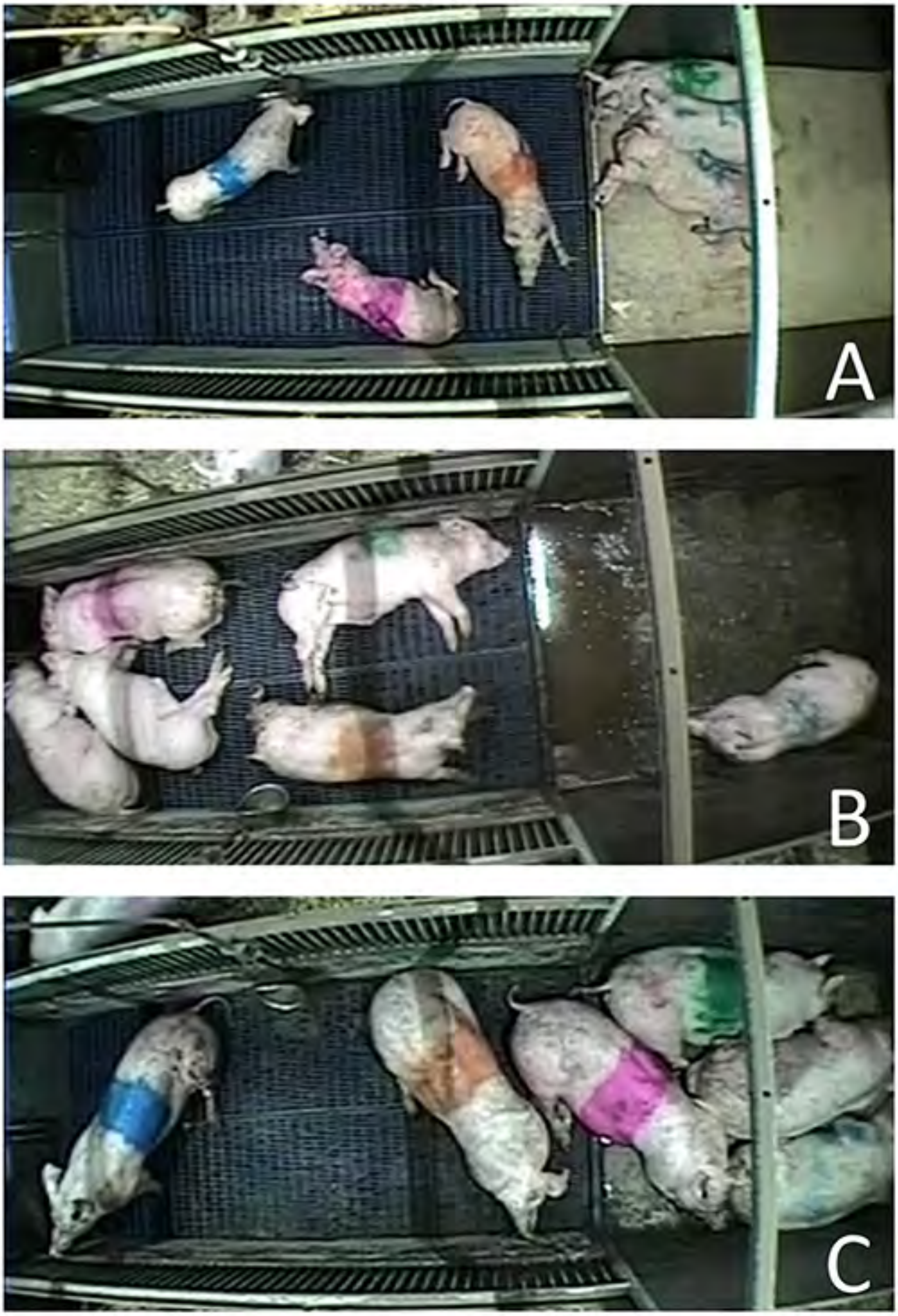

Figure 3.8 - Example frames of young detection model (A), test video (B), and old detection model (C).

These one-sided switches are not well-described in the literature (Li et al., 2009). An advantage of this type of switches is that it is easier to trace back in tracking data since a new ID is created in the process and usually this new ID only has a limited track length. However, it still remains an issue in tracking data. Removing these false positives based on short track length seems a viable way to correct for ID switches.

The algorithm used in this study showed is a first step to measure resilience in future applications. Individual activity or variation in individual activity under stress is a potential indicator of resilience (Cornou and Lundbye-Christensen, 2010; Nordgreen et al., 2018; van der Zande et al., 2020; van Dixhoorn et al., 2016). The algorithm presented estimated bounding boxes and connected them between frames with assigned IDs. When the trajectory is lost, a human observer needs to assign the trajectory to the right ID. Using this algorithm for six pigs, the human observer needs to correct on average the IDs 10 times per hour for enriched housed pigs, and 22 times per hour for barren housed pigs. For a commercial management system, this would still be too labour intensive, but for research purposes this is possible. To improve performance further, multiple sensors should be integrated to achieve high accuracy with less labour (Wurtz et al., 2019). To recognize damaging behaviour using proposed algorithm is challenging due to the low occurrence of such behaviour. Posture estimation could be integrated in proposed algorithm since these behaviours occur regularly. However, for research purposes, this algorithm allows tracking activity of a larger number of individual animals in a non-invasive manner. From location data of every frame, distance moved could be calculated.

3.5 Conclusions

The aim of this study was to investigate the potential of state-of-the-art CV algorithms using YOLOv3 and SORT for pig detection and tracking for individual activity monitoring in pigs. Results showed that individual pigs could be tracked up to 5.3 h in an enriched environment with maintained identity. On average, identity was maintained up to 24.8 min without manual corrections. Using annotations of a combination of younger and older animals had the best performance to detect pigs in both the barren and the enriched environment. The tracking algorithm performed better on pigs housed in an enriched environment compared to pigs in a barren environment, probably due to the lower stocking density. The tracking algorithm presented in this study outperformed other studies published to date. The better performance might be due to the different detection method used, variation in environment, time of day or the size of the training data used. Thus, based on tracking-by-detection algorithm using YOLOv3 and SORT, pigs can be tracked in different environments. The tracks could in future applications be used as an estimate to measure resilience of individual pigs, by recording activity, proximity to other individuals and use of space under varying conditions.

3.6 Acknowledgements

The authors thank Séverine Parois for carrying out the experiment and Manon van Marwijk and Monique Ooms for putting up the cameras and collecting the videos.

Chapter 4

A multi-suckling system combined with an enriched housing environment during the growing period promotes resilience to various challenges in pigs

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Abstract

Little is known about the impact of social and environmental enrichment on improving livestock resilience, i.e., the ability to quickly recover from perturbations. We evaluated the effect of an alternative housing system (AHS) on resilience of pigs, as compared to conventional housing (CONV). The AHS consisted of multi-litter housing during lactation, delayed weaning, extra space allowance and environmental enrichment at all times. We assessed recovery to a 2 h-transport challenge, an LPS injection, 2 h-heat stress and a biopsy wound in 96 pigs. Additionally, indicators of long-term "wear and tear" on the body were determined. AHS pigs had better physiological recoveries with quicker returns to baseline in the transport and LPS challenges, showed lower cortisol accumulation in hairs and lower variance in weight gain over the experimental period compared to conventionally-housed (CONV) pigs. They also had higher levels of natural antibodies binding KLH than CONV pigs. Their response to heat stress revealed a different strategy compared to CONV pigs. Taken together, AHS pigs appear to be more resilient and experience less chronic stress. Enhancing welfare by provision of social and environmental enrichment that better meets the behavioural needs of pigs seems to be a promising approach to improve their resilience.

4.1 Introduction

In current husbandry systems, most pigs seem to cope with the metabolic demands of rapid, efficient growth combined with the multiple acute and chronic stressors they are exposed to, such as vaccination, pathogenic load, regrouping, and suboptimal housing conditions. The burden of cumulative stress, however, may cause "wear and tear" on the animals and reduce their resilience, i.e., their capacity to recover from challenges. Resilience can be defined as the capacity of an animal to be minimally affected by a disturbance or to rapidly return to its normal state (Colditz and Hine, 2016). As poor resilience may manifest itself as an increased risk to develop behavioural and health problems (Brunberg et al., 2016; Scheffer et al., 2018), optimizing resilience is important for the performance and welfare of farm animals (Colditz and Hine, 2016; Döring et al., 2014; Guy et al., 2012). Studies on farm animals have mainly focussed on enhancing resilience through genetic selection (Berghof et al., 2019b; Colditz and Hine, 2016), while limited research has been conducted on the effect of the environment in which the animals are reared and kept.

Enrichment is defined as a modification in the environment that improves the biological functioning of animals (Newberry, 1995). Numerous studies have shown that social and/or environmental enrichment promotes natural behaviour (Ko et al., 2020; Oostindjer et al., 2011a, 2011b; van de Weerd et al., 2003; van de Weerd and Ison, 2019; C. H. Yang et al., 2018) and the development of appropriate social skills (Chaloupková et al., 2007; D'Eath, 2005; Ferguson, 2014; Kutzer et al., 2009; Pitts et al., 2000), reduces damaging behaviour (Beattie et al., 1995; Bolhuis et al., 2005; van Nieuwamerongen et al., 2015), leads to a more optimistic emotional state (Douglas et al., 2012), and reduces stress at weaning (Campbell et al., 2013; Oostindjer et al., 2014a, 2011b; van Nieuwamerongen et al., 2015; Verdon et al., 2016). Less is known, however, about the impact of enrichment on resilience in pigs. It has been suggested, however, that raising animals in housing conditions that promote the satisfaction of their essential behavioural needs may improve resilience (Colditz and Hine, 2016; Fraser, 1988; Ko et al., 2020; Rault et al., 2018; van Dixhoorn et al., 2016).

Recent studies suggest that loss of resilience is characterized by a slow recovery after exposure to a challenge (Scheffer et al., 2018). The recovery rate could thus serve as a putative indicator of (the loss of) resilience. Animals in a state of poor resilience may need only a small disturbance to collapse into a health- or behavioural crisis (Hodgson et al., 2015; Scheffer et al., 2009, 2001). This seems to be the case in, for instance, the occurrence of tail biting in pigs where only a seemingly small change in e.g., climatic conditions or feed can result in a severe outbreak of this injurious behaviour. It has been hypothesized that moving towards the threshold of change from one (normal) state into the other (problematic) state, also referred to as a 'tipping point', can also be predicted by changes in the dynamics of patterns of generic parameters (Scheffer et al., 2012, 2009). In pigs these could, for instance, be weight gain or activity patterns. In line with this, some variables reflecting changes in activity patterns following a porcine reproductive and respiratory syndrome virus (PRRSV) infection were related to morbidity and mortality of pigs (van der Zande et al., 2020). However, the dynamic indicators mentioned are not pertinent for determining resilience in all cases. The use of more common indicators of recovery from challenge measurable in blood, saliva or as behavioural changes might be useful, as animals develop a combination of physiological and behavioural responses when they encounter perturbations to restore homeostasis (Etim et al., 2013). Signs of improved resilience might then be explored through multiple and diverse indicators of recovery from challenges.

A challenge can be defined as an episodic situation where animals may experience acute stress after their external or internal environments change abruptly (Colditz and Hine, 2016). A challenge is characterized by the duration of stimuli, the frequency of their occurrence and the predictability of the onset by the individual. Every response to a challenge has distinct features like response peak, duration, onset and recovery (Colditz and Hine, 2016).

In the current study, resilience of pigs kept in an alternative system was compared to that of pigs kept under conventional commercial conditions. The alternative system differs profoundly from conventional conditions. It consists of multi-litter housing during lactation, allowing piglets to mingle with multiple litters during the socialization period, delayed weaning, extra space allowance and provision of rooting substrates. The alternative system resembles the natural situation of pigs more and therefore better meets the behavioural needs of pigs. It was hypothesized that this difference in social and environmental conditions that support the execution of important natural behaviours will result in improved general resilience, i.e., an overall ability to deal with all kinds of unpredictable challenges (Carpenter et al., 2012). Resilience was probed in depth by measuring the speed of recovery from a range of (mild) challenges. The series of perturbations selected were known to be challenging for pigs, and similar to events that often occur - either successively or simultaneously - in commercial pig husbandry conditions. Chronic indicators of stress, for instance cortisol in hairs, were also measured to capture "wear and tear" on the body, as well as fluctuations in weight development. To assess a full picture of the general resilience of pigs, challenges varied in nature, strength and duration to explore each aspect of recovery.

4.2 Material and Methods

Established principles of laboratory animal use and care, and the Dutch law on animal experiments were followed. They comply with the European Directive 2010/63/EU on the protection of animals used for scientific purposes. The Animal Care and Use Committee of Wageningen University approved the experiment (AVD1040020186245). All methods applied in the study were performed in accordance with the ARRIVE guidelines and regulations. Due to the difference in housing system according to the treatment group, the investigators were aware of the treatment of the animals when collecting samples. However, lab analyses of tear staining measurements on pictures, behavioural observations during the transport challenge, respiratory rate and metabolic chamber parameters during the heat stress challenge, wound healing areas on pictures and scores related to organs during dissections were done blindly. The sample size estimation was based on the rise in cortisol levels using results obtained in pigs subjected to transport stress (Carpenter et al., 2012) ($\alpha = 5\%$, power = 80%, SD = 2.42, $\delta = 2.8$).

4.2.1 Animals

A total of 144 Tempo × Topigs-20 pigs ($n = 71$ females; $n = 73$ males) were used during the experiment, spread over three batches ($n = 48$ pigs per batch). Piglets were offspring from 24 multiparous sows. During lactation, half of the sows and their piglets were housed in a conventional farrowing pen (CONV) (mean ± SD; sow parity = 4.2 ± 1.8) and the other half in an alternative group housing system (AHS) (parity = 4.0 ± 1.7) at the Swine Innovation Centre

(Sterksel, The Netherlands). The piglets were not castrated, nor were their tails docked or teeth clipped. Average birth weight was similar for piglets from both systems: 1.46 ± 0.28 kg for the CONV and 1.44 ± 0.27 kg for the AHS.

4.2.2 Housing systems

4.2.2.1 From birth to nine weeks of age

Piglets were raised in two different housing systems (similar to van Nieuwamerongen et al. (2015)). The AHS comprised of five farrowing pens of 3.2 × 2.2 m (mix of solid 2.2 × 2.2 m and slatted floor 1.0 × 2.2 m), adjacent to a communal area of 11.1 × 2.80 m (solid floor). Next to the communal area, were a dunging (2.8 × 3.3 m, slatted floor) and a feeding area (4.2 × 3.3 m, solid floor). Enrichment was provided in the form of four jute bags (110 × 80 cm) and during the first week a slice of straw was added to the farrowing pens (approximately 2.5 kg per pen). One week before the expected farrowing date, five sows per batch were put in this system. Two days before the expected day of farrowing, sows were moved to a farrowing pen and confined in a farrowing crate (1.9 × 0.6 m). Two days after farrowing, they were allowed to access the full system again. Newly born piglets were kept with their own litter in the farrowing pens for one week, after which they could access the entire system and mingle with the other litters. Piglets were provided with a heated piglet nest next to the farrowing pens (0.7 × 1.6 m), with a temperature of 33–35 °C (day 1 till day 7), 29–31 °C (day 7 till day 25) and 23–26 °C (day 25 till weaning). Piglets were fed in round bowls until five weeks of age and from a sensor-controlled automatic feeder (Rondomat, from three weeks of age). Besides this, the piglets could participate in feeding with the sows, that were fed in a large trough placed on the floor. Ingestion of solid feed was stimulated with the use of intermittent suckling from week 5 of age onwards. AHS piglets were weaned at an average of 62.6 ± 1.9 days and a body weight of 26.6 ± 4.9 kg. They received a starter diet from 35 days onwards.

In the CONV system, piglets were kept in farrowing pens of 2.8 × 1.8 m until weaning. Sows were confined in a crate (1.9 × 0.6 m). The floor consisted of metal slats within the crate. There was a solid floor of 1.2 × 0.3 m with heating lamp for the piglets and the remaining area consisted of plastic slats. Piglets received additional creep feed in the farrowing pens from one week after birth.

CONV piglets were weaned at 27.4 ± 1.2 days of age and 8.7 ± 1.3 kg. After weaning, CONV piglets were housed with their litter mates in nursery pens of 3.18 × 1.0 m (0.40 m2 per piglet) for five additional weeks with a chain and a jute bag as enrichment. They received a commercial weaner diet for ten days after weaning and a starter diet, similar to that provided to AHS piglets, from 35 days onwards.

Lights were on from 07:00 h till 19:00 h in both systems, giving the sows and piglets a 12 h light regime with 115 Lux. Besides that, the AHS had natural daylight through two windows. Transition between day and night light settings was done progressively in 10 min. Ambient temperature was 23 °C in both systems. Water was available ad libitum in both systems.

4.2.2.2 From nine weeks of age onwards

After weaning of the AHS piglets at approximately nine weeks of age, all piglets were moved to the Carus research facilities in Wageningen, the Netherlands. They were mixed in groups of six unfamiliar piglets originating from the same system. Litter, sex and weight were balanced between pens. Piglets were selected based on their sex (50:50% male and female) and weight at birth, in order to choose piglets representative of the full litter. Per litter, six piglets were selected: two piglets with a birth weight between the minimum weight of the litter + 10% and the 1st weight quartile (light); two piglets with a birth weight between the 1st and 3rd quartile (medium); two piglets with a birth weight between the 3rd quartile and the maximum weight − 10% (heavy).

AHS pigs were housed in a 2.40 × 4.67 m pen, i.e., double the size of a conventional pen (1.87 m2 per pig), enriched with deep straw, peat and sawdust bedding, which was replenished regularly (2.5 kg of straw and 30 L of sawdust every day, 22.5 L peat every week). Besides that, AHS pigs were provided with a handful of hay, alfalfa or cardboard egg trays once a week, and a chain, jute bag or rope (rotation every week). They were also provided with one extra toy (either a biting ball on a chain, a free chewing ball for dogs, a tyre dog toy, a porcichew® toy, a green MS Schippers Bite cylinder®, or a green MS Schippers Cross®) that was changed every two days to preserve the attractiveness of the toy. CONV pigs were housed in standard pens of 1.20 × 4.67 m with conventional space allowance (0.93 m2 per pig), with a partly solid and partly slatted floor without substrate. CONV piglets were provided with a ball and a chain with screws, that were not changed. AHS and CONV pens were placed alternately in the rooms. Pigs were all fed the same feed (a standard commercial diet for growing pigs) ad libitum from a single pig feeder and water was available ad libitum.

The light regime was similar to that before nine weeks of age, giving the pigs 115 Lux in the pens during the day (from 7:00 h to 19:00 h; 5000 K ultraviolet A at an intensity of 42, 2700 K at 60) and 30 Lux during the night (5000 K ultraviolet A at an intensity of 3, 2700 K at 0). The transition between the day and night rhythm was done progressively for 10 min. No natural day light was available. Temperature was kept at 23 °C for the first two days, then at 22 °C for the two subsequent days and at 21 °C onwards.

4.2.3 Challenges

Resilience of the pigs in the two housing systems was assessed by following the recovery process of the animals after submission to different successive challenges in the following order: a 21 h-isolation challenge (not detailed in this paper), a 2 h-transport challenge, an LPS injection to induce a sickness response, a 2 h-heat stress challenge and a wound. **Figure 4.1** summarizes the order and time of the challenges described in this paper. Order of exposing pigs to the challenges was always balanced for housing system. Four animals per pen (Focals, two males and two females) were exposed to the experimental challenges, while two other pigs served as companions (i.e., the two pigs that deviated most from the average pen weight).

4.2.3.1 Transport challenge

At the age of 83.0 ± 1.7 days (weight: 41.6 ± 5.5 kg), 94 focal pigs experienced a 2 h transport. At this point, one of the 96 focal pigs was a tail biter (CONV housing). It was euthanized using an injection of pentobarbital (Euthasol® Vet) and replaced by a companion pig after the transport challenge. Neither of those two pigs was exposed to the transport challenge. Pigs were loaded in a trailer with a thin layer of sawdust on the floor and were transported from 8:00 to 10:00

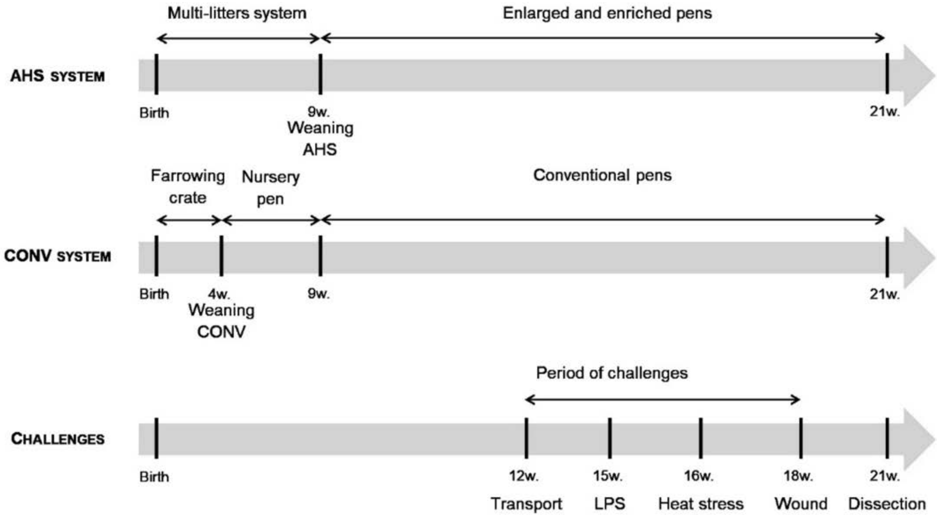


Figure 4.1 - Schematic view of the experiment from birth till dissection of pigs housed in an alternative (AHS) or conventional (CONV) system.

h. All pigs from one batch (from both housing systems) were mixed in the same trailer, which created both a metabolic and social challenge. To follow the recovery of the animals, blood samples were drawn 24 h before the transport challenge (baseline), when they came back from the 2 h-transport (referred to as 0 h), and at 3 h, 24 h and 48 h after the end of transport.

The day before the transportation, pigs were marked with paint to individually track their behaviour in the trailer during the 2 h-transport challenge. Number of aggressive acts (either a push, a bite, a knock, a mount or a fight) and lying behaviour were continuously scored from video by a single trained observer using the software Observer 14.2 (Noldus Information Technology, Wageningen, The Netherlands). The percentage of time spent lying was averaged over 30-min intervals. The total number of aggressive acts and the total number of posture changes were summed over the 2 h period. Because poor lighting in the trailer caused difficulties to observe the pigs before sunrise, the first half-hour of batch 1 was excluded from analysis.

4.2.3.2 LPS challenge

At the age of 104.4 ± 1.7 days (weight: 60.0 ± 7.5 kg), 92 focal pigs were injected in the ear vein with 2 µg of LPS/kg of body weight (LPS sigma L4391 Escherichia coli O111 B4). Four focal pigs were not subjected to the challenge as they were sick and on antibiotic treatment at the day of challenge. For time constraints, the pigs were challenged in two different groups on two consecutive days, balanced for housing system. Pigs were injected in their home pen in one of their ears. Blood samples were collected at 24 h before (baseline) and 1 h, 3 h, 5 h and 24 h after the injection. At each of these time points, rectal temperature was measured before taking a blood sample. Pigs were habituated to rectal temperature measurements in the weeks prior to the challenge to prevent a temperature change related to handling stress.

4.2.3.3 Heat stress challenge

At the age of 111.0 ± 1.9 days (weight: 68.3 ± 6.9 kg), the 96 focal pigs were group-wise subjected to a heat stress challenge. Due to time and space constraints, the pigs were tested on two different days. The focal animals were moved per pen to climatic respiration chambers (4.5 m × 2.5 m). This was done the day before the challenge at 13:00 h to habituate them to the new environment. The focal animals were housed in pens of 3.5 m × 1.8 m (1.6 m2 per pig) with a partly solid and partly slatted floor. Feed and water were available ad libitum. A ball on a chain was provided as a toy. The light schedule was similar to that of the regular room. The ambient temperature was set at 21 °C. During the heat challenge, the temperature raised in 2 h (from 8:30 to 10:30 h) from 21 to 35 °C, stayed at 35 °C for two extra hours and returned to 21 °C in the following 2 h. Humidity was set at 50%. The following day, at 8:00 h, the pigs were moved back to their original room.

From 13:00 h on the day before heat stress until 8:00 h on the day of the heat stress challenge, heat production (kJ/kg$^{0.75}$/day), activity (counts), O_2 consumption (L/kg$^{0.75}$/day), CO_2 production (L/kg$^{0.75}$/day) and CH_4 production (L/climate respiration chamber/day) were measured every 5 min as described before (Geverink et al., 2004). Average values per relevant time period (baseline during the day, baseline during the night from 20:00 to 8:00 h (based on 2 days, the day before and the day after the challenge), during the 2 h temperature increase, during the 2 h period of temperature at 35 °C, and during the 2 h temperature decrease was calculated. At the individual level, the respiration rate per min was measured by counting belly movements of each animal for a period of 30 s and by doubling the amount obtained. Respiratory rates were measured three times on the day before the challenge at 12:00 h in their home pen; at 15:00 h and 16:00 h in the climatic chambers; the day of the heat challenge every 30 min between 8:00 and 15:00 h and at 16:00 h; the day after the challenge at 8:00 h in the climatic chambers before leaving, and at 12:00 h in their home pen. Feed intake was determined for a period of 19 h from entering until leaving the respiratory chambers.

4.2.3.5 Wound healing challenge

At the age of 123.0 ± 1.7 days (weight: 88.5 ± 13.3 kg), the 96 focal pigs were subjected to a fat biopsy to study their wound healing. A wound with a diameter of 0.7 cm and a length of 3.5 cm was created in the fat from the neck by a penetrating gun as described by Baes et al. (2013). Briefly, the pigs were restrained with a nose sling during the procedure. The location sampled was cleaned with betadine soap before the biopsy. Between each pig, the needle of the gun was cleaned with a solution of 70% ethanol. After the biopsy was done, the wound was sprayed with betadine to prevent infection.

The wound healing process was followed by measuring the perimeter of the wound from photographs taken, without restraining the pigs, by a digital camera daily for 10 days and on day 15 after the biopsy. If needed, the wound was cleaned with water before taking a picture. Measurements were made by a single trained person, blind to treatment, using the ImageJ software (Schneider et al., 2012) to delimit the wound perimeter. The length of a sticker placed close to the wound was used as a scale to standardize the measurements.

4.2.4 Indicators measured during the challenges

4.2.4.1 Weights

All pigs were weighed 24 h before and 24 h after the start of each challenge. Relative weight change was estimated as follows: $\frac{(Final\ weight - initial\ weight)}{Initial\ weight}$.

4.2.4.2 Blood samples

The animals were restrained with a nose sling during the blood sampling procedures. Blood samples were collected from the jugular vein. The blood (10 ml) was distributed as follows: 4 ml in EDTA tubes, 4 ml in heparin tubes and 2 ml in serum tubes. The EDTA and heparin tubes were centrifuged at 1500g for 10 min at + 4 °C. The serum samples were kept at ambient temperature for 30 min and thereafter were centrifuged at 1500g for 10 min at 4 °C. Plasma and serum were stored at − 20 °C until laboratory analyses.

Cortisol assays were performed in EDTA samples using the cortisol RIA kit from Immunotech (Beckman-Coulter, ref IM1841, Czech Republic). Glucose concentrations were measured in EDTA samples using a GOD-PAP kit (Hoffmann-La Roche, Switzerland). Non-Esterified Fatty Acids (NEFA) concentrations were measured in serum samples using a NEFA kit (WAKO Chemicals GmbH, Germany). Haptoglobin levels in heparin plasma were measured using the kit PHASE TM Haptoglobin Assay from Tridelta Development Limited (ref TP-801, Ireland). Titers of IgG and IgM in heparin samples binding keyhole limpet hemocyanin (KLH), myelin basic protein (MBP) and phosphoryl choline-conjugated to Bovine Serum Albumin (PC-BSA) were measured as described previously40. For IgG and IgM determination, only the sampling points 24 h pre-challenge, 24 h post-challenge and 48 h post-challenge were used. Urea concentrations were measured in EDTA samples using the enzymatic colorimetric test (ref 10506, HUMAN Gesellschaft für Biochemica und Diagnostica mbH, Wiesbaden, Germany).

4.2.4.3 Hair samples

The animals were shaved at 11 weeks of age before the start of the period with challenges, and at 18 weeks of age simultaneously with the biopsy, to determine the accumulation of cortisol over this period. The shaving area of about 225 cm^2 was located at the same spot for all piglets to avoid potential bias related to the body part. The location was close to the hip of the animals, at the connection between the abdominal area and the hind leg. A one-use surgical razor was used for each animal. The experimenter wore gloves to avoid contamination of the samples. At 11 weeks, only the right side of the animals was shaved, while two sides were shaved at 18 weeks. This enabled to distinguish the effect of the period of challenges between 11 and 18 weeks (regrowth of the right side between the two shaving points) from the full life of the animals from birth onwards (left side never shaved). Samples were stored in aluminium foils at room temperature in the dark until analysis.

Hairs were washed three times: once in PBS and twice in isopropanol to remove any dirt and dust, and were dried for 96 h at 37 °C. To extract the cortisol from the hairs, the samples were first cut with scissors in small pieces before being grinded with a tissue lyser. The cortisol from the hair powder was then extracted with methanol for 24 h. The extract was dried in a speedvac for 3 h and dissolved in a phosphate buffer. Cortisol concentration was determined using the high sensitivity salivary cortisol ELISA kit (ref 1-3002) from Salimetrics (Pennsylvania, USA).

4.2.4.4 Skin lesions

Skin lesions were scored 24 h before and 24 h after the start of each challenge according to the Welfare Quality Assessment Protocol® for pigs41, except for the wound healing challenge, when it was done simultaneous with the biopsy. Briefly, the body of the pig was divided into five separate regions: 1—ears, 2—front (head to the back of the shoulder), 3—middle (back of the shoulder to the hind quarters, 4—hind quarters, and 5—legs (from the accessory digit upwards). A scratch longer than 2 cm was considered as one lesion, and two parallel scratches with up to 0.5 cm space in between were considered to be one lesion. Wounds smaller than 2 cm were considered as one lesion. Bleeding wounds, healed wounds of more than 5 cm, as well as deep and open wounds of more than 5 cm, were never observed. The number of lesions from the different body regions were summed to create a global score.

4.2.4.5 Tear staining

Photographs of the left eye of each focal pig were taken eight times during the experiment to measure tear staining. This was done 24 h before and 24 h after transport, LPS and heat stress challenges, right before the biopsy and before slaughter. Measurements were made on photographs by a single experienced person, blind to treatment, using the ImageJ software (Schneider et al., 2012) to delimit the tear perimeter. The length of the iris was used as a scale to standardize the measurements. All the brownish areas on the direct periphery of the eye (bottom of the upper eyelid, top of the lower eyelid, internal and external corners) were recorded (DeBoer et al., 2015). The variable analyzed was the cumulative area covered by the stain.

4.2.4.6 Pathological examination at slaughter

At the age of 140.0 ± 1.9 days (weight: 95.2 ± 8.5 kg), the 96 focal pigs were exsanguinated after an electrical stunning for dissection purpose. The judging of pathological changes was done blind to the housing treatment of the pigs. The heart was examined for pericarditis, the lungs for pneumonia and pleurisy, and the stomach for lesions and ulcers. The heart of the pigs was assessed for pericarditis with a score of 0 (no pericarditis) to 3 (severe pericarditis) (Scott et al., 2006). Lung lesions for pneumonia were scored with a scale ranging from 0 (no lesion) to 28 (total pulmonary consolidation) (Merlot et al., 2013). Pleurisy was scored for the entire lungs ranging from 0 (no adhesion between the lobes) to 4 (lungs fully adherent to the thoracic cavity). Stomach wall damage at the pars oesophagus was assessed with a score of 0 (normal pars oesophagea, no hyperkeratosis or lesions) to 5 (hyperkeratosis and more than 10 lesions, ulcer or occlusion of the oesophagus into the stomach) (adapted from Hessing et al. (1992)).

4.2.5 Statistical analyses

Statistical analyses were performed with the software R 4.0.3. (R Core team, 2013). The variables tear staining, serum NEFA concentration and hair cortisol concentration were normalized by logarithmic transformation. Areas under the recovery curves (AUC) were approximated from repeated measurements using the trapezoidal rule $\sum_{k=0}^{N}\left(\frac{f(x_{k-1})+f(x_k)}{2}\right)\times \Delta\, xk$, where $f(x)$ is a function and Δxk is the length of the k^{th} subinterval. The resilience indicator (ln(variance) based on body weight deviations was estimated using the same formulas as in the paper of Berghof et al. (2019b).

On focal pigs only, linear mixed models were used for all variables measured once (relative growth, tear and skin lesions measured 24 h after the biopsy and before slaughter, post-mortem examinations, AUC, latencies of behaviours, total numbers of aggressive acts and posture changes during the transport challenge) with the function lmer from the R package “lme4”, except for frequencies of behaviours and scores of skin lesions and post-mortem measurements. For these variables, generalized mixed models with a Poisson distribution and Log link function were used and the function glmer from the R package “lme4”. In these models, Housing and Sex (boar versus gilt) were fixed effects, and Pen and Batch were random effects. For the repeated variables, repeated linear mixed models or repeated generalized mixed models with Housing, Sex, Time and the housing × time interaction as fixed effects, Pen and Batch as random effects, and Pig as a repeated variable were used. For hair cortisol, the Side of shaving and the Status of the animal (focal versus companion) were also included as fixed effects.

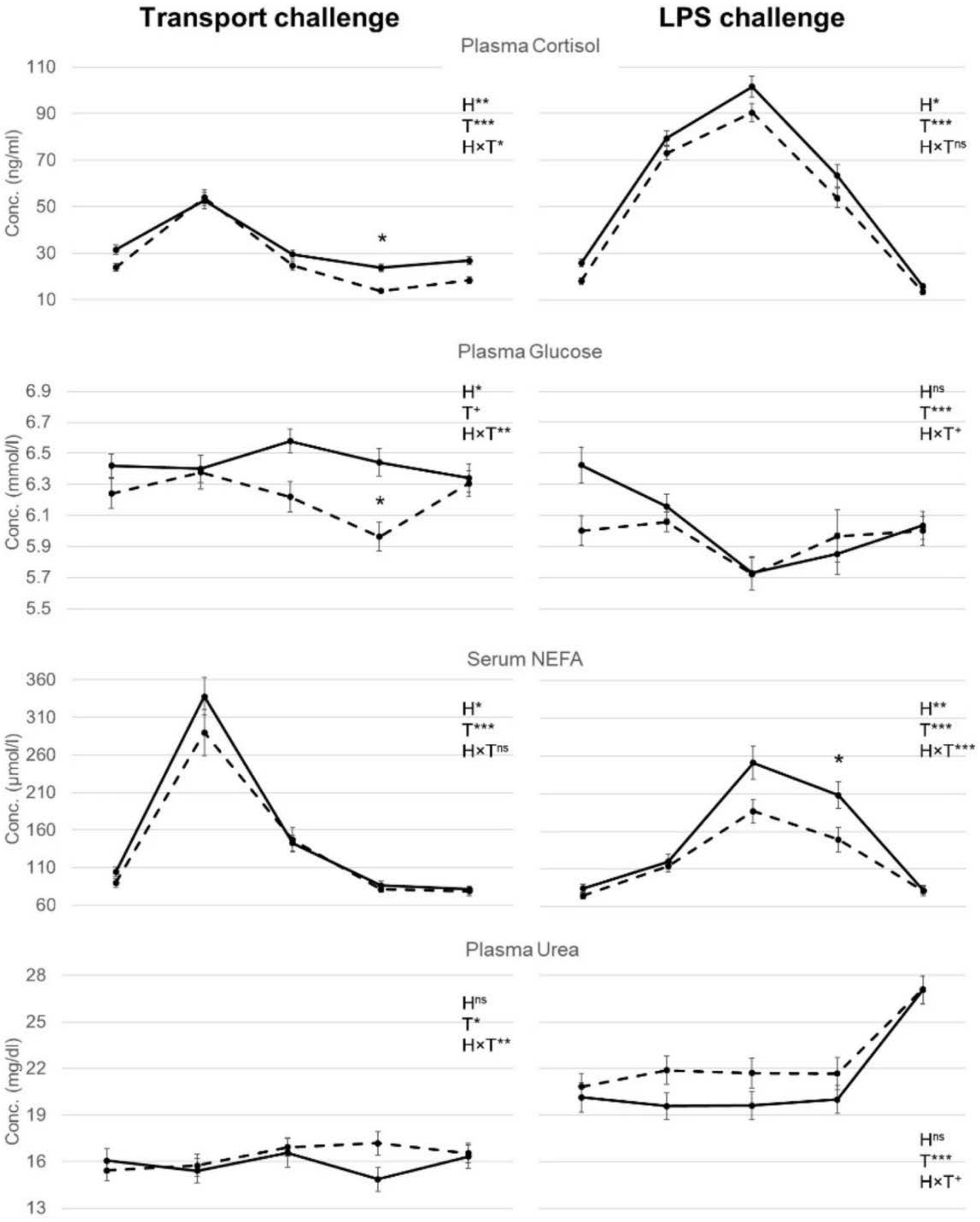
For the measurements of the heat challenge at the group level, Housing, Time period and housing × time period were used as fixed effects and Batch as a random effect. For the feed intake in the climatic respiration chambers, Housing was used as a fixed effect and Batch as a random effect.

P-values below 0.05 were considered as significant effects and below 0.1 as tendencies. When a significant effect was found, pairwise comparisons between groups were made with the emmeans function of the emmeans package from R, including a Tukey correction. Data are presented as means ± SEM.

4.3 Results

4.3.1 Transport challenge

Figure 4.2 shows blood parameter concentrations around transport. Plasma cortisol levels were affected by housing ($p = 0.0035$), time ($p < 0.0001$) and their interaction ($p = 0.035$), with lower cortisol concentrations at + 24 h in AHS compared to CONV pigs. AHS pigs also had a lower AUC of cortisol ($p = 0.0010$). Glucose levels were affected by housing ($p = 0.0047$), tended to be affected by time ($p = 0.075$), and were affected by the housing × time interaction ($p = 0.0094$). AHS pigs showed a drop in glucose at + 24 h compared to + 2 h and + 48 h, and had lower glucose concentrations at this time point than CON pigs. AUC of glucose was also lower for AHS pigs ($p = 0.0023$). NEFA levels increased during transport ($p < 0.0001$) and were higher for CONV than for


Transport challenge
LPS challenge
Plasma Cortisol
Conc. (ng/ml)
110
90
70
50
30
10
H**
T***
H×T*
*
H*
T***
H×Tns
Plasma Glucose
Conc. (mmol/l)
6.9
6.7
6.5
6.3
6.1
5.9
5.7
5.5
H*
T+
H×T**
*
Hns
T***
H×T+
Serum NEFA
Conc. (µmol/l)
360
310
260
210
160
110
60
H*
T***
H×Tns
H**
T***
H×T***
*
Plasma Urea
Conc. (mg/dl)
28
25
22
19
16
13
Hns
T*
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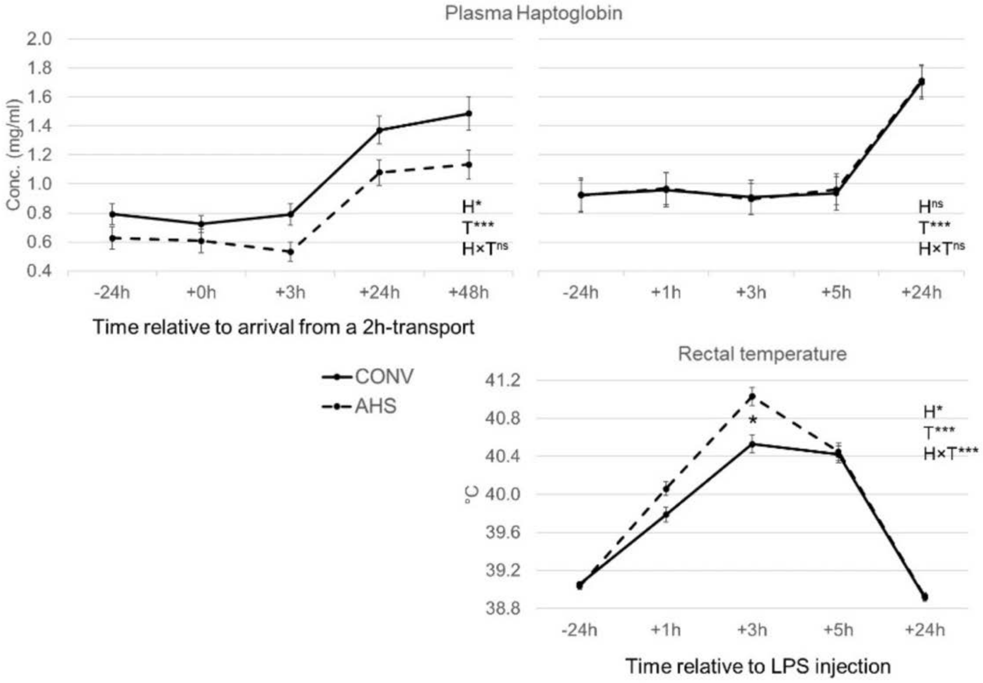


Figure 4.2 - Plasma cortisol, plasma glucose, serum NEFA, plasma urea and plasma haptoglobin for both the transport and LPS challenge, and rectal temperature for the LPS challenge, of pigs housed in an alternative (AHS, dotted line) or conventional (CONV, solid line) system. *H* housing effect, *T* time effect, *HxT* housing × time interaction; ***$p < 0.001$; **$p < 0.01$, *$p < 0.05$, $^{+}p < 0.10$, ^{ns} non-significant. In case of interaction effects, within-timepoint differences between housing system are indicated by *. Please note, different Y-axis scaling. For the transport challenge, t = 0 h corresponds to the arrival of the pigs to the farm after the 2 h-transport.

AHS pigs ($p = 0.047$). AUC of NEFA was unaffected by housing. The course of urea levels over time ($p = 0.021$) was affected by housing (housing × time effect, $p = 0.0088$), without significant pairwise differences. The AUC of urea was unaffected by housing. Haptoglobin levels were affected by time ($p < 0.0001$), with increasing levels from + 3 h onwards, and were lower in AHS than in CONV pigs ($p = 0.026$). The AUC of haptoglobin was also lower for AHS pigs ($p = 0.021$).

Titers of IgM binding KLH were increased (time effect, $p = 0.0003$) 48 h post-challenge (5.33 ± 0.12) compared to 24 h before (5.02 ± 0.12) and 24 h after (5.07 ± 0.12) and were higher in AHS (5.42 ± 0.13) than in CONV pigs 4.86 ± 0.13, $p = 0.0002$). KLH-IgG titers ($p < 0.0001$) were higher in AHS than in CONV pigs too ($AHS = 5.11 \pm 0.08$, $CONV = 4.70 \pm 0.08$), without a time effect. Similarly, PC-BSA-IgM titers were higher in AHS pigs ($p = 0.0081$; $AHS = 7.32 \pm 0.13$, $CONV = 6.85 \pm 0.13$), but were unaffected by time. PC-BSA-IgG titers were unaffected by housing but showed a time effect ($p = 0.011$), with higher levels at + 24 h (5.50 ± 0.47) than at − 24 h (5.22 ± 0.47) and + 48 h (5.34 ± 0.47). MBP-IgM titers were higher ($p = 0.0012$) at 48 h post-challenge (7.27 ± 0.14) than 24 h before (6.99 ± 0.14) and 24 h post-challenge (6.84 ± 0.14) and were unaffected by housing. MBP-IgG titers were higher ($p = 0.0001$) + 24 h post-challenge (5.12 ± 0.25) compared to 24 h before (4.71 ± 0.25) and 48 h after (4.51 ± 0.25) and were unaffected by housing. None of the antibody titers were affected by the housing × time interaction.

The percentage of time spent lying over time ($p < 0.0001$; **Figure 4.3**) was affected by housing (housing × time effect, $p = 0.0097$), without significant pairwise differences. AHS pigs showed more posture changes ($p = 0.011$; $AHS = 3.67 \pm 0.11$, $CONV = 3.41 \pm 0.11$). The number of skin lesions was affected by the housing × time interaction ($p = 0.012$), but pairwise differences showed no differences between AHS pigs before or after the challenge, and in both housing systems lesions increased after transport (AHS: − 24 h $= 2.42 \pm 0.19$, + 24 h $= 3.65 \pm 0.19$; CONV: − 24 h $= 2.46 \pm 0.19$, + 24 h $= 3.52 \pm 0.19$). AHS pigs tended to show more aggressive interactions than CONV pigs ($p = 0.083$; $AHS = 2.04 \pm 0.23$, $CONV = 1.50 \pm 0.23$). CONV pigs gained relatively more weight during the challenge than AHS pigs ($p = 0.017$; $AHS = 2.3\% \pm 0.8$ of their original body weight, $CONV = 3.4\% \pm 0.8$). Tear staining did not change in response to the challenge and was not affected by housing.

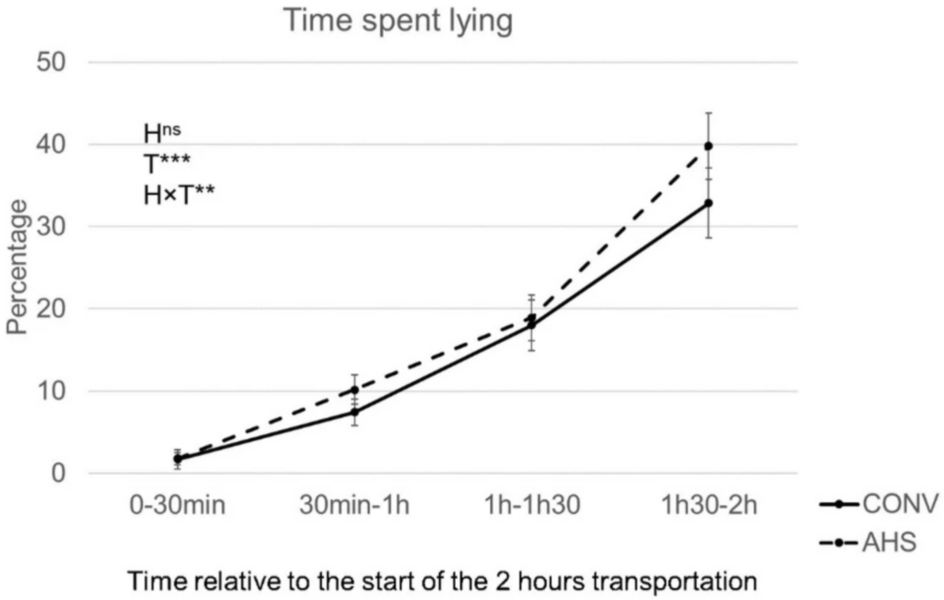


Figure 4.3 - Time spent lying during the transport challenge of pigs housed in an alternative (AHS, dotted line) or conventional (CONV, solid line) system. H housing effect, T time effect, HxT housing × time interaction; ***$p < 0.001$; **$p < 0.01$, ^{ns} non-significant. In case of interaction effects, within-timepoint differences between housing system are indicated by *.

4.3.2 LPS challenge

Figure 4.2 shows blood parameter concentrations around the LPS challenge. Cortisol levels and the AUC of cortisol were lower for AHS than for CONV pigs ($p = 0.030$ and $p = 0.038$, respectively), and cortisol levels peaked at + 3 h (time effect, $p < 0.0001$). Glucose levels dropped at + 3 h (time effect, $p < 0.0001$), but both levels and AUC of glucose were unaffected by housing. NEFA levels were affected by housing ($p = 0.0068$), time ($p < 0.0001$) with a peak at + 3 h, and by the housing × time interaction ($p = 0.0007$) with higher levels for CONV than for AHS pigs at + 5 h. The AUC of NEFA was larger for CONV than for AHS pigs ($p = 0.025$). Urea was affected by time only

($p < 0.0001$), with a sudden increase 24 h post challenge. Haptoglobin started to increase at + 24 h ($p < 0.0001$), and was unaffected by housing.

Rectal temperature was affected by housing ($p = 0.034$), time ($p < 0.0001$) and their interaction ($p < 0.0001$). Temperatures increased till + 3 h before going back to baseline levels at + 24 h. At + 3 h, AHS pigs had higher temperatures than CONV pigs.

KLH-IgM was affected by housing ($p < 0.0001$) and the housing × time interaction ($p = 0.027$) with higher titers for AHS pigs than CONV pigs both at − 24 h (AHS = 6.34 ± 0.1, CONV = 5.78 ± 0.1) and at + 24 h (AHS = 6.38 ± 0.10, CONV = 5.64 ± 0.10) and a decrease after injection for CONV pigs only. Titers of KLH-IgG were higher in AHS (5.70 ± 0.17) than in CONV pigs (5.32 ± 0.17, $p = 0.028$) and were also affected by time ($p = 0.016$) with increasing values post-challenge (− 24 h = 5.46 ± 0.15, + 24 h = 5.56 ± 0.15). PC-BSA-IgM was affected by the housing × time interaction ($p = 0.014$), without significant pairwise differences (− 24 h, AHS = 7.71 ± 0.14, CONV = 7.62 ± 0.14; + 24 h, AHS = 7.88 ± 0.14, CONV = 7.51 ± 0.14). PC-BSA-IgG increased over time ($p = 0.0049$, − 24 h = 5.78 ± 0.47, + 24 h = 5.89 ± 0.47), but was unaffected by housing or its interaction with time. Time also affected titers of MBP-IgM ($p = 0.023$, − 24 h = 7.53 ± 0.22, + 24 h = 7.76 ± 0.22) and MBP-IgG ($p = 0.005$, − 24 h = 4.63 ± 0.13, + 24 h = 4.47 ± 0.13), but housing did not.

Skin lesions (AHS = 2.44 ± 0.89, CONV = 2.54 ± 0.88) and tear staining (AHS = 0.68 ± 0.094, CONV = 0.62 ± 0.057) did not change in response to the challenge, and were unaffected by housing. Also, relative weight change was unaffected by housing (AHS = 0.99 ± 0.40%, CONV = 0.66 ± 0.40%) (data not shown).

4.3.3 Heat stress challenge

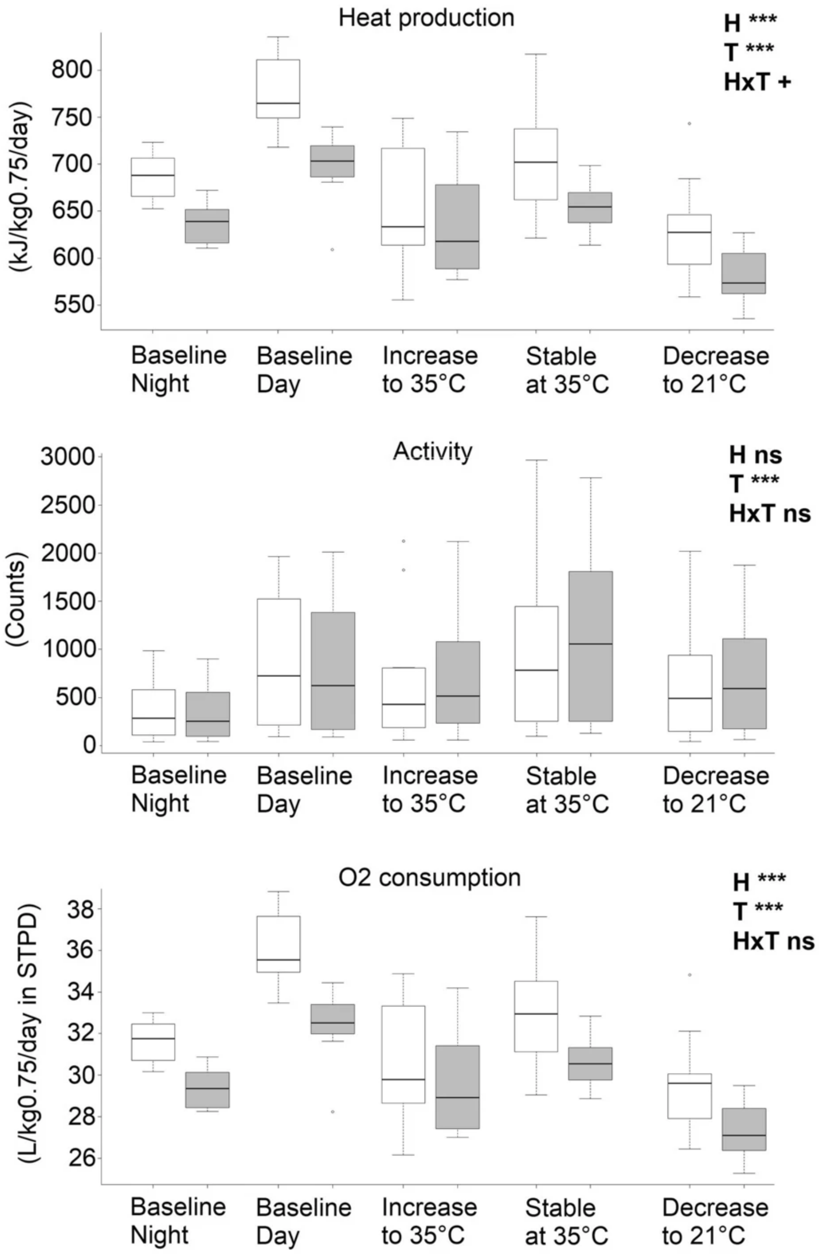
Figure 4.4 shows the O_2 consumption, CO_2, CH_4 and heat production and activity as assessed in the climatic respiration chambers per time period of interest. A more detailed graph is shown in **Figure S4.1** (in supplementary information). All variables were affected by time period ($p < 0.0001$). Baseline O_2 consumption and CO_2 and heat production were lower during the night than during the day. During the 2 h heating up of the chambers, levels of these variables went down, as compared to baseline day levels. The subsequent 2 h period at 35 °C showed an increase in O_2 consumption and CO_2 and heat production again, but levels did not reach baseline day levels. During the 2 h cooling down of the chamber, pigs showed the lowest levels of O_2 consumption and CO_2 and heat production. Methane production was lower during the heating up, the 2 h 35 °C period and the cooling down, than during the baseline levels of the day and the night (which did not differ from each other).

CONV pigs produced more heat ($p < 0.0001$), more O_2 ($p < 0.0001$), CO_2 ($p = 0.00013$) and CH_4 ($p < 0.0001$), irrespective of time period, as there were no housing × time interactions, and housing effects were already found at baseline.

The baseline activity was lower at night than at day. Compared to baseline day levels, activity was lower during the heating up, increased during the 35 °C period and decreased again during the cooling down. Activity levels were not affected by housing.

Respiratory rate (RR) and the related AUC as measured at the individual level were affected by housing ($p < 0.0001$ and $p = 0.00081$, respectively), by time ($p < 0.0001$) and their interaction ($p < 0.0001$). From 30 min after the 35 °C was reached till 1h30 after the cooling process was started post-challenge, AHS pigs had a higher RR than CONV pigs (**Figure 4.5**).

CONV pigs gained relatively more weight during the challenge period than AHS pigs ($p = 0.043$; AHS = 0.58% ± 0.29 of their pre-challenge body weight, CONV = 1.41% ± 0.29). Effect of housing on feed intake (AHS 13.5 ± 0.44 kg; CONV 14.4 ± 0.44 kg) did not reach significance ($p = 0.058$). Skin lesions were higher post-challenge than pre-challenge ($p = 0.0031$; − 24 h = 2.78 ± 0.050 and + 24 h = 2.88 ± 0.050), suggesting an increase in the number of aggressive events. The heat stress challenge caused an increase of the tear staining area ($p = 0.029$; − 24 h = 0.56 ± 0.12 arb. unit, + 24 h = 0.79 ± 0.055 arb. unit).


Heat production
(kJ/kg0.75/day)
800
750
700
650
600
550
H ***
T ***
HxT +
Baseline Night
Baseline Day
Increase to 35°C
Stable at 35°C
Decrease to 21°C
Activity
(Counts)
3000
2500
2000
1500
1000
500
0
H ns
T ***
HxT ns
Baseline Night
Baseline Day
Increase to 35°C
Stable at 35°C
Decrease to 21°C
O2 consumption
(L/kg0.75/day in STPD)
38
36
34
32
30
28
26
H ***
T ***
HxT ns
Baseline Night
Baseline Day
Increase to 35°C
Stable at 35°C
Decrease to 21°C

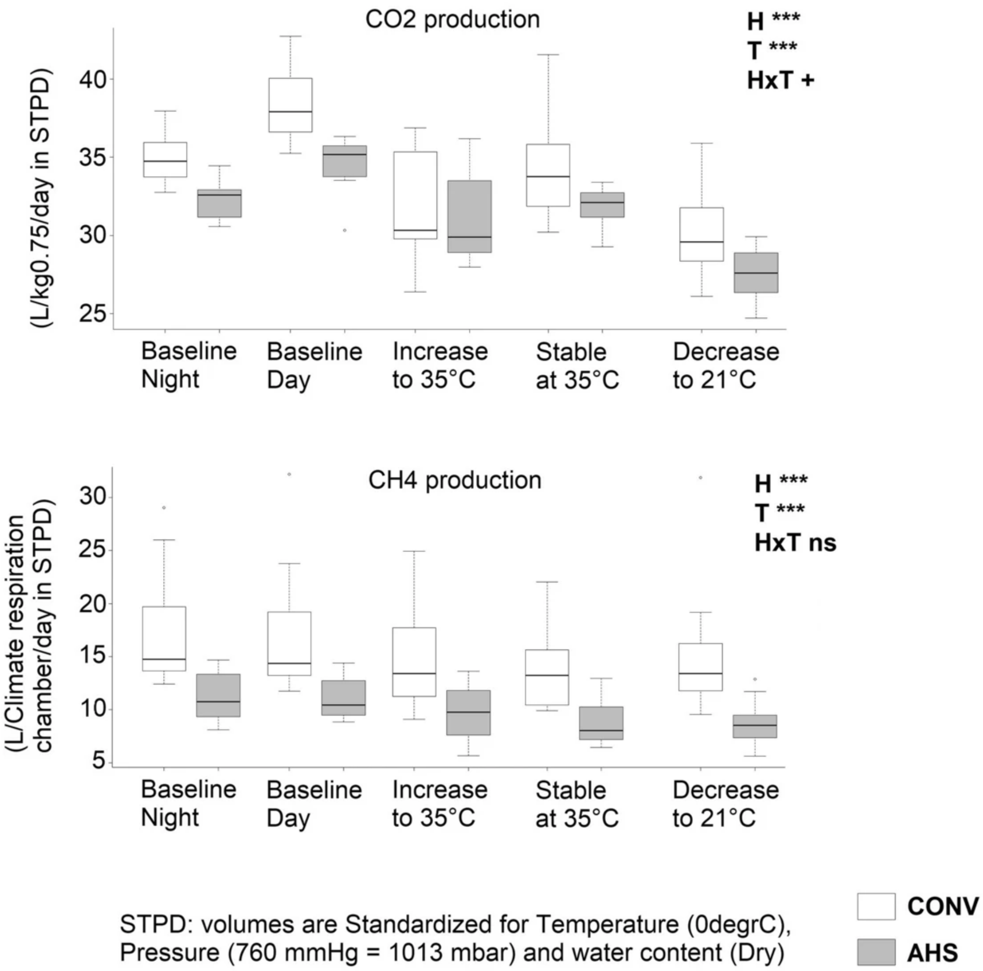


Figure 4.4 – Heat production, O_2 consumption, CO_2 production, CH_4 production and activity during the heat stress challenge of pigs housed in an alternative (AHS, in grey) or conventional system (CONV, in white). ***H*** **housing effect,** ***T*** **time effect,** ***HxT*** **housing × time interaction; ***$p < 0.001$; + $p < 0.10$; ^{ns} non-significant.**

4.3.4 Wound healing challenge

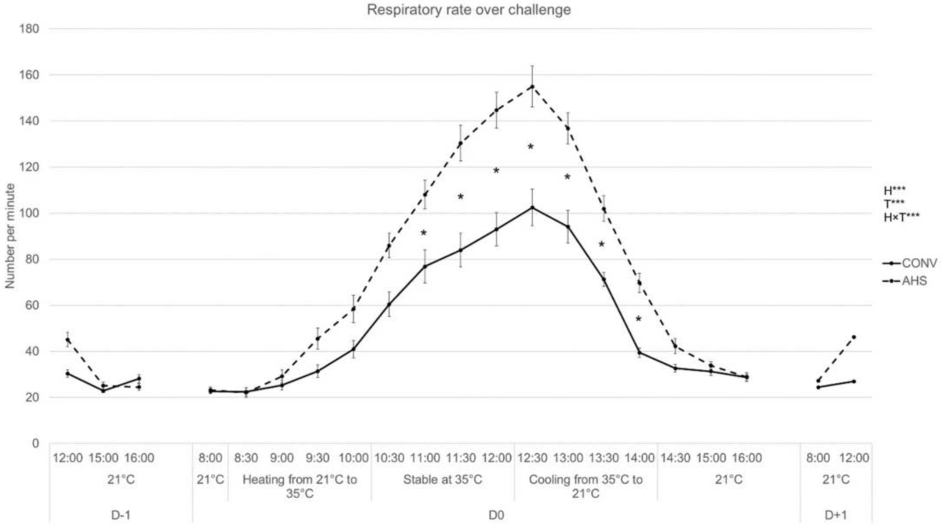


Figure 4.5 – Respiratory rate during the heat stress challenge of pigs housed in an alternative (AHS, dotted line) or conventional (CONV, solid line) system. *H* housing effect, *T* time effect, *HxT* housing × time interaction; ***p < 0.001; *p < 0.05. In case of interaction effects, within-timepoint differences between housing system are indicated by *.

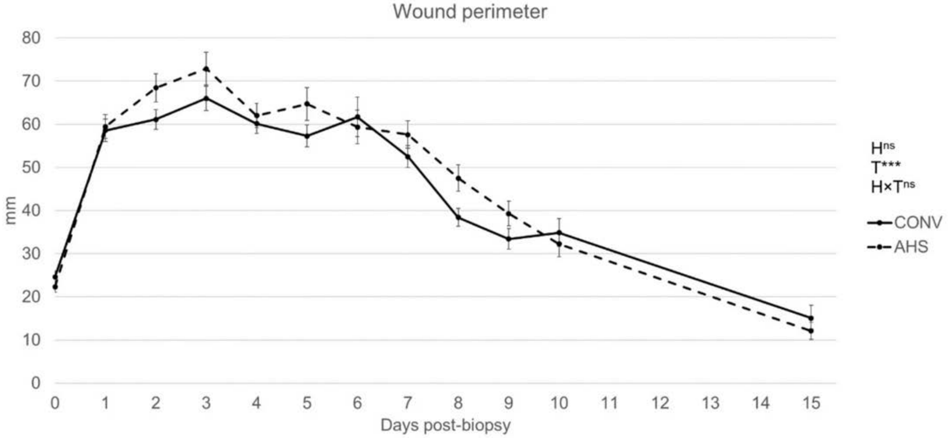


Figure 4.6 – Wound perimeter as a reflection of the wound healing process of pigs housed in an alternative (AHS, dotted line) or conventional (CONV, solid line) system. *H* housing effect, *T* time effect, *HxT* housing x time interaction; ***p<0.001; ^{ns} non-significant.

The healing perimeter of the biopsy was affected by time ($p < 0.0001$), with an increase up to three days post-challenge followed by a progressive decrease over time (**Figure 4.6**), but was unaffected by housing. AHS pigs presented more skin lesions on the day of the challenge ($p = 0.0012$; AHS = 2.96 ± 0.17, CONV = 2.53 ± 0.16). Tear staining measured right before wound healing challenge, possibly indicating the stress experienced the previous days, was higher for CONV pigs compared to AHS pigs.

4.3.5 Growth, accumulation of cortisol in hairs, and post-mortem assessment

Body weight gain over time ($p < 0.0001$) was not affected by housing. However, the Ln(variance) of the weights of CONV pigs was higher than that of AHS pigs ($p = 0.018$; AHS = − 1.90 ± 0.22 arb. unit, CONV = − 1.58 ± 0.22 arb. unit). Tear staining was affected by time only ($p < 0.0001$) with lower values at 11 and 12 weeks than in the weeks thereafter. The concentration of cortisol in hairs was affected by time ($p < 0.0001$), housing ($p < 0.0001$) and their interaction ($p < 0.0001$). At 11 weeks of age, hair cortisol levels were similar for CONV and AHS pigs. However, in CONV pigs, cortisol concentration in hairs increased profoundly between 11 and 18 weeks of age, while in AHS pigs it decreased over time, resulting in a treatment difference at 18 weeks of age (**Figure 4.7**). The side of shaving at 18 weeks of age and the status of the animal (focal versus companion) had no effects on the cortisol concentration in hairs. At slaughter, AHS pigs had more skin lesions ($p = 0.014$; AHS = 2.83 ± 0.17, CONV = 2.45 ± 0.17). Pericarditis (only one CONV pig affected) and pleurisy (9 CONV pigs and 4 AHS pigs had a score of 1) hardly occurred. Scores of pneumonia (17.4 ± 1.1) and gastric lesions (2.92 ± 0.13) were not affected by housing.

4.3.6 Sex effects

Only the significant sex effects are described below and summarized in **Table 4.1**. Boars had higher levels and AUC of glucose than gilts in both the transport and LPS challenge, and lower levels and AUC of urea levels. Rectal temperature during the LPS challenge was higher for gilts. Boars changed their posture more often, showed more aggressive acts, and presented more skin lesions during transport. Skin lesions measured at the LPS, heat stress and wound challenge were also higher for boars. During the heat challenge, boars had a higher RR than gilts as well as higher relative weight gain. Around the wound challenge, gilts recovered slower than boars, but they had a smaller tear staining area. At slaughter, the pneumonia score was higher for boars compared to gilts.

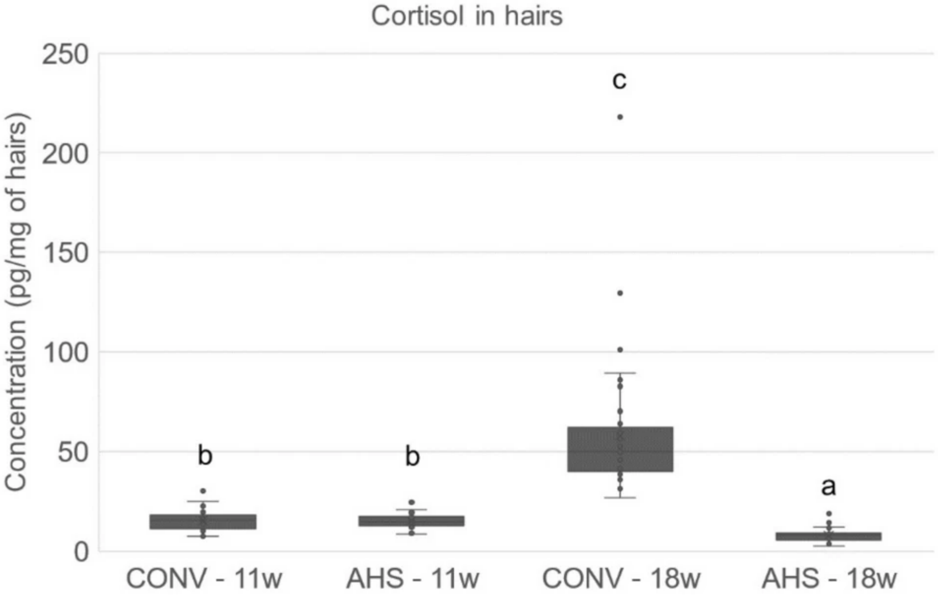


Figure 4.7 – Cortisol concentration in hairs measure before (11 weeks of age) and at the end (18 weeks of age) of the period of challenges in pigs housed in alternative (AHS) or conventional (CONV) system. Letters were attributed for significantly different values a<b<c; $p < 0.0001$.

Table 4.1 – Significant effects of sex (Boar versus Gilt) on variables measured during various challenges (lsmeans ± SEM).

Challenge	Variable	Boar	Gilt	p value
2 h-Transport	Plasma glucose (mg/100 ml)	116 ± 2.3	112 ± 2.3	0.0020
	Plasma urea (mmol/l)	2.38 ± 0.2	3.00 ± 0.2	< 0.0001
	Aggression (n)	2.60 ± 0.43	1.45 ± 0.43	< 0.0001
	Posture changes	3.66 ± 0.1	3.42 ± 0.1	< 0.0001
	Skin lesions (n)	3.19 ± 0.19	2.84 ± 0.19	< 0.0001
LPS injection	Plasma glucose (mg/100 ml)	110 ± 1.9	106 ± 1.9	0.022
	Plasma urea (mmol/l)	3.18 ± 0.12	4.15 ± 0.12	< 0.0001
	Rectal temperature (°C)	39.8 ± 0.05	39.9 ± 0.05	0.039
	Skin lesions (n)	2.60 ± .080	2.38 ± 0.081	0.0095
Heat stress	Respiratory rate (n/min)	59.0 ± 2.6	52.1 ± 2.6	0.0017
	Relative growth (%)	1.45 ± 0.25	0.54 ± 0.25	0.00067
	Skin lesions (n)	2.95 ± 0.065	2.71 ± 0.066	0.0091
Wound healing	Healing perimeter[a] (mm)	46.4 ± 3.4	50.7 ± 3.4	0.0058
	Skin lesions (n)	2.80 ± 0.14	2.69 ± 0.14	0.020
	Tear staining (arb. unit)	0.79 ± 0.14	0.42 ± 0.07	0.026
Dissection	Pneumonia score (arb. unit)	3.49 ± 0.61	3.41 ± 0.61	0.028

[a] Healing perimeter: perimeter of the wound caused by biopsy.

4.4 Discussion

In this study, resilience was assessed by subjecting pigs to a series of challenges. Pigs from the alternative housing system (AHS) were expected to have an improved resilience compared to pigs from conventional housing (CONV). This hypothesis was based on the AHS promoting socialization, providing a more gradual and prolonged weaning transition, and ample provision of environmental enrichment and space in the AHS, to support the expression of important species-specific behaviours. This putative improved resilience would then be demonstrated in a faster recovery from the challenges, and lower expression of indicators reflecting poor welfare and "wear and tear" on the body over the complete experimental period.

As hypothesized, AHS pigs showed a faster recovery and/or a lower response than CONV pigs in several physiological indicators around the 2 h-transport (cortisol, glucose and non-esterified fatty acids, NEFA), and the LPS challenge (cortisol and NEFA). Only in the AHS pigs, the elevation of corticosteroid production following stressful conditions caused a drop in glucose, which will turn the body into a catabolic state, activating the use of alternative fuels such as NEFA (Martínez-Miró et al., 2016). Transport is one of the most well-known stressors in pig production, involving handling, loading, and packing in a truck (Rioja-Lang et al., 2019), and usually leading to a rise in cortisol (Salamano et al., 2008; Sutherland et al., 2014, 2009). A less pronounced cortisol response to transport in AHS pigs is in line with a previous study in which pigs kept in enriched and larger pens showed a lower increase after transport (Geverink et al., 1999) or no increase at all (de Jong et al., 2000b). Similar to other studies, transport also increased the levels of haptoglobin, an acute phase protein (Salamano et al., 2008; Sutherland et al., 2014, 2009). This increase could both reflect acute stress (Cray et al., 2009), or, alternatively, skin inflammation caused by the lesions induced during fighting at transport. The latter is less likely, however, as deep, large or bleeding wounds were never observed. Although the dynamics of the haptoglobin response did not differ between housing systems, AHS pigs showed overall lower levels of haptoglobin than CONV pigs, which corresponds with other studies on enriched housed pigs (Reimert et al., 2014a; Scollo et al., 2013; Scott et al., 2006).

The LPS challenge induces a sickness response in pigs, which is associated with increased cortisol levels, a fever response, decreased activity, and reduced feed and water intake (Clouard et al., 2015; de Groot et al., 2007; Pastorelli et al., 2012), mimicking the response to infection. Although AHS pigs showed a less pronounced response and/or faster recovery in cortisol and NEFA, their peak in rectal temperature at 3 h post LPS injection was higher, with a similar area under the curve. Developing fever may be an efficient strategy to fight infection by stimulating proliferation of immune cells and limiting micro-organism proliferation (Pecchi et al., 2009). A number of studies suggest indeed that enriched housed pigs combat infections more efficiently. Pigs provided with larger living space had lower lung lesions induced by *M. hyopneumonia* (Hennig-Pauka et al., 2019). Similarly, pigs housed in enriched social and environmental conditions demonstrated a faster viral clearance compared to pigs raised in barren pens, and less lung lesions (van Dixhoorn et al., 2016) when exposed to a PRRSV—*Actinobacillus pleuropneumoniae* co-infection. Disease challenges can generate pain and general fatigue, compromising animal welfare (Hart and Hart, 2019; Scott, 2013). Moreover, it has been shown recently that induction of a sickness response (by LPS) was a risk factor for the expression of damaging behaviours like tail biting (Munsterhjelm et al., 2019). Tail biting is a common problem in pig farming leading to impaired welfare and health, as well as production loss. Taken together, the more favourable response to the LPS challenge with quicker recovery in the AHS pigs may thus have important welfare consequences.

We expected a faster wound healing in AHS pigs than in CONV pigs. However, no effects of housing system were found, while a better wound healing has been demonstrated for pigs receiving cognitive enrichment (Ernst et al., 2006).

Heat stress is metabolically demanding for pigs, affecting carbohydrate and lipid metabolism, e.g., reflected in an increased NEFA concentration and respiratory rate (Marco-Ramell et al., 2011). AHS and CONV pigs differed in responses to the heat challenge, as AHS pigs showed a lower growth rate and tended to eat less while being in the climate respiration chambers. During heat stress periods, reducing feed intake has been shown to be a good strategy to minimize metabolic heat production (Pearce et al., 2013). It cannot be excluded, though, that the lower feed intake and weight gain in AHS pigs were related to the larger difference in environmental quality between the climate respiration chambers and home pens for those pigs compared to the CONV pigs, due to the lack of bedding and extra toys. Switching from enriched to barren environments resulted in frustration, apathy and more aggressive interactions in previous studies (Latham and Mason, 2010; Melotti et al., 2011). During the challenge, AHS pigs showed a higher respiratory rate than CONV pigs. It might be that the CONV pigs developed a different thermoregulatory strategy during the heat wave. Even though not scored systematically, more manure and urine were seen on the floor of the metabolic chambers with CONV pigs, which were possibly used to wallow in, as observed in other studies (Aarnink et al., 1996; Huynh et al., 2005). During the heat stress, pigs were expected to increase heat production and consume more O_2 due to the increase of respiratory rate trying to dissipate the extra heat (Sanz Fernandez et al., 2015). In this study, however, O_2 consumption was unchanged during the heat wave but increased post-entrance to the chamber and right after the heat wave, which was unrelated to an increased general activity. Those two peaks might reflect a delayed response or recuperation to stressful events: the transport to the metabolic chamber on one side, and the heat stress on the other side. As expected, CH_4 production decreased during the heat wave, probably due to a reduction in feed intake (Lorschy, 2005; Sanz Fernandez et al., 2015). Activity followed an expected pattern with lower intensity during the night compared to the day. However, the highest intensity was observed when temperature was 35 °C, which might be related to frequent changing of the lying postures from belly to the side due to the pigs' discomfort. Both activity and heat production showed a peak right after entrance to the chambers, probably related to exploration of the new environment, and right after the heat wave, which might be due to compensatory feed intake. Heat production was higher at night, during the day, and when temperature was at 35 °C for CONV pigs compared to AHS pigs. Overall, the CONV pigs also consumed more O_2 and produced more CH_4 and CO_2 during the day. These differences between CONV and AHS pigs might point to a higher metabolic rate in CONV pigs. This could be in line with the study from Matuszewich and Yamamoto (2003) and de Jong et al. (1999), who demonstrated hyperthermia in rats and pigs, respectively, and suggested that this was a reflection of long-lasting effects of chronic stress. An increased standard metabolic rate, determined by O_2 consumption, was also observed in brown trout following a social stressor (Sloman et al., 2000). More research is needed on a potential difference in metabolic rate in pigs in diverging housing conditions, as this would also be relevant to sustainability of pig farming.

Stressors, via their effects on the hypothalamic–pituitary–adrenal axis and sympathetic autonomous nervous system, affect the immune system (Elenkov and Chrousos, 2002). Natural antibodies (NAb) are important as a first line of defence against pathogens due to their poly-specificity (Coutinho et al., 1995), and part of them, natural auto-antibodies, also bind to self-antigens. It has been shown that infection (Luo et al., 2017), but also acute stressors, such as regrouping (Reimert et al., 2014a) and transport (Luo et al., 2017), can change NAb levels in pigs, including those binding KLH, MBP and phosphorylcholine (PC). In our study, levels of several natural (auto)antibodies changed after transport and LPS injection. Unlike a previous study, these changes were generally not depending on housing system, except for IgM binding KLH after the LPS challenge, which increased more in AHS pigs. However, irrespective of time of sampling, AHS pigs showed higher levels of IgM binding PC, a cell wall component which is exposed after cell damage and inflammation, around the transport challenge, and higher KLH-IgM and KLH-IgG levels at both the transport and LPS challenge. Also in another study, enriched housed pigs showed higher levels of antibodies binding KLH (Reimert et al., 2014a), although others found the opposite (Luo et al., 2020a; van Dixhoorn et al., 2016). KLH is a large glycoprotein with different epitopes recognized by heterogeneous NAbs. Increased KLH NAb levels might reflect better resilience, as favourable associations between KLH NAbs and disease resistance and survival have been demonstrated in chickens (Berghof et al., 2019b; Star et al., 2007; Sun et al., 2011; Wondmeneh et al., 2015). Similarly, in pigs, a recent paper reported that pigs reaching market age (a proxy for survival) had higher IgG NAb binding KLH (Chen et al., 2020). However, little is known about the benefits or drawbacks to have high levels of antibodies and the conclusions need to be taken with caution.

It was expected that a better resilience to the challenges would also be reflected in indicators of "wear and tear" on the body over the complete experimental period. Indeed, AHS pigs had lower cortisol accumulation in hairs from 11 to 18 weeks of age, i.e., over the weeks in which they were exposed to challenges, than CONV pigs, reflecting lower accumulative stress. This could partly be due to the chronic stress of suboptimal housing in CONV pigs, as lack of environmental enrichment induces changes in the HPA-axis (de Jong et al., 2000b). CONV pigs also presented larger tear staining areas than AHS pigs when measured around 18 weeks of age. Tear staining, which may reflect chronic stress and therefore a poorer welfare (DeBoer et al., 2015; Telkänranta et al., 2015). Lastly, in spite of the fact that they gained less weight during the transport and heat stress challenge, AHS pigs had a lower variance of their weight development over time. The lower weight gain around the transport challenge might be related to a higher general activity, with more posture changes and aggressive interactions. Regarding the heat stress challenge, the decrease of feed intake, as an adaptive strategy to reduce heat production, might explain the lower weight gain at this period. In other studies, a lower variance of their weight development has been associated with improved resilience (Berghof et al., 2019b). Thus, this overall indicator would be in accordance with all the other ones and might indicate a higher resilience in the AHS pigs. It should be noted, though, that this did not lead to a difference in damage to lungs and stomach as assessed post-mortem.

Contrary to expectations, AHS pigs had a higher number of skin lesions when measured before the wound challenge and before slaughter, which may reflect more aggressive interactions, even though the overall number of skin lesions was low. Increased aggressiveness was also noticed for pigs enriched pre-weaning compared to barren ones at weaning and mixing (Melotti et al., 2011). However, in the same study, the authors found an opposite result when the enrichment was delivered post-weaning. Other studies showed a reduction of aggression post-weaning when piglets received pre-weaning enrichment and early mingling (Ko et al., 2020; Salazar et al., 2018; Verdon et al., 2019). In the current study, piglets were mixed in a bigger group during transport than what they were used to and had to adapt to transportation. This specific context makes the comparison with existing studies difficult.

As expected, boars showed more aggression (Bünger et al., 2015) which is likely related to their concentrations of sexual steroids (Giammanco et al., 2005). The current study also exposed differences between males and females regarding resilience. Boars showed increased levels of glucose, while gilts had higher urea concentrations, reflecting more protein metabolism, in both the transport and

LPS challenge. This indicates that both sexes differ in their metabolic response to stress. Gilts also had higher rectal temperatures during the LPS challenge and presented a slower wound healing. At the end of the experiment, they had less tear staining and lower pneumonia scores. Those differences might reveal different strategies to fight against challenges. Others also found that females and males seem to react differently to stress depending on the nature of the stressor, its duration, the age of the individual and the behavioural or physiological phenotype considered (Fallon et al., 2020; Hodes and Epperson, 2019). More research is needed on sex effects on resilience.

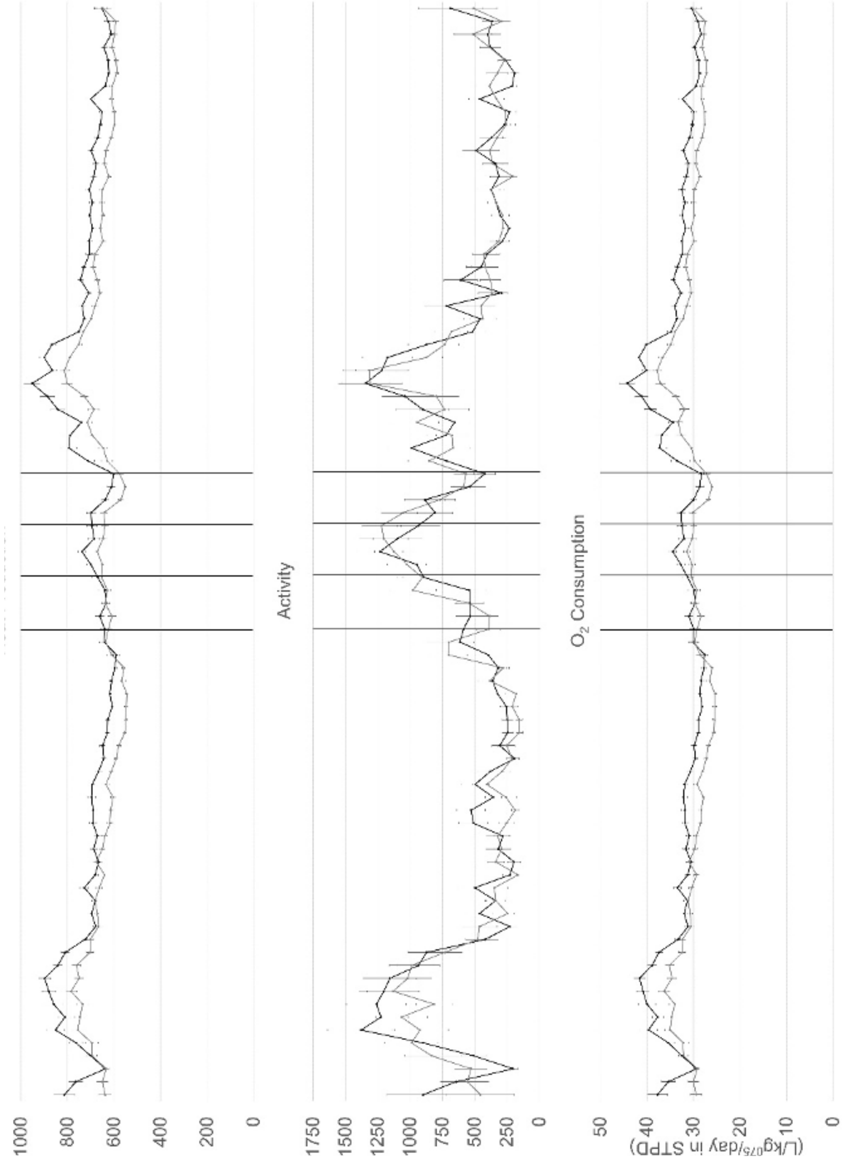
4.5 Conclusion

Pigs raised and kept in an alternative housing system as compared with those in a conventional system showed better physiological recoveries from challenges, as well as less signs of long-term "wear and tear" on the body. Providing social and environmental enrichment that promotes socialization, the expression of species-specific behaviours and, overall, the satisfaction of their essential needs appears to be a promising approach to favour resilience in pigs. Further research is needed to confirm enhanced resilience when welfare is promoted via increased opportunities to show natural behaviour.

4.6 Acknowledgements

The authors would like to thank the farm staff at the Swine Innovation Centre (Sterksel, the Netherlands) and at Carus research facilities (Wageningen, the Netherlands) for daily animal care and the help in the data collection. They would also like to address a special thanks to the technicians and laboratory persons from the Adaptation Physiology group: Joop Arts, Rudie Koopmanschap, Bjorge Laurenssen, Manon van Marwijk and Monique Ooms. They are also grateful for the invaluable help of the students involved in the project: Marith Booijen, Tao Cui, Jori Noordenbos, Marloes van Beurden, Sandra van Iwaarden and Shiv Vasa.

4.7 Supplementary information



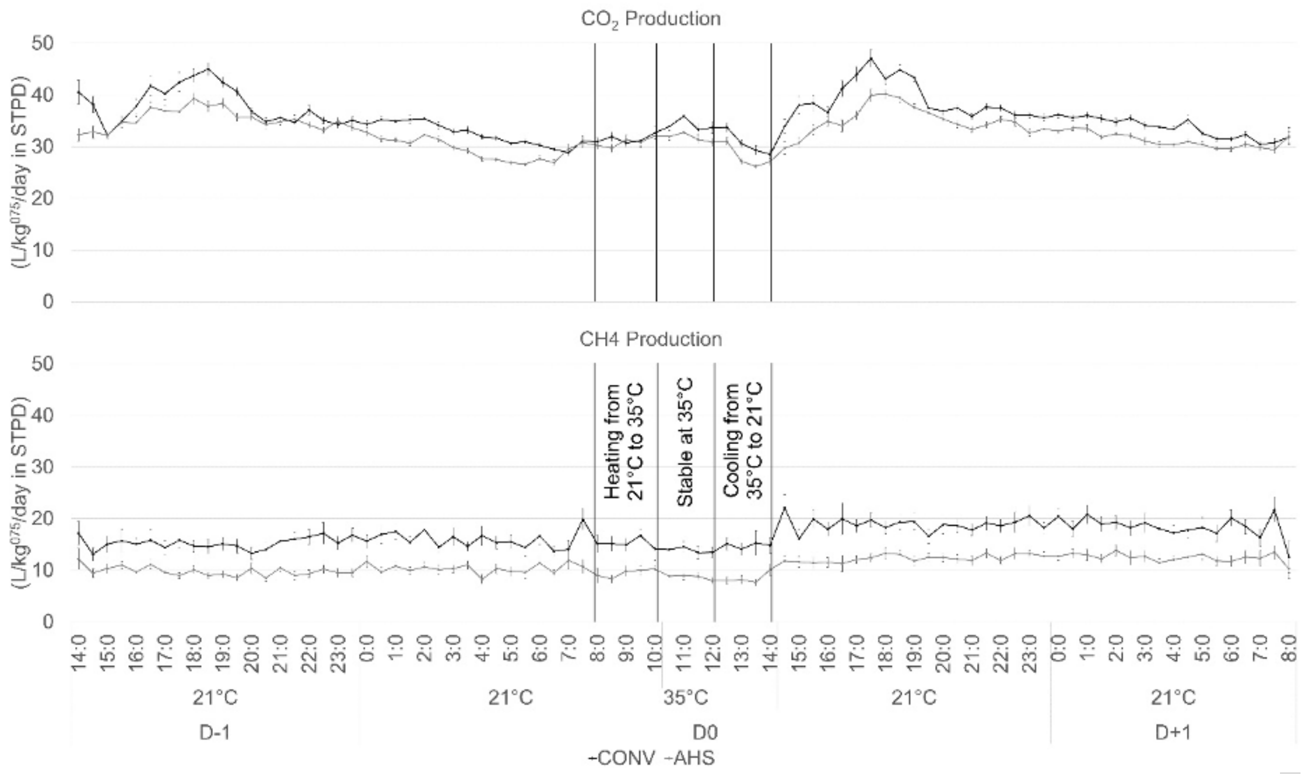


Figure S4.1 – Heat production, O_2 consumption, CO_2 production, respiratory quotient, CH_4 production and activity during the heat stress challenge of pigs housed in an alternative (AHS, grey line) or conventional system (CONV, black line).

Chapter 5

Estimation of resilience parameters following LPS injection based on activity measured with computer vision

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Abstract

Resilience could be referred to as the animal's ability to successfully adapt to a challenge. This is typically displayed by a quick return to initial metabolic or activity levels and behaviours. Pigs have distinct diurnal activity patterns. Deviations from these patterns could potentially be utilized to quantify resilience. However, human observations of activity are labour intensive and not feasible in practice on a large scale. In this study, we show the use of a computer vision tracking algorithm to quantify resilience based on activity patterns following a lipopolysaccharide (LPS) challenge, which induced a sickness response. We followed 121 individual pigs housed in barren or enriched housing systems, as previous work suggests an impact of housing on resilience, for eight days. The enriched housing consisted of delayed weaning in a group farrowing system and extra space compared with the barren pens and environmental enrichment. Enriched housed pigs were more active pre-injection of LPS, especially during peak activity times, than barren housed pigs (49.4 ± 9.9 vs. 39.1 ± 5.0 meter/hour). Four pigs per pen received an LPS injection and two pigs a saline injection. LPS injected animals were more likely to show a dip in activity than controls (86% vs. 17%). Duration and Area Under the Curve (AUC) of the dip were not affected by housing. However, pigs with the same AUC could have a long and shallow dip or a steep and short dip. Therefore the AUC:duration ratio was calculated, and enriched housed pigs had a higher AUC:duration ratio compared to barren housed pigs (9244.1 ± 5429.8 vs 5919.6 ± 4566.1). Enriched housed pigs might therefore have a different strategy to cope with an LPS sickness challenge. However, more research on this strategy and the use of activity to quantify resilience and its relationship with physiological parameters is therefore needed.

5.1 Introduction

Several modern animal welfare definitions incorporate the animal's ability to successfully cope with challenges in the environment (i.e., maintain homeostasis), and to reach a mental state that the animal experiences as positive (Mellor, 2016; Ohl and van der Staay, 2012). Successful adaptation to a challenge result in a relatively swift recovery to baseline parameters, and this could be referred to as resilience (Colditz and Hine, 2016). Resilient animals are able to recover quickly from a challenge. This quick recovery implies a shorter exposure to a challenging situation that may impair welfare.

Pigs have a distinct diurnal activity pattern, where they usually have two activity peaks during the day (one in the morning and one in the afternoon), and are, on commercial farms, approximately 70% of their time relatively inactive (Fraser, 1985; Maselyne et al., 2014). Deviations from this rhythm might be used to determine the level of resilience of the animals (Aubert, 1999; van der Zande et al., 2020). Several studies showed deviations from daily activity patterns in both directions following a challenge. For instance, injection of a hypothalamic stress hormone (Corticotropin-releasing factor or CRF) increases locomotor activity in pigs (Salak-Johnson et al., 2004), whereas sickness often causes a decrease in active behaviour (more lying, less standing) (Escobar et al., 2007; Munsterhjelm et al., 2019; Nordgreen et al., 2018). These deviations from daily activity patterns might be influenced by the housing conditions or the sex of the animal. van Dixhoorn et al. (2016) showed that housing pigs in an enriched environment, with increased space allowance, rooting materials and early-life access to non-littermates, reduced disease susceptibility to co-infection of PRRSV and *A. pleuropneumoniae* (APP), as reflected in a quicker clearance of the PRRSV virus, and a lower probability to develop lung lesions in response to the APP infection that followed. In addition, barren housed pigs showed a stronger decrease in activity than enriched housed pigs the day after infection with *A. pleuropneumoniae*. This suggests a better resilience to a sickness challenge when enrichment is provided.

Quantification of behavioural changes through human observations could be time consuming and subjective, especially when the number of animals to observe increases. Computer vision has the capacity to automatically track individual pigs in a fast and objective manner (Alameer et al., 2020b; Psota et al., 2020a; van der Zande et al., 2021). Based on the continuous location data of each individual pig generated by a computer vision tracking algorithm, the distance moved can be calculated. Distance moved is, in turn, a quantitative measure for activity. A computer vision tracking algorithm is, therefore, able to quantify the diurnal activity rhythm and its corresponding deviations at the level of the individual pig.

Lipopolysaccharide (LPS) endotoxin, a component of the cell wall of gram-negative bacteria, is a well-known agent used in different sickness models. The sickness response includes physiological changes like fever, but also behavioural changes, referred to as 'sickness behaviour', such as lethargy, anorexia and decreased social motivation (Aubert, 1999; Weary et al., 2009). Sickness behaviour involves deviations from the diurnal activity rhythm (Llamas Moya et al., 2008; Munsterhjelm et al., 2019; Nordgreen et al., 2018; Parois et al., 2022a; Veit et al., 2021). An LPS challenge is a suitable sickness model to verify whether a computer vision tracking algorithm is able to detect potential deviations from a diurnal activity rhythm in a controlled and measurable manner. Most studies using an LPS challenge in pigs observe the animals for 24 hours or less (Johnson and von Borell, 1994; Llamas Moya et al., 2006; Parois et al., 2022a). However, Munsterhjelm et al. (2019) observed a change in social behaviour even 40 hours after physiological signs of sickness had dissipated. So even without physiological signs of sickness, the behaviour could still be affected by an LPS injection, even after several days post-challenge. Therefore, this study focused on the assessment of individual and group activity rhythms for eight days around an LPS challenge in barren and enriched housed pigs. We hypothesized to find a clear dip in activity following LPS injection as compared with controls. The aim of this study was to quantify resilience to LPS injection effects using derivatives from location data generated by a computer vision tracking algorithm and to investigate the possible effect of housing conditions and sex on resilience parameters.

5.2 Material and Methods

The protocol of the experiment (AVD1040020186245) was approved by the Animal Care and Use Committee of Wageningen University & Research (Wageningen, The Netherlands) and in accordance with the Dutch law on animal experimentation, which complies with the European Directive 2010/63/EU on the protection of animals used for scientific purposes.

5.2.1 Animals

A total of 144 Topigs-20 x Tempo pigs (n = 71 females; n = 73 males) divided over three batches (n = 48 pigs per batch) were used (same animals as Parois et al., (2022a)). Half of the pigs originated from a conventional farrowing pen (CONV) and the other half from an alternative group housing system (AHS) at the Swine Innovation Centre (Sterksel, The Netherlands). See below for details. None of the pigs were castrated, tail docked or teeth clipped.

5.2.2 Housing systems

5.2.2.1 From birth to nine weeks of age

Piglets were raised in two different housing systems, both at VIC Sterksel (similar to van Nieuwamerongen et al (2015)). The AHS consisted of five farrowing pens of 3.2 × 2.2 m (mix solid and slatted floor), adjacent to a common area of 11.1 × 2.80 m (solid floor), dunging area (2.8 x 3.3m, slatted floor) and a feeding area (4.2 × 3.3 m, solid floor). Four jute bags were provided in the common area as enrichment. One week prior to the expected farrowing date, sows were moved to the AHS system, and a slide of straw was provided in the farrowing pens. Two days before the expected farrowing date, the sows were isolated in a farrowing pen and confined to a crate. They regained access to the full AHS system two days post farrowing. The piglets gained access to the full system one week post farrowing. Heated piglet nests were attached to the farrowing pen (0.7 x 1.6 m), with a temperature of 33-35°C (day 1 till day 7), 29-31°C (day 7 till day 25) and 23-26°C (day 25 till weaning). Piglets were fed in round bowls (until five weeks of age) and from a sensor-controlled automatic feeder (Rondomat, from three weeks of age). Sows were fed in a large trough placed on the floor, where piglets could participate in feeding. Ingestion of solid feed was stimulated with the use of intermittent suckling to facilitate gradual weaning from four weeks of age onwards, i.e., from day 28-34 sows were separated from the piglets from 7:00 h till 17:00 h in a separate area, and from day 35 onwards, sows could voluntarily access both this area and the AHS by stepping over a flexible partition. AHS piglets were weaned at an average of

62.6 ± 1.9 days and a bodyweight of 26.6 ± 4.9 kg. They received a starter diet from 35 days onwards.

In the CONV system, sows and their piglets stayed in the farrowing pens of 2.8 × 1.8 m until weaning. Sows were confined in crates with metal slats within the crate. Outside the crate was a part with a solid floor (1.2 x 0.3 m) with a heating lamp, and the remaining area was plastic slats. Piglets received additional creep feed in the farrowing pens from one week after birth. Piglets were weaned at average 27.4 ± 1.2 days of age and a bodyweight of 8.7 ± 1.3 kg. After weaning, eight piglets were housed with littermates in nursery pens of 3.18 × 1.0 m (0.40 m^2 per piglet) for five additional weeks with a chain and jute bag as enrichment. A commercial weaner diet was provided for ten days after weaning, and a starter diet, similar to that provided to AHS piglets, from 35 days onwards.

Lights were on from 7:00 h to 19:00 h in both systems, giving the sows and piglets a 12- hour light regime with 115 Lux. In addition, the AHS had natural daylight in the system. The transition between day and night light settings was done progressively in 10 min. The ambient temperature was set to 23°C in both systems. Water was available ad libitum in both systems.

5.2.2.2 From nine weeks of age onwards

After weaning of the AHS piglets at nine weeks of age, all piglets were moved from VIC Sterksel to the Carus research facilities in Wageningen, the Netherlands, where they were mixed in groups of six unrelated piglets originating from the same system. Litter, sex and weight were balanced between pens. Four animals per pen (focals, two males and two females) were exposed to experimental challenges, i.e., isolation, transport, LPS, heat, and wound healing, of which isolation and transport took place before the LPS challenge (for further details see Parois et al. (2022a)), while two other pigs, with the most deviating body weight from the pen average, served as controls.

CONV pigs were housed in standard barren pens 1.20 x 4.67 m with conventional space allowance (0.93 m^2 per pig), with a solid and slatted floor without substrate. A ball with chain and a chain with screws were attached to the pen wall as enrichment. AHS pigs were housed in an enriched pen, double the size of a conventional pen, namely 2.40 x 4.67 m (1.87 m^2 per pig). The pen was enriched with deep straw, peat and sawdust bedding, which was replenished regularly (2.5 kg of straw and 30 L of sawdust every day, 22.5 L peat every week). In addition to the bedding, AHS pigs were provided with hay, egg trays or alfalfa once a week and a chain, jute bag or rope (rotation every week), plus one extra toy out of six toys, which was rotated every two days. Enriched and barren pens were placed alternately in the rooms. All pigs had *ad libitum* access to water and to a standard commercial diet for growing pigs from a single pig feeder.

All pens were equipped with an RGB camera (Velleman: 1lux/f2.0) mounted perpendicular (top-down) above the pen. Videos from three barren pens were not used because the full pen was not visible due to a tilted camera. Videos were recorded with a frame rate of 25 FPS for 24 hours per day during the entire experiment. The resolution of the videos was 352 by 288 pixels. The total duration of the recorded video material was approximately 4600 hours.

The light regime was similar to that before nine weeks of age, giving the pigs 115 Lux in the pens during the day (from 7:00 h to 19:00 h; 5000K ultraviolet A at an intensity of 42, 2700K at 60) and 30 Lux during the night (5000K ultraviolet A at an intensity of 3, 2700K at 0). The transition between the day and night rhythm was done progressively for 10 min. No natural daylight was available. The temperature was kept at 23°C for the first two days, then at 22°C for the two subsequent days and at 21°C onwards.

5.2.3 LPS challenge

All focal pigs underwent multiple challenges described in Parois et al. (2022a). This study only uses data around the LPS challenge. Briefly, 92 focal pigs, four pigs per pen (two males and two females), at the age of 104.4 ± 1.7 days (weight: 60.0 ± 7.5 kg) were injected in the ear vein with 2 µg of LPS/kg of body weight (LPS sigma L4391 *Escherichia coli* O111:B4, dose and strain based on (Clouard et al., 2015)). The animals were restrained using a nose-sling and LPS was injected using a catheter. Four animals were not injected with LPS due to health issues, and these were not included in this study. The other 48 control pigs, two pigs per pen (one male and one female), received a sterile saline injection in the same location as the LPS injected pigs. Due to time constraints, the pens were divided into two groups, balanced for the housing system, and injected on two consecutive days. Blood samples were collected at 24h before and 1h, 3h, 5h and 24h after the injection to characterize the physiological response to the challenge (Parois et al., 2022a), which is not reported here. To minimize the effect of human interventions on distance moved, data of the whole pen were removed from 1 minute before until 4 minutes after the start of LPS injection and blood sampling. Videos from 3 days prior to injection starting at 7:00 till 4 days post-injection ending at 18:00 were selected to measure the distance moved (see below).

5.2.4 Computer vision algorithm

The computer vision tracking algorithm for object detection and tracking developed by van der Zande et al. (2021) was used to analyse the recorded videos. In short, this algorithm was trained on 4,000 annotated images originating from the same experiment (but different days) as described above. The You Only Look Once version 3 (YOLOv3) algorithm was used to detect pigs in their home pens (Redmon and Farhadi, 2018). The barren environment had a region of interest (ROI) zone manually defined, since pigs from neighbouring pens were also visible due to the smaller size of the pen (**Figure 5.1**). Detections outside the pre-defined ROI zone were removed. For both environments, the numeric data for the detected bounding boxes was cleaned by removing false positive (FP) detections. FP detections were identified when more than six 'pigs' were detected within the frame. Only six pigs were housed per pen, so the six bounding boxes with the highest detection probability were kept for each processed frame. After the initial removal of extra detections, all bounding boxes with a probability of detecting a pig lower than 0.5 were removed to ensure that all random detections were deleted.

5.2.5 Frame rate

Based on earlier experience (van der Zande et al., 2021), there was inconsistency from the tracking algorithm on how precise the bounding boxes were placed in a frame, even though the pigs themselves were not moving (e.g., sleeping). For calculating distance moved, this would lead to a considerable overestimation of activity. By not using all the 10 FPS of the video, some of the noise could also be deleted. To find the optimal frame rate, a gold standard dataset was annotated using the computer vision annotation tool CVAT (Sekachev et al., 2019). To

Figure 5.1 – Example of the region of interest (ROI) indicated with the red horizontal lines. Detections above the upper red horizontal line and detections below the lower red horizontal line were outside the ROI and therefore removed.

create the gold standard, every fouth frame was manually annotated, and CVAT interpolated the frames in between to minimize the noise an annotator could introduce by manually drawing bounding boxes for every frame. All the frames of in total 12 videos of three minutes each were annotated, balanced for enriched and barren housing, and high and low activity levels where in a low activity video at least five pigs were not moving and high activity had at least five pigs moving. The developed computer vision algorithm then analysed these exact same 12 videos. Distance moved was calculated as mentioned below using different frame rates (10, 5, 1, 0.5, 0.25 and 0.1 FPS) and performance was compared using Pearson correlation.

5.2.6 Distance moved and rolling mean

Distance moved was calculated between the centroids of the bounding boxes, assigned to the same individual between two consecutive frames. The distance (d) between two frames was calculated using Pythagorean theorem using the following formula,

$$d = \sqrt{(x_{\mathrm{t+1}} - x_{\mathrm{t}})^2 + (y_{\mathrm{t+1}} - y_{\mathrm{t}})^2}$$

where d is the distance moved, x_{t} the x-coordinate of frame t, $x_{\mathrm{t+1}}$ the x-coordinate of frame t+1, y_{t} the y-coordinate of frame t, and $y_{\mathrm{t+1}}$ the y-coordinate of frame t+1.

Pigs have a distinct diurnal pattern in their activity (Fraser, 1985). To correct for this diurnal rhythm, the rolling mean of distance moved with a window of 24 hours was used (ten Thij et al., 2020). The data contained missing data, for example, around blood sampling or when pigs were visually obstructed. A maximum of 25% missing values in 24 hours was still considered as a reliable mean.

5.2.7 Resilience parameters

Based on the rolling mean using a 24-hour window, resilience parameters could be quantified (**Figure 5.2**). During the three-day baseline, the mean and maximum error (i.e., maximum deviation observed from the mean) were calculated for each individual. The mean and boundary of the error were applied to post-LPS injection observations and represented their original state, i.e., state before injection. Changes in activity were identified by finding the longest consecutive stretch of data points below the boundary starting not later than 24 hours post-injection of LPS. From this change (i.e., a dip), several resilience parameters could be quantified, namely a binary trait of showing a dip in activity or not, the time from the injection to the start of the dip (referred to as onset of the dip), the recovery time from injection to the end of the dip, and the Area Under the Curve (AUC) of the dip to the boundary (**Figure 5.2**). When no measurable dip was observed, the onset of the dip was set to the maximum value, whereas

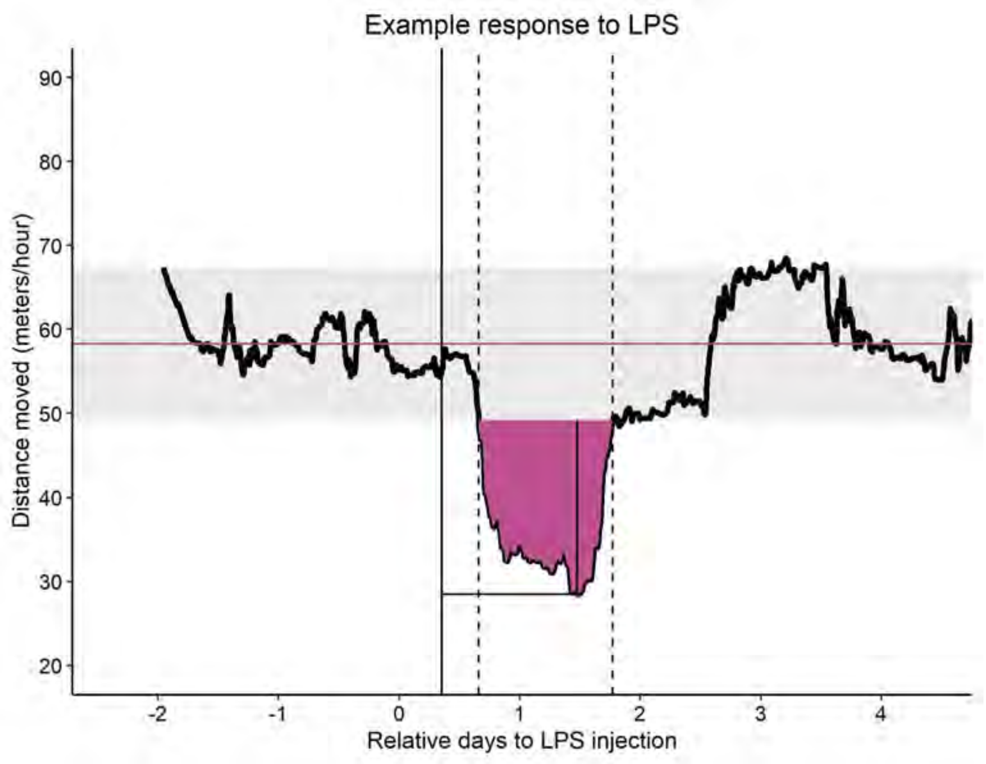


Figure 5.2 – Example of rolling mean of distance moved with 24-hour window of an LPS injected animal. The black points are the rolling means. The purple solid horizontal line is the mean distance moved of the baseline observations before injection. The light grey area around the mean is the maximum error (i.e., maximum deviation observed from the mean) during the baseline observations. The black solid vertical line indicates the LPS or saline injection. The black dashed vertical lines represent the start and end of a dip in activity (i.e., observation below the grey area). The purple filled area represents the Area Under the Curve (AUC) of the dip in activity. The black solid vertical line in the AUC area indicates the maximum decrease and the horizontal black line indicates the time to reach this point.

the other resilience parameters were set to zero (e.g., duration of the dip was zero when no dip was observed). The AUC could be the same for a short deep dip and a long shallow dip, therefore the ratio of AUC and duration of the dip was calculated. The ratio represents the severity per time unit (for pigs with a dip only). In addition, the time to reach the maximum percentual decrease was calculated. The maximum percentual decrease was calculated by:

$$\frac{(lowest\ distance\ moved\ of\ the\ dip - bottom\ of\ baseline\ boundary)}{bottom\ of\ baseline\ boundary}$$

5.2.8 Statistical analysis

Statistical analyses were performed with the software R 4.1.0. (R Core team, 2013). Pearson correlations were calculated between the distance moved according to the gold standard and the tracking algorithm at different frame rates, to assess the optimal frame rate for assessing distance moved. Resilience parameters were analysed using (generalized) linear mixed models with the function 'glmer' and 'lmer' from the R package 'lme4'. All parameters, except the ratio of AUC and maximum percentual decrease, were log-transformed to obtain normality.

The effect of housing, sex and their interaction on average distance moved and maximum error during the baseline was analysed with a linear mixed model. Concerning the response to LPS, first, the effect of the challenge treatment (LPS injected *vs.* control) on the probability to show a dip was analysed using a generalized linear mixed model with binary distribution and logit link function. Due to an unequal distribution of control and LPS injected animals in the probability of showing a dip (much lower in controls), we created a subset of the data with only LPS injected animals. The effect of housing, sex, and their interaction on the resilience parameters (time from the injection to start of the dip, the duration of the dip, the recovery time from injection to the end of the dip on time from the injection to start of the dip, the duration of the dip, the AUC of the dip to the boundary, the ratio of AUC and duration, the time to reach the lowest distance moved of the dip (i.e. time to reach the maximum decrease in activity) and the maximum percentual decrease) were analysed with a linear mixed model using the subset of LPS injected animals only. The time from injection to start of the dip (onset of the dip) and ratio of AUC were analysed for the pigs with a dip in activity only.

Pen and batch were included as random effects in each model. P-values below 0.05 were considered as significant effects, and P-values below 0.1 as tendencies. When a significant effect was found, comparisons between groups were made with the 'emmeans' function from the R package 'emmeans', including a Tukey correction.

5.3 Results

5.3.1 Validation of distance moved

Figure 5.3 shows the correlation between distance moved calculated based on the manually annotated gold standard and based on the tracking algorithm at different FPS rates (dashed line: "Gold standard vs. tracking algorithm"). The correlation between both increased when fewer frames were used to calculate distance moved and seemed to reach a plateau from 0.5 FPS onwards. In other words, potential noise due to micro-movement of the bounding boxes was removed when decreasing the frame rate. To verify whether too much information was lost, the correlation within the gold standard was evaluated (**Figure 5.3**; solid line: "Gold standard (different frame rates)"). We assumed that the gold standard at 10 FPS was the 'true gold standard', since it contained most information. The correlations between the distance moved based on the 'true gold standard' and distance moved using lower frame rates were calculated (e.g., a correlation at 2 FPS is the correlation between distance moved of the gold standard at 10 FPS and of the gold standard at 2 FPS). This correlation dropped significantly after 0.5 FPS, indicating a loss of accuracy in distance moved. The intersection indicates an optimal frame rate, namely 0.5 FPS or 1 frame every 2 seconds, to calculate distance moved. This frame rate was used for further calculations.

According to van der Zande et al. (2021), this computer vision algorithm had a different performance between the two housing systems and overestimation was expected due to micro-movements of the bounding boxes. Not all overestimation was removed by lowering the frame rate to 0.5 FPS. During the low activity, activity in the enriched environment was overestimated 34.1 fold and in the barren environment 21.8 fold, compared to the annotated gold

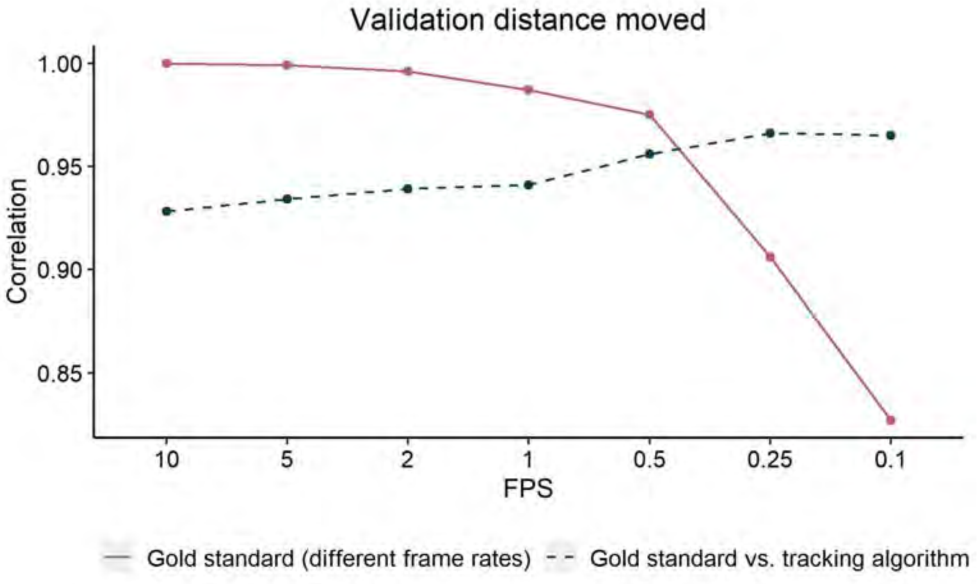


Figure 5.3 – Validation of distance moved using different frame rates. 'Gold standard (different frame rates)' displays the correlation between distance moved of the gold standard at 10 frames per second (FPS) with different frame rates to calculate distance moved in the gold standard (i.e., correlation within the gold standard). 'Gold standard vs. tracking algorithm' displays the correlation between distance moved of the gold standard and the tracking algorithm with corresponding frame rates (i.e., correlation between gold standard and tracking algorithm).

standard using 10 FPS. This overestimation only occurred in the low activity videos and not in the high activity videos. During the high activity, the overestimation was 1.05 fold in the enriched environment and 1.06 fold in the barren environment.

5.3.2 Baseline differences

Figure 5.4 shows the distribution of average distance moved during a 24-hour period pre-injection for both housing conditions. The mean distance moved during this baseline period was higher in the enriched housing system ($P < 0.001$) and tended to be affected by the Sex x Housing interaction ($P < 0.1$).

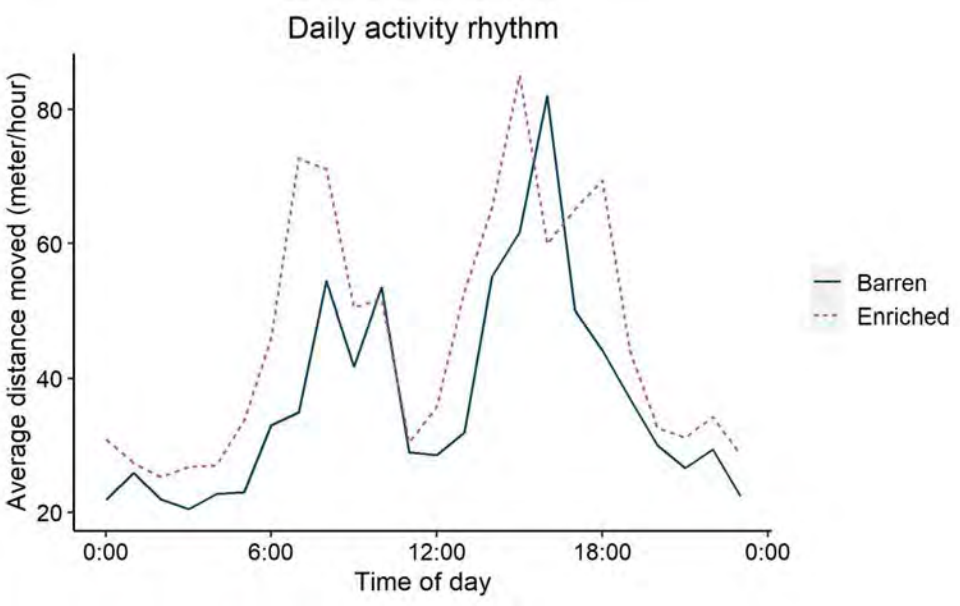


Figure 5.4 – Average daily activity rhythm per hour during one day grouped by housing system. The solid blue line represents the barren housing system and the purple dashed line the enriched housing system.

Post hoc pairwise comparison of this tendency showed that the difference between sexes was only present within the enriched housing system ($P < 0.05$), where boars showed a higher distance moved than gilts, but not within the barren housing system (**Figure 5.5A**). The maximum error during the baseline was affected by the housing system ($P < 0.05$) and sex ($P < 0.01$). Enriched pigs had a larger maximum error during the baseline than barren pigs, and boars larger than gilts. The interaction between housing and sex on maximum error was not significant (**Figure 5.5B**).

5.3.3 Response to LPS

As illustrated in **Figure 5.6** and in the Supplementary material, LPS injected and control pigs showed different activity patterns following LPS or saline injection. The probability of showing a dip in activity was significantly higher when

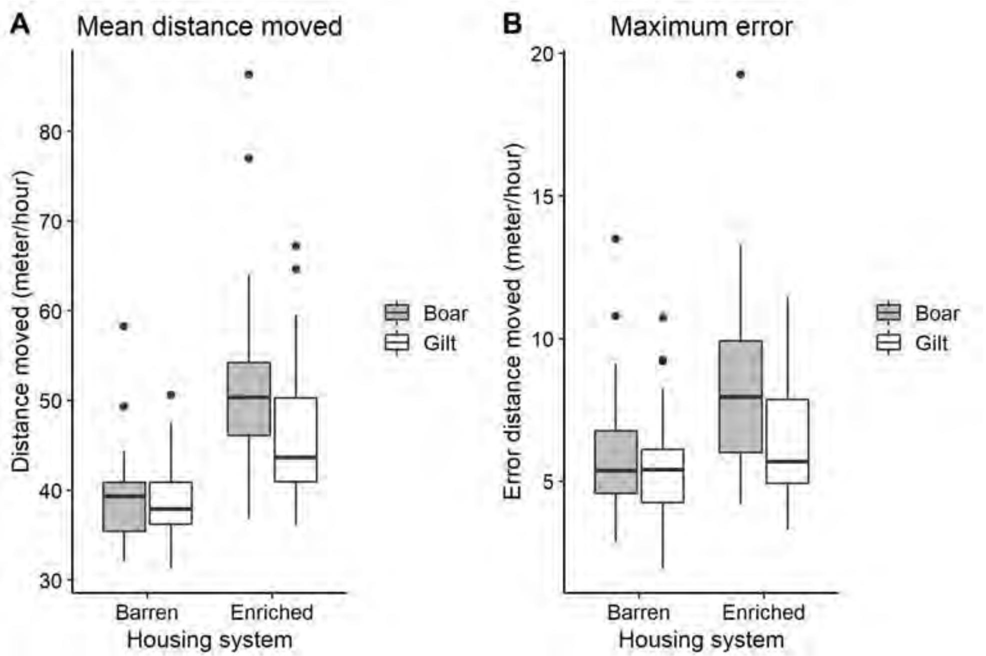


Figure 5.5 – A: Boxplot of the mean distance moved, based on the rolling window, during the baseline grouped by housing system and sex. B: Boxplot of the maximum error of the mean distance moved (i.e. maximum deviation observed from the mean), based on the rolling window, during the baseline grouped by housing system and sex.

injected with LPS compared to a saline injection ($P < 0.001$): 69 out of 80 pigs injected with LPS (86%) versus 7 out of 41 (17%) control animals showed this dip. Further characteristics on the dip will be presented on only LPS injected animals due to the imbalanced presence of the dip in activity between LPS injected animals and control.

The onset of the dip (8.6 ± 4.8 h, calculated based on LPS injected animals with a dip in activity), duration of the dip (30.1 ± 24.4 h), recovery time (37.5 ± 25.5 h), and AUC (267,394 ± 295,623 days*m/h) were not affected by housing or sex (**Figures 5.7A, 5.7B, 5.7C, 5.7D**). The AUC could be the same for a short deep dip and a long shallow dip, so the ratio of the AUC and the duration was calculated. This ratio reflects the severity of the dip per time unit (only for the LPS injected pigs showing a dip in activity). The ratio of AUC and duration was

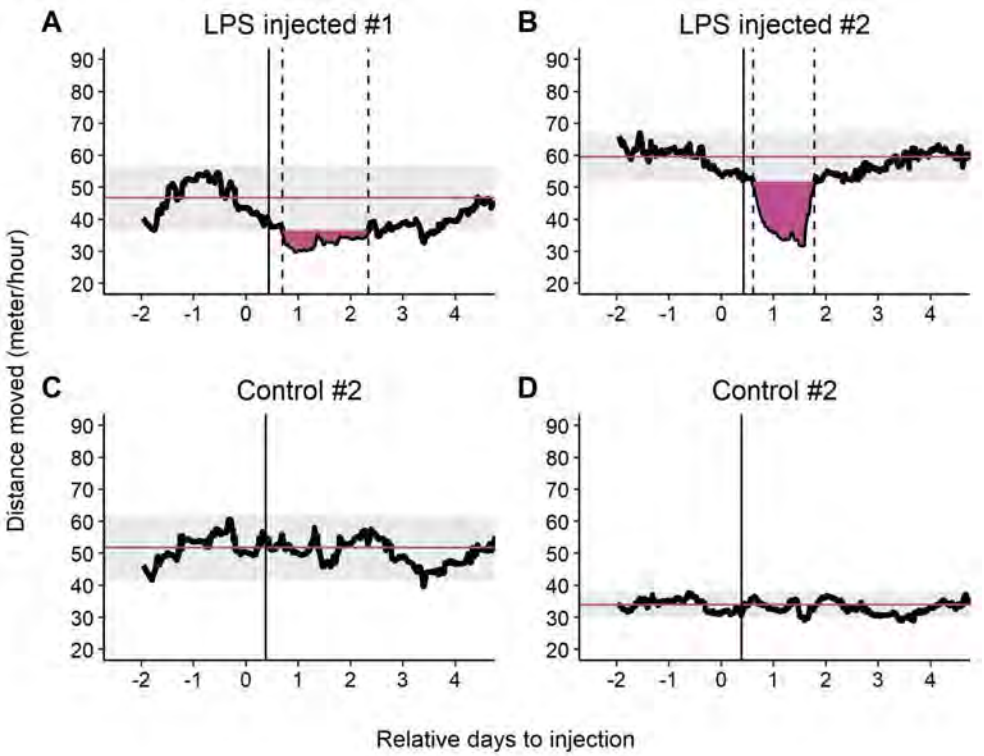
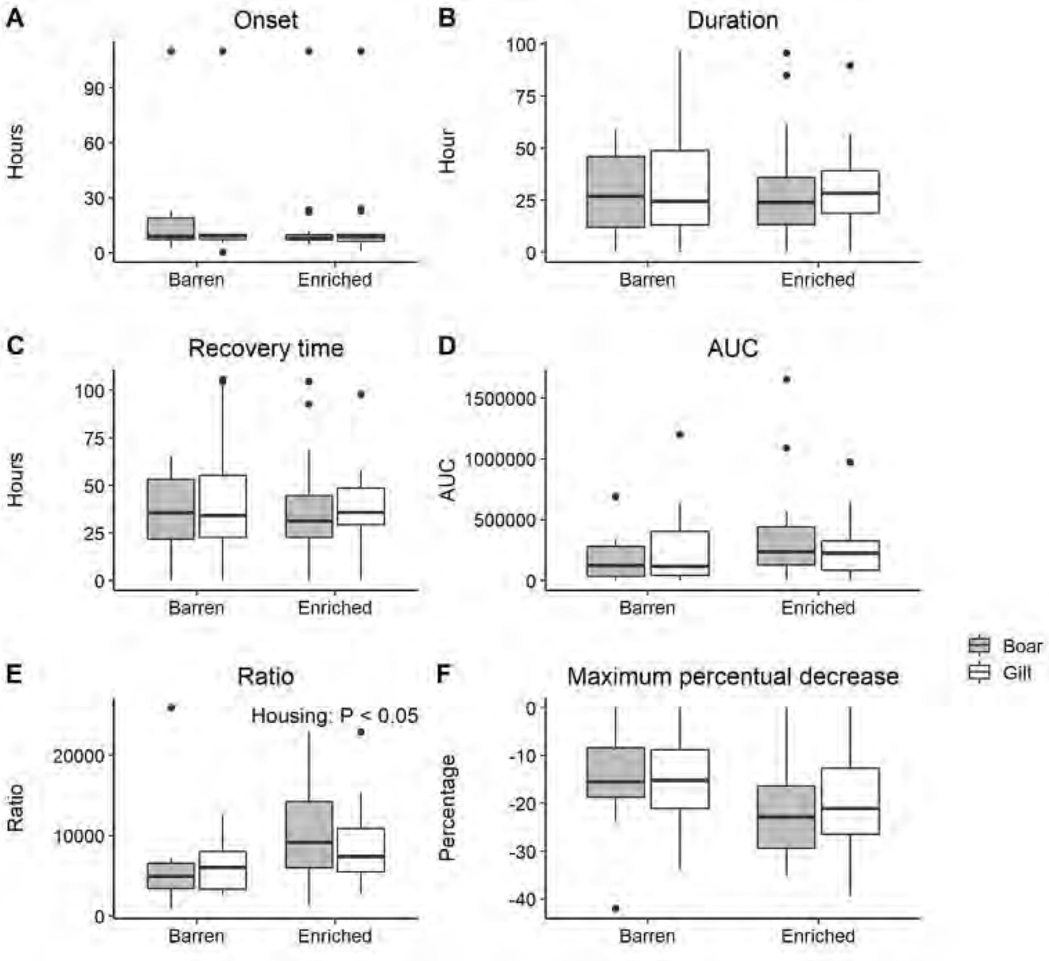


Figure 5.6 – Example of rolling mean of distance moved with 24-hour window of LPS injected animals (A&B) and control animals injected with saline (C&D). The black points are the rolling means. The purple solid horizontal line is the mean of the baseline observations before injection. The light grey area around the mean is the maximum error (i.e., maximum deviation observed from the mean) during the baseline observations. The black solid vertical line indicates the LPS or saline injection. The black dashed vertical lines represent the start and end of a dip in activity (i.e., observation below the grey area). When the black dashed vertical lines are absent indicates no dip in activity was observed. The purple filled area represents the Area Under the Curve (AUC) of the dip in activity.


A
Onset
Hours
0
30
60
90
Barren
Enriched
B
Duration
Hour
0
25
50
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50
75
100
Barren
Enriched
D
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AUC
0
500000
1000000
1500000
Barren
Enriched
E
Ratio
Housing: P < 0.05
Ratio
0
10000
20000
Barren
Enriched
F
Maximum percentual decrease
Percentage
0
-10
-20
-30
-40
Barren
Enriched
Boar
Gilt

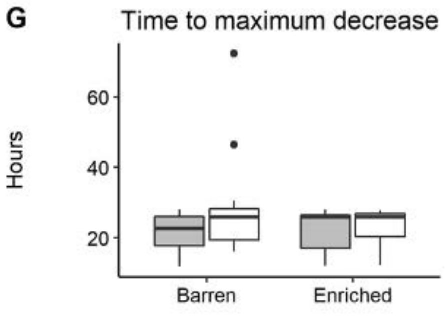


Figure 5.7 – Boxplots of resilience parameters following a lipopolysaccharide (LPS) challenge grouped by housing and sex. Only animals with an LPS injection were used in these boxplots. A: Boxplot of the time from injection to the onset of the dip in activity. B: Boxplot of the duration of the dip in activity. C: Boxplot of the time from injection to the end of the dip in activity (i.e., recovery time). D: Boxplot of the Area Under the Curve (AUC) of the dip in activity. E: Boxplot of the ratio of AUC and duration of the dip in activity. F: Boxplot of maximum percentual decrease of the dip in activity. G: Boxplot of the time to reach the maximum percentual decrease of the dip in activity.

affected by housing ($P < 0.05$), where enriched housed pigs (9,740 ± 5,485) had a higher ratio than barren housed pigs (6,184 ± 4,613) (**Figure 5.7E**).

The maximum percentual decrease (i.e., maximum deviation of the dip in activity, expressed as a percentage from the lower boundary) was not affected by housing or sex within the LPS injected animals (-17.9 ± 10.9 %) (**Figure 5.7F**). The time to reach this maximum decrease was not affected by housing, but tended to be affected by sex ($P < 0.1$). Within the LPS injected animals, gilts tended to take longer to reach the maximum deviation (25.5 ± 10.4 h) compared to boars (22.1 ± 5.3 h) (**Figure 5.7G**).

5.4 Discussion

The aim of this study was to quantify recovery of normal activity patterns as an indicator of resilience to an LPS sickness challenge, using location data from a computer vision tracking algorithm, and to study the impact of housing and sex on the recovery of these patterns.

5.4.1 Validation of distance moved

We transformed location data to distance moved using a reduced frame rate of 0.5 FPS to remove potential noise due to micro-movements of the bounding boxes. However, a reduced frame rate did not remove all the noise. The tracking algorithm showed an overestimation in low activity videos of up to 34.1 fold in the enriched environment, and 21.8 fold in the barren environment. This overestimation was only observed when there was no visible movement of the pigs. This overestimation is mainly caused by the inaccuracy of the tracking algorithm. The difference in overestimation between the housing systems could be due to the bedding material in the enriched housing system, where animals could be (partly) hidden by the bedding material, making them less visible and harder to follow from frame to frame for the tracking algorithm.

Pigs that moved less than five pixels in the annotated gold standard, moved on average 74.5 pixels (approximately 1 meter) according to the tracking algorithm. The videos used for the gold standard were 3 minutes each, so in other words, about 20 meters of noise per hour was generated during inactivity when using the tracking algorithm. Lind et al., (2005) also reported overestimation in non-moving pigs. They compared the distance moved of non-moving pigs to a stationary object of the same size, and the estimated distance travelled of the pig was twice as high compared to the stationary object. This suggests that the bounding box recorded entire body movements without the animal actually moving around. To only measure the distance moved without body movement while standing still, we recommend a variable frame rate with 0.5 FPS as a basis, where the distance between frame t and t+1 needs to exceed a threshold for the frame to be used. When there is no activity, the threshold will not be met, and frame t+1 could be skipped so no noise is added to the dataset. Effectively, only frames with activity will be used to calculate distance moved, and the overestimation could be removed using this method.

5.4.2 Baseline activity

This unique dataset showed higher baseline activity (activity pre-challenge) and baseline variation in activity in pigs kept in an enriched environment compared to a barren environment. In the literature, it has been found that pigs housed on straw bedding show higher activity (Day et al., 2008; Luo et al., 2020b). Previously we mentioned an overestimation of distance moved by the tracking algorithm during inactive periods only. Therefore, during inactive periods we could not differentiate between actual movement or noise. During active periods, the tracking algorithm did not overestimate the distance moved. So in these periods, enriched pens were indeed more active than barren pens (**Figure 5.4**). During active periods, this (true) difference in activity between pigs in enriched and housing could be explained by the availability of bedding leading to increased exploration (Bolhuis et al., 2005) and more space allowance per animal (Day et al., 2008).

Boars tended to move more during the baseline period within the enriched housing system. A previous study showed that entire males of approximately the same age, housed in either single-sex or mixed-sex groups, showed more aggression and mounting than gilts (Holinger et al., 2015), which are highly active behaviours. The tracking algorithm only estimated the location of individual pigs, and not the behaviours performed. So future work would be needed to establish if this higher activity in male pigs is a result from aggression or related to other behaviours.

5.4.3 Response to LPS

From previous literature, we know that LPS induces symptoms such as fever, anorexia, somnolescence and a reduction in general activity, often referred to as a sickness response, of which the behavioural symptoms reflect sickness behaviour (Johnson and von Borell, 1994). Indeed, almost all LPS injected animals (86%) showed decreased activity post-injection, whereas only a minor part of the saline-injected control pigs did (17%). It is unknown why some control pigs showed a dip in activity too. Possibly, these animals were affected by the inactivity of their LPS injected pen mates. Pigs tend to synchronize their behavioural activities, which is at least partially driven by social facilitation of behaviour (Docking et al., 2008). It could be hypothesized that, since four of their pen mates were inactive due to sickness, some control pigs adjusted their behaviour to the majority of the group. Additionally, control pigs may also have been influenced by their pen mates' negative affective states, i.e., emotional contagion (Reimert et al., 2015, 2013). It has been shown that following a negative treatment of one pig, this pig, but also all of its pen mates that had not been exposed to this negative event, showed more lying behaviour (Reimert et al., 2017).

This study showed different parameters that could reflect sickness behaviour, namely the time between injection and onset of the dip in activity, the duration of the dip, the AUC of the dip, the ratio of the AUC and duration of the dip, the maximum percentual decrease in the dip and the time to reach this maximum decrease. The computer vision tracking algorithm is thus able to quantify a sickness response.

Physiological responses to LPS show varying dynamics. In the pigs from this study, the cortisol response peaked at 3 hours post-injection, and this was still not back to basal level at 5 hours post-LPS injection, whereas at the next sampling point, 24 hours post-injection, basal levels were seen again (Parois et al., 2022a). This pattern of recovery in cortisol is in line with Sali et al. (2021). Using a similar dose of LPS, others showed a rise in cortisol levels for at least seven hours post-injection (de Groot et al., 2007). As in other studies (Clouard et al., 2015; de Groot et al., 2007; Luo et al., 2022), pigs from this study had increased body temperatures until at least 5 hours post-injection and had returned to basal levels 24 hours post-injection with no additional measurements between five- and 24-hours post-injection (Parois et al., 2022a). Clouard et al. (2015), however, showed a return to basal body temperature at nine hours post-injection. Other studies found a more prolonged lasting physiological response to LPS. Recently Nordgreen et al. (2018) found a decrease in noradrenaline and an increase in serotonin in different parts of the brain still 72 hours after LPS injection. Veit et al. (2021) showed that LPS injected pigs had lower concentrations of tryptophan (the precursor of serotonin) in their plasma 72 hours after injection. Our results show that changes in behaviour may last for days.

This study showed that pigs injected with LPS started to decrease their activity below basal level 8.6 ± 4.8 hours post-injection and that this dip lasted on average for 30.1 ± 24.4 hours. The peak response in sickness behaviour, with a decrease of 17.9 ± 10.9 % in activity, was 25.5 ± 10.4 hours post-LPS injection. The onset of this dip in activity is later compared with Llamas Moya et al. (2008), demonstrating a significant reduction in activity levels from one-hour post-injection onwards, which lasted for three hours. However, it should be noted that this previous study used a higher dose of LPS and intraperitoneal injection instead of intravenously. Studies using a similar dose injected intravenously showed an increased latency to approach a human, which lasted for at least six hours post-injection (de Groot et al., 2007), and in one study, the latency to approach peaked at three hours and ended about nine hours post-injection (Clouard et al., 2015). In addition, two studies observing active behaviours for six hours (Veit et al., 2021) and 72 hours (Nordgreen et al., 2018), reported decreased activity between 3-5h and 3-4h, respectively, after LPS injection. Thus, these studies demonstrate a change in behaviour or behavioural motivation that seems to start and end earlier than the dip in activity in this study. Munsterhjelm et al. (2019) reported a dip in social and damaging behaviour, like tail and ear biting, on the day of injection, and a tendency for lower activity, but did not specify during which hours of the day this was. Intriguingly, in the latter study, social and damaging behaviours reached levels above baseline from two days post-injection. This study observed sickness behaviour later and for a longer period of time. The method to measure activity differs between our study and that of Munsterhjelm et al. (2019). We used tracking data and applied a rolling mean with a 24-hour window to measure activity, whereas the previously mentioned study used scan sampling to observe the activity. A rolling mean with a large window causes a delay in observing an effect, which might explain the difference in observing sickness behaviour. In the simulation showed in **Figure 5.8**, the delay to the onset of the dip is five hours, and to recovery is 19 hours compared to raw data. Without the use of a rolling window, the onset of the dip in activity and time to recovery become closer to values reported in other studies.

5.4.4 Sex and housing effects on recovery in activity following LPS injection

The only sex effect on recovery in activity found in this study was on the time to the maximum decrease in activity during the dip, where gilts tended to take longer to reach this point compared to boars. Most studies reported results on sickness behaviour on gilts only (Clouard et al., 2015; de Groot et al., 2007; Munsterhjelm et al., 2019; Nordgreen et al., 2018; Veit et al., 2021) or did not report sex effects (Clouard et al., 2015).

It has been shown that housing conditions that better meet their behavioural needs and that have the potential to improve welfare, i.e., enriched environments, may improve the resilience of pigs (Luo et al., 2020a; van Dixhoorn et al., 2016). Concerning the pigs used in this study, Parois et al. (2022a) showed that pigs housed in the enriched environment recovered faster and/or had a lower response to a two-hour transport challenge than barren housed animals in several metabolic indicators (cortisol, glucose and non-esterified fatty acids). Enriched housed animals also showed a less pronounced response and/or faster recovery in cortisol and non-esterified fatty acids.

Regarding sickness behaviour, no effects of housing were found on the duration of the dip, its Area Under the Curve (AUC) or the time to recover to normal activity levels. This seems not in line with the reported effects of housing on the physiological responses in the same pigs (Parois et al., 2022a). However, housing did have an effect on the ratio between the AUC and the duration of the dip. This ratio between AUC and duration reflects the severity of the dip in activity per time unit. Enriched housed animals had a higher ratio than barren housed animals. This coincides with a stronger fever response, i.e., a higher peak but similar AUC, for the enriched housed animals in this study, as reported by Parois

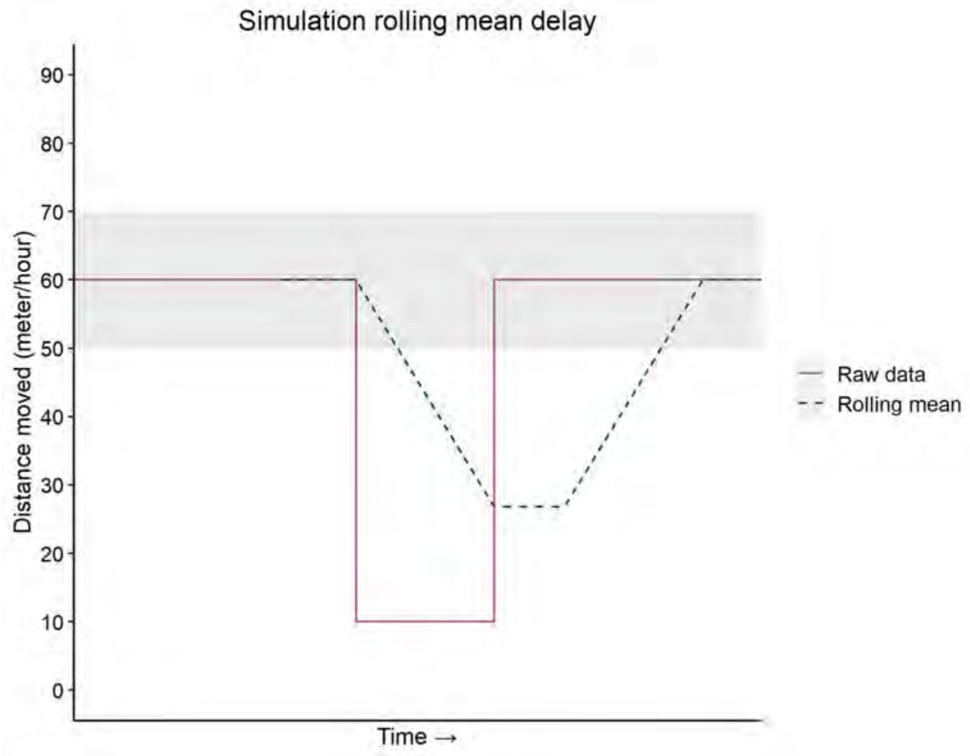


Figure 5.8 – Simulation of a possible delay in detection of a dip in activity using a rolling mean with a large window. The solid purple line indicates raw distance moved, assuming an immediate response to inactivity after LPS injection. The dashed blue line represents the rolling mean with a large window of the raw data. The light grey area represents the baseline area. Below this boundary is defined as a dip in activity. The dashed line crossed down the boundary five hours later than the raw data and crossed up 19 hours later than the raw data.

et al. (2022a). However, housing did not affect the maximum percentual decrease, which reflects a higher response, or the other parameters, so the effect on the AUC:duration ratio should be interpreted with caution. More research is needed to investigate the relationship between a stronger fever reaction and decrease in activity.

This study showed a clear difference in the presence of a dip in activity following an LPS injection or a saline injection. The individual tracking algorithm could thus be used to measure resilience in pigs, since the dip and its corresponding characteristics could be estimated using the novel technology. Nevertheless, the strong indication of housing effects on response to the LPS challenge as found by Parois et al. (2022a) was not established in this study so far. As data were generated from the same animals, it is a strong indication that the sickness behaviour response represents a different aspect than the physiological responses measured. More research on the use of activity to quantify resilience and its relation to physiological parameters is needed.

5.5 Conclusion

In conclusion, it was shown that distance moved calculated from a computer vision tracking algorithm captures the change in activity following an LPS sickness challenge and the recovery thereof, which could indicate resilience to this challenge. Differences in characteristics of the sickness response were identified between LPS injected animals and control animals. Enrichment did not affect the duration or magnitude of the activity change, but showed an increased ratio between the magnitude and duration, indicating a higher intensity of the dip in activity which could reflect a different strategy to cope with the sickness challenge. More research is needed to validate activity as a proxy for resilience to LPS.

5.6 Acknowledgements

The authors would like to thank Manon van Marwijk, Bjorge Laurenssen, Monique Ooms, Ilona van den Anker, Sandra van Iwaarden, and the caretakers of Carus, especially Arjan van Dolderen, for the help during experimental work. In addition, we would like to thank Egbert van Nes for his valuable input for this study.

5.7 Supplementary figures

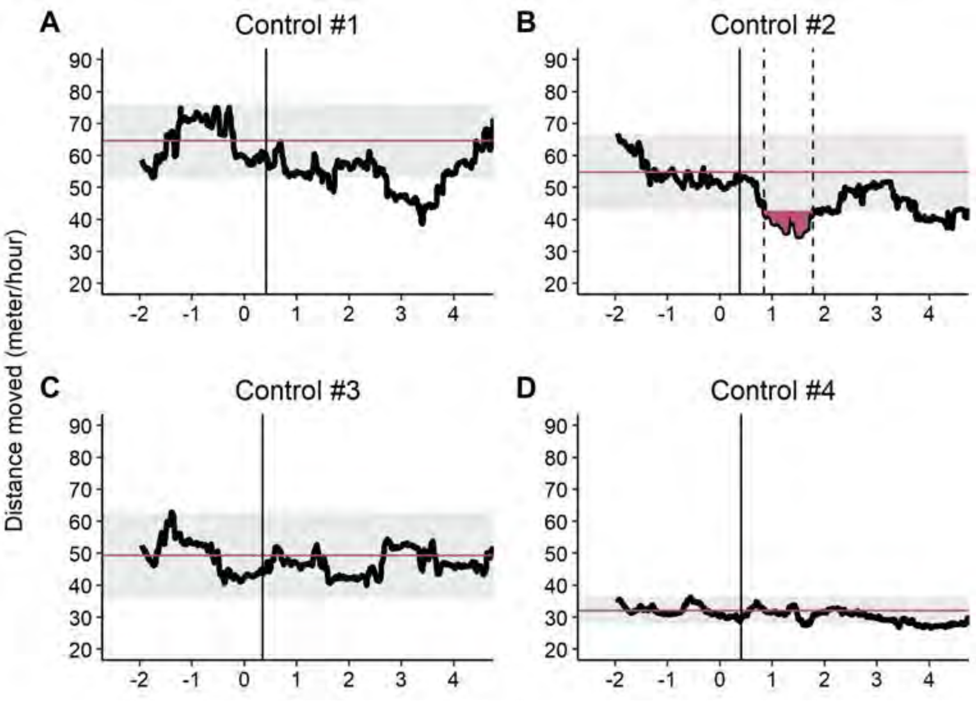
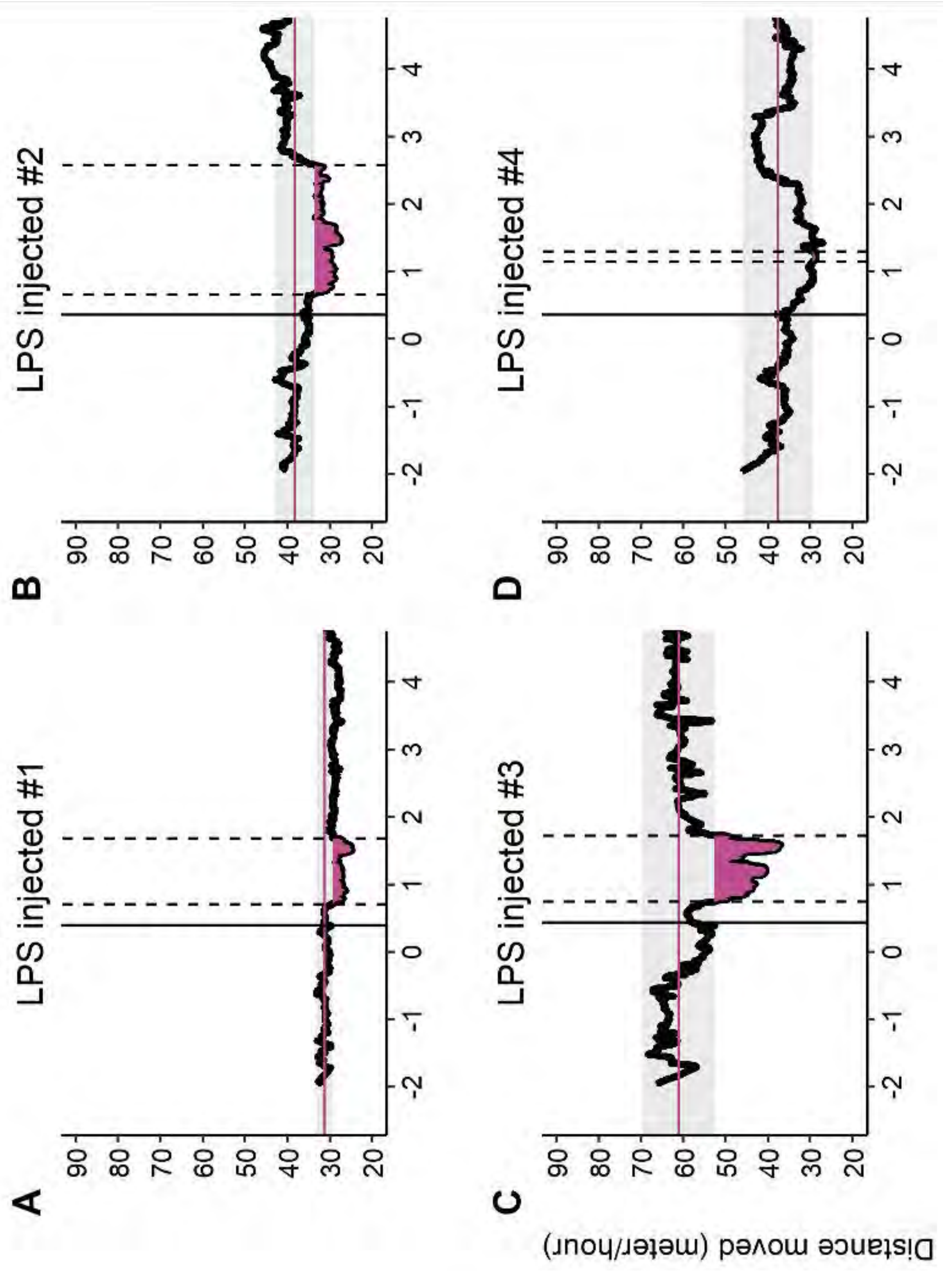


Figure S5.1 – Examples of rolling mean of distance moved with 24-hour window of control animals injected with saline. The black points are the rolling means. The purple solid horizontal line is the mean of the baseline observations before injection. The light grey area around the mean is the maximum error (i.e., maximum deviation observed from the mean) during the baseline observations. The black solid vertical line indicates the LPS or saline injection. The black dashed vertical lines represent the start and end of a dip in activity (i.e., observation below the grey area). When the black dashed vertical lines are absent, this indicates no dip in activity was observed. The purple filled area represents the Area Under the Curve (AUC) of the dip in activity. A: A dip in activity resulting from an LPS challenge was not allowed to start later than 24 hours post injection. The dip observed in panel A started later than 24 hours and is therefore not considered as a dip in activity resulting from injection. B: Example of a control animal with a dip in activity. C: Example of a control animal with a high baseline mean and error. D: Example of a control animal with a low baseline mean and error.


A
LPS injected #1
B
LPS injected #2
C
LPS injected #3
D
LPS injected #4
Distance moved (meter/hour)
90
80
70
60
50
40
30
20
-2
-1
0
1
2
3
4

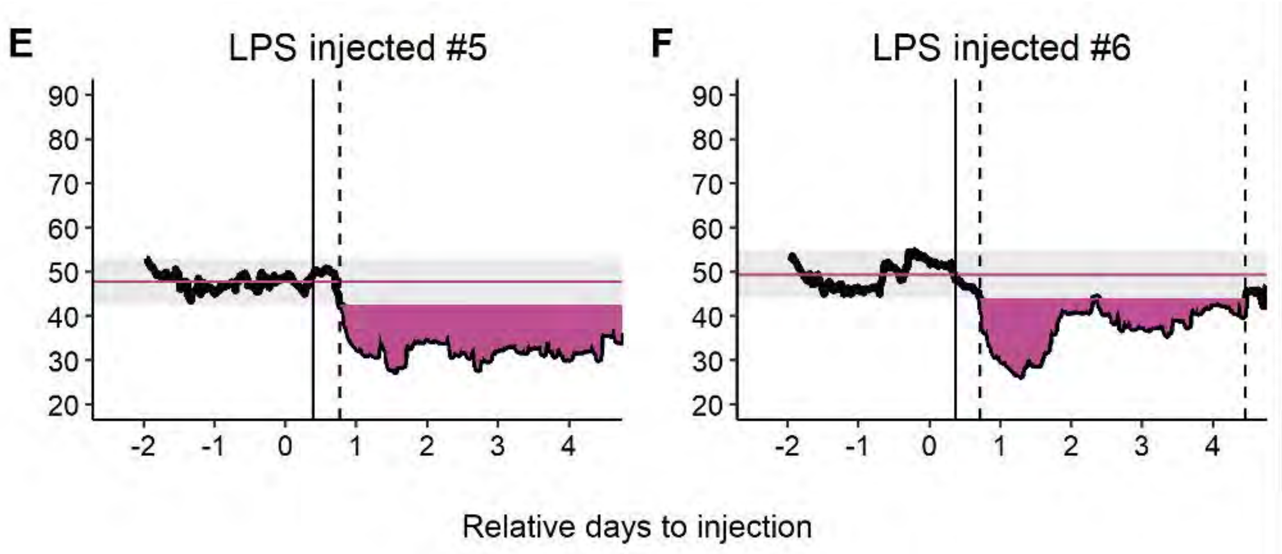


Figure S5.2 – Examples of rolling mean of distance moved with 24-hour window LPS injected animals. The black points are the rolling means. The purple solid horizontal line is the mean of the baseline observations before injection. The light grey area around the mean is the maximum error (i.e., maximum deviation observed from the mean) during the baseline observations. The black solid vertical line indicates the LPS or saline injection. The black dashed vertical lines represent the start and end of a dip in activity (i.e., observation below the grey area). When the black dashed vertical lines are absent indicates no dip in activity was observed. The purple filled area represents the Area Under the Curve (AUC) of the dip in activity. A: Example of a low baseline mean and error and a shallow dip in activity. B: Example of a medium baseline mean and error, with a long and shallow dip in activity. C: Example of a strong decrease in activity. D: Example of a very short dip in activity. E: Example of an individual that did not recover to baseline activity within the dataset. F: Example of a long recovery time.

Chapter 6

Adding activity monitoring to physiological parameters to assess resilience to an LPS challenge in pigs: An explorative study

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Abstract

Pigs respond physiologically and by changes in behaviour to restore homeostasis after an infection. However, it is unknown whether the behavioural response is related to physiological changes, and could be used as an alternative to characterize the sickness response. We explored this relationship by assessing physiological and activity parameters following LPS injection, a known sickness model in pigs, housed either under conventional conditions (CONV) or in an alternative system with group farrowing, delayed weaning and enrichment (AHS). Surprisingly, activity during the three baseline days before the LPS challenge affected the Area Under the Curve (AUC), which represents both the strength and duration of a response, of several physiological parameters. AUC of glucose and haptoglobin were lower in more active individuals, and, particularly in CONV pigs the AUC of NEFA was higher and urea AUC was lower with increasing baseline activity. Also the deviation in baseline activity affected the AUC of several physiological variables in a housing dependent manner. Possibly, more active animals have an inherently different metabolic state that affects their sickness response. In addition, a higher AUC of cortisol was associated with a larger AUC of the dip in activity post-injection, a stronger percentual decrease. Also a stronger glucose depletion (smaller AUC) was associated with a longer duration and larger AUC of the activity dip, and a higher percentual decrease. Additionally, a factor analysis was conducted on AUC, baseline and peak levels of the physiological variables, and factor scores were related to activity variable. Scores of Factor 1, on which baseline cortisol and haptoglobin and +24h haptoglobin loaded strongly, were negatively correlated with AUC and duration of the dip. Taken together, our explorative study reveals a limited number of weak to moderate correlations between characteristics of the change in activity and the physiological variables measured following LPS injection. This implies that non-invasive behavioural observations capture another aspect of the sickness response than physiological parameters.

6.1 Introduction

During their lifetime, pigs are exposed to several challenges, among which infectious diseases, which are hard to avoid even with high bio-security levels and hygiene protocols. For both the benefit of the farmer and the pigs, it is desired that the animals are resilient to such challenges. Resilience can be referred to as the capacity of an animal to be minimally affected by a disturbance and to rapidly return to its normal state (Colditz and Hine, 2016). Poor resilience is characterized by a slow recovery after exposure to a challenge (Scheffer et al., 2018). Pigs respond physiologically and by changes in behaviour to restore homeostasis (Parois et al., 2022a; van der Zande et al., 2022). These responses might reflect the recovery from a perturbation.

Lipopolysaccharide (LPS) endotoxin, a component of the cell wall of gram-negative bacteria, is a well-known agent used in various sickness models. LPS injection induces a typical sickness response, i.e., a suite of immunological, metabolic and behavioural changes, among which fever, lethargy and anorexia (Aubert, 1999). Pigs injected with LPS showed elevated cortisol levels for several hours post injection compared to saline-injected pigs (Luo et al., 2022; Nordgreen et al., 2018), a decrease in blood glucose concentrations (Luo et al., 2022; Parois et al., 2022a) and an increase in blood lactate (Luo et al., 2022) and Non-Esterified Fatty Acids (NEFA) concentrations (Parois et al., 2022a). Recent studies indicate that housing and management conditions of pigs can affect their resilience to sickness as induced by LPS injection (Luo et al., 2022; Parois et al., 2022a) or by infection (van Dixhoorn et al., 2016), which was revealed by physiological responses. Physiological parameters are, however, labour intensive to collect, process and analyse. In addition, handling and blood sampling are invasive procedures which may include restraining of the animals or their removal from the home pen, and cause stress to the animals. Behavioural responses could be a potential alternative to characterize the sickness response in pigs, which would be valuable both for assessing the impact of environmental conditions on resilience, as well as for detecting sickness in pig husbandry practice. The sickness response includes behavioural changes, collectively referred to as 'sickness behaviour', among which decreased activity post-injection (van der Zande et al., 2022). This behavioural sickness response is a strategy intended to support the metabolic and physiological changes and help to fight the pathogen (Aubert, 1999). Wilson et al. (2016) observed the recovery of pigs admitted to a hospital pen by recording the time spent in different postures. They suggested that assessment of changes in behaviour is a good method to measure recovery.

With human observers, collecting behavioural responses related to a challenge is time consuming. Computer vision has the potential to obtain behavioural data with less human effort (Gentz et al., 2020). We recently developed a computer vision algorithm to track the activity of pigs at the individual level and used this to characterize the changes in activity following LPS injection (van der Zande et al., 2022). However, it is unknown whether this behavioural response in a sickness model is related to physiological changes, and could be used as an alternative to characterize the sickness response, potentially replacing physiological measurements. Therefore, this study explores the relation between physiological and behavioural responses to an LPS-induced sickness response, using data collected in Parois et al. (2022a) (blood cortisol, glucose, NEFA, urea, haptoglobin and rectal temperature) and van der Zande et al. (2022) (activity). We hypothesized that the activity changes in pigs following LPS injection would reflect the physiological changes detected in these animals. In addition, we studied the relation between baseline activity levels and the physiological response to LPS.

6.2 Material and Methods

Established principles of laboratory animal use and care and the Dutch law on animal experiments were followed. These comply with the European Directive 2010/63/EU on the protection of animals used for scientific purposes. The Animal Care and Use Committee of Wageningen University approved the experiment (AVD1040020186245).

6.2.1 Animals

The same animals were used as described in Parois et al. (2022a) and van der Zande et al. (2022).To summarize, a total of 144 Tempo × Topigs-20 pigs (n = 71 females; n = 73 males) were used during the experiment, spread over three batches (n = 48 pigs per batch). During lactation, half of the sows and their piglets were housed in a conventional farrowing pen (CONV) (mean ± SD; sow parity = 4.2 ± 1.8) and the other half in an alternative group housing system (AHS) (parity = 4.0 ± 1.7) at the Swine Innovation Centre (Sterksel, the Netherlands). The piglets were not castrated, neither were their tails docked or teeth clipped.

6.2.2 Housing

6.2.2.1 From birth to nine weeks of age

Piglets were raised in two different housing systems (similar to van Nieuwamerongen et al. (2015), as described in Parois et al. (2022a) and van der Zande et al. (2022)). In brief, the AHS consisted of five farrowing pens of 3.2 x 2.2 m, adjacent to a communal area of 11.1 x 2.8 m, a dunging area of 2.8 x 3.3 m and a feeding area of 4.2 x 3.3 m. One week before the expected farrowing date, five sows per batch were moved to this system. Two days before the expected farrowing date, sows were moved to the farrowing pens and confined in a farrowing crate. Two days after farrowing, they were allowed to access the full system again. Newly born piglets were kept with their own litter in the farrowing pens for one week, after which they could access the entire system and mingle with the other litters. AHS piglets were weaned at an average of 62.6 ± 1.9 days and a body weight of 26.6 ± 4.9 kg.

In the CONV system, piglets were kept in farrowing pens of 2.8 × 1.8 m until weaning. Sows were confined in a crate (1.9 × 0.6 m) one week before the expected farrowing date and during the entire lactation period. CONV piglets were weaned at 27.4 ± 1.2 days of age and 8.7 ± 1.3 kg. After weaning, CONV piglets were housed with their litter mates in nursery pens of 3.18 × 1.0 m (0.40 m^2 per piglet) for five additional weeks with a chain and a jute bag as enrichment.

6.2.2.2 From nine weeks of age onwards

After weaning of the AHS piglets at approximately nine weeks of age, all piglets were moved to the Carus research facilities in Wageningen, the Netherlands. They were mixed in groups of six unrelated piglets originating from the same system. Litter, sex and weight were balanced between pens. Piglets were selected based on their sex (50:50% male and female), and weight in order to choose piglets representative of the full litter.

AHS pigs were housed in a 2.4 x 4.7 m pen (1.86 m^2 per pig), enriched with deep straw, peat and sawdust bedding, which was replenished regularly. Besides that, AHS pigs were provided with a handful of hay, alfalfa or cardboard egg trays once a week and a chain, jute bag or rope (rotation every week). They were also provided with one extra toy, which was changed every two days to preserve the attractiveness of the toy. CONV pigs were housed in standard pens of 1.2 × 4.7 m (half the size of AHS pens) with conventional space allowance (0.93 m^2 per pig), with a partly solid and partly slatted floor without substrate. CONV piglets were provided with a ball and a chain with screws, which were not changed. Pigs were all fed the same feed (a standard commercial diet for growing pigs) ad libitum from a single pig feeder and water was available ad libitum. Lights were on between 7:00 h and 19:00 h.

6.2.3 LPS challenge

Resilience of the pigs was assessed by following the recovery process of the animals after exposure to different successive challenges. This study only uses data around the LPS challenge. Before the LPS challenge pigs had been exposed to a 21 h-isolation challenge (Parois et al., 2022b) and a 2 h-transport challenge (Parois et al., 2022a). Four animals per pen (Focals, two males and two females) were exposed to the LPS challenge, while two pen mates were used as controls and injected with saline. This study focuses on the LPS-treated animals only.

At the age of 104.4 ± 1.7 days (weight: 60.0 ± 7.5 kg), 92 focal pigs were injected in the ear vein with 2 μg of LPS/kg of body weight (LPS sigma L4391 *Escherichia coli* O111 B4). Four focal pigs were not subjected to the challenge as they were sick and on antibiotic treatment at the day of challenge. For time constraints, the pigs were challenged in two different groups on two consecutive days, balanced for housing system. Pigs were injected in their home pen, while restrained with a nose sling. Blood samples were collected at 24 h before (baseline) and 1 h, 3 h, 5 h and 24 h after the injection of LPS. At each of these time points, rectal temperature (RT) was measured before taking a blood sample. Pigs were habituated to RT measurements in the weeks prior to the challenge to prevent a temperature change related to handling stress.

6.2.4 Indicators measured during the LPS challenge

6.2.4.1 Blood samples

Blood samples were collected from the jugular vein. The blood (10 ml) was distributed as follows: 4 ml in EDTA tubes, 4 ml in heparin tubes and 2 ml in serum tubes. The EDTA and heparin tubes were centrifuged at 1500g for 10 min at + 4 °C. The serum samples were kept at ambient temperature for 30 min and thereafter were centrifuged at 1500g for 10 min at 4 °C. Plasma and serum were stored at − 20 °C until laboratory analyses.

Cortisol assays were performed in EDTA samples using the cortisol RIA kit from Immunotech (Beckman-Coulter, ref IM1841, Czech Republic). Glucose concentrations were measured in EDTA samples using a GOD-PAP kit (Hoffmann-La Roche, Switzerland). Non-Esterified Fatty Acids (NEFA) concentrations were measured in serum samples using a NEFA kit (WAKO Chemicals GmbH, Germany). Haptoglobin levels in heparin plasma were measured using the kit PHASE TM Haptoglobin Assay from Tridelta Development Limited (ref TP-801, Ireland). Urea concentrations were measured in EDTA samples using the enzymatic colorimetric test (ref 10506, HUMAN Gesellschaft für Biochemica und Diagnostica mbH, Wiesbaden, Germany).

6.2.4.2 Individual tracking

Using the individual tracking computer vision algorithm of van der Zande et al. (2021), individual activity was estimated. A full description of the method can be found in van der Zande et al. (2022). In brief, home pen activity was recorded for each individual pig from three-days prior to injection starting at 7:00 h (baseline) till four days post-injection ending at 18:00 h. The tracking algorithm generated bounding boxes for each of the six pigs per pen. Using videos recorded at 0.5 frames per second (FPS), distance moved between two consecutive frames was calculated using Pythagorean Theorem. Pigs have a distinct diurnal pattern in their activity (Fraser, 1985), with peaks in early morning and late afternoon. To correct for this diurnal rhythm, the rolling mean of distance moved with a window of 24 hours was used (Ten Thij et al., 2020). During the three-day baseline period, the mean and maximum error (i.e., maximum deviation observed from the mean) was calculated for each individual. The mean and boundary of the error represented their original state, i.e., state before injection, and were applied to the four days post-injection. A dip in activity was identified by finding the longest consecutive stretch of observations below the boundary starting no later than 24 hours post-injection. Based on the dip in activity, several putative resilience parameters could be calculated: the duration of the dip in activity, the area under the curve (AUC) of the dip, the ratio of the AUC and duration, the maximum deviation from the boundary, and the time to this maximum deviation.

6.2.5 Statistical analysis

Statistical analyses were performed with the software R 4.1.0 (R Core team, 2013). AUC of cortisol, RT, NEFA, urea, glucose and haptoglobin (physiological AUC variables) were approximated from repeated measurements using the trapezoidal rule $\sum_{k=0}^{N} (\frac{f(x_{k-1})+f(x_k)}{2}) \times \Delta x_k$, where $f(x)$ is a function and Δx_k is the length of the k-th subinterval. The baseline level was set to the measurement before the LPS challenge and peak level was the highest measurement after the challenge. Glucose decreased after the LPS challenge, therefore its minimum value at the peak of the response was used. Please note, for urea and haptoglobin the maximum level may not have been reached yet at the last sampling point (see Parois et al., 2022a), so these are not referred to as maximum but as urea +24h and haptoglobin +24h.

To explore the relationship between the physiological response and activity changes following the LPS injection, linear mixed models were used with the function lmer from the R package “lme4”. To test whether the pre-challenge baseline activity would affect the AUCs of the physiological variables, pre-challenge baseline activity was added as covariate to the model, i.e., the physiological AUC variables were the dependent variables. To test post-challenge activity response, the physiological AUC variables were added as covariate and the activity parameters related to the response to the challenge were the dependent variables. In these models, housing (AHS vs. CONV) and sex (boar vs. gilt) were included as fixed effects, and pen and batch were included as random effects. These effects were included in the models to correct for, as they affected some of the variables. The interaction between activity and housing (pre-challenge) and physiological AUC variables and housing (post-challenge) was also included but was only kept in the model when significant. The decision to enter the activity or physiological variable as a covariate in the model with housing and other fixed effects, or to use it as the dependent variable, was based on timing (e.g., measurements of baseline activity occurring before the challenge and hence physiological response to the challenge) and on the fact that housing affected baseline activity and physiological responses, which was accounted for in the models. AUC NEFA, dip duration, AUC of the activity dip and time to maximum deviation from baseline activity were log transformed, when they were the dependent variable.

Table 6.1 – Loadings of physiological variables measured around a LPS challenge (residuals corrected for housing) on the factors with an eigenvalue above one that were extracted by factor analysis with orthogonal Kaiser-Varimax rotation.

Variable	Factor 1	Factor 2	Factor 3	Factor 4
AUC cortisol	0.01	0.87	-0.13	-0.36
Basal cortisol	0.91	0.04	0.02	0.04
Peak cortisol	0.11	0.84	-0.05	-0.08
Peak rectal temperature	0.23	0.35	0.16	-0.27
Basal NEFA	0.18	0.1	-0.02	0.12
Peak NEFA	0.09	0.28	-0.12	-0.43
Basal urea	0.05	-0.06	0.97	0.06
Urea +24h	-0.01	-0.04	0.75	-0.19
Basal glucose	0.14	-0.17	-0.16	0.35
Minimum glucose	0.03	-0.11	-0.11	0.86
Basal haptoglobin	0.97	-0.01	0.06	0.03
Haptoglobin +24h	0.88	0.13	-0.04	-0.1
Eigenvalues	*3.07*	*2.44*	*1.93*	*1.06*
% of variance explained	*20%*	*20%*	*17%*	*15%*

An additional approach to explore the potential relationship between the physiological and activity variables while correcting for housing effects was to calculate correlations using residuals of the variables produced in a general linear model with housing as fixed effect. AUC NEFA was log transformed when residuals were obtained. To reduce the number of physiological variables, first a factor analysis with orthogonal Kaiser-Varimax rotation was performed on the residuals of the AUCs, baseline levels and peak levels. Variables with a communality estimate below 0.3 were excluded from the factor analysis (AUC RT, basal RT, AUC NEFA, AUC urea, AUC glucose, AUC haptoglobin). Based on Kaiser's criterium, factors with an eigenvalue above one were retained, resulting in four factors (**Table 6.1**). Spearman correlations between factor scores of each individual pig and activity variables were then calculated.

P-values below 0.05 were considered as significant effects and below 0.1 as tendencies. Data are presented as means ± standard deviation.

6.3 Results

6.3.1 Descriptive statistics

Table 6.2 shows the means and standard deviations of both physiological and activity variables.

Table 6.2 – Mean and standard deviation (SD) of physiological and activity variables measured around a LPS challenge.

Variables	Mean	SD
AUC cortisol ((ng/ml) x h)	2217.9	636.6
AUC RT (°*C* x h)	1903.2	14.3
AUC NEFA ((μmol/l) x h)	5560.6	2083.2
AUC urea ((mg/dl) x h)	176.5	46.4
AUC glucose ((mmol/l) x h)	5230.0	398.2
AUC haptoglobin ((mg/ml) x h)	53.6	36.1
Mean baseline activity (m/h)	44.3	9.5
Error baseline activity (m/h)	6.6	2.6
AUC activity dip ((m/h) x h)	267393.5	295623.1
Duration activity dip (h)	30.1	24.4
AUC:duration ratio	8296.8	5407.3
Maximum deviation activity (%)	-0.17	0.1
Time to maximum deviation (h)	23.7	8.3

6.3.2 Pre-injection activity

Figure 6.1 displays the relationship between the mean activity measured during the three-days baseline period before LPS injection and the AUC of the responses of different physiological parameters. With mean activity added to the model, the AUC of cortisol (P = 0.03) was affected and the AUC of NEFA (P = 0.09) tended to be affected by housing system, where AHS pigs had lower AUCs of cortisol and NEFA than CONV pigs. The mean activity during the baseline affected the AUC of glucose (P = 0.02) and haptoglobin (P = 0.03). Pigs with a higher baseline activity had a smaller AUC of glucose (i.e., larger dip), and AUC of haptoglobin. The effect of baseline activity on AUC of NEFA (P = 0.03) depended on housing with a stronger positive relationship for CONV animals than for AHS pigs (0.027 ± 0.011 vs. 0.00032 ± 0.005, interaction effect, P = 0.03). Also for AUC of urea, the effect of baseline activity (P = 0.004) depended on housing (interaction effect, P = 0.06), with a stronger decrease in urea AUC with increasing basal activity levels in CONV pigs, but less so in AHS pigs (-3.45 ± 1.31 vs. -0.77 ± 0.57). There was no effect of basal activity on the AUC of cortisol or RT.

Supplementary **Figure S6.1** displays the relationship between the error during the baseline, i.e., the maximum deviation from the mean activity during the three days before the challenge, and the AUC of the responses of different physiological parameters. The baseline error influenced the AUC of NEFA (P = 0.02), urea (P = 0.008), and glucose (P = 0.04) and tended to influence the AUC of haptoglobin (P = 0.07) (Supplementary **Figure S6.1**). Cortisol AUC increased (162.34 ± 61.88 , P = 0.02) while glucose (-34.51 ± 16.88, P = 0.04), urea (-9.34 ± 4.10, P = 0.008) and haptoglobin AUC (-3.04 ± 1.68, P = 0.07) decreased with increasing levels of error during baseline activity. Error during the baseline affected AUC cortisol (P =0.02), but the effect depended on housing (interaction effect, P = 0.02), as error during baseline only significantly affected the AUC of cortisol of the CONV pigs (162.34 ± 61.9, P = 0.01), but not of the AHS pigs (0.69 ± 31.08, P = 0.98). The AUC of NEFA tended to be affected by housing (P = 0.09), error in activity during baseline (P = 0.02) and their interaction (P = 0.01). NEFA AUC increased with increasing levels of the error in activity during baseline in CONV (0.1 ± 0.03, P = 0.007), but not in

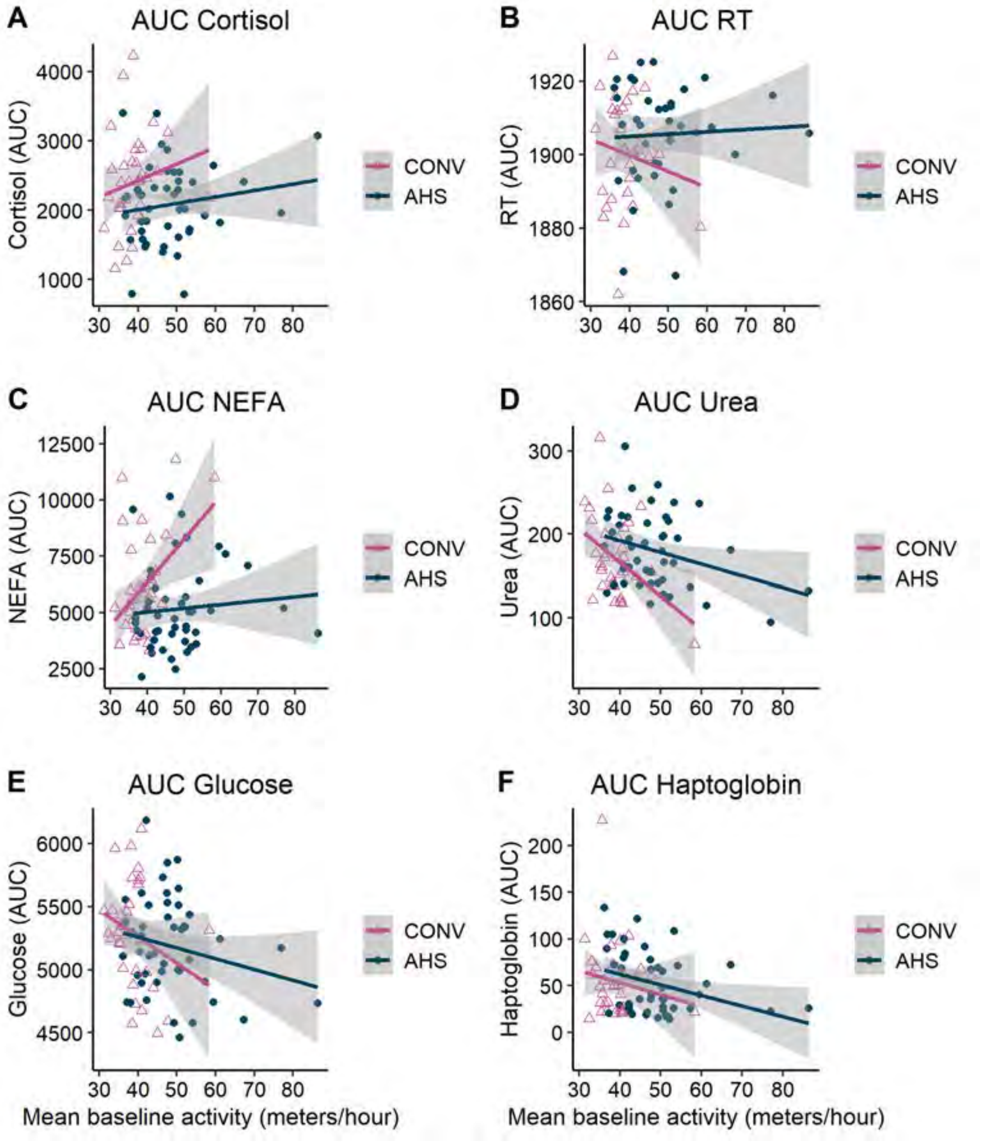


Figure 6.1 – Area under the curve (AUC) cortisol (A), Rectal temperature (RT) (B), AUC Non-Esterified Fatty Acids (NEFA) (C), AUC urea (D), AUC glucose (E), AUC haptoglobin (F) for both pigs housed in a conventional (CONV, purple open triangles) and alternative (AHS, dark blue solid circles) housing system related to mean baseline activity. Please note, different Y-axis scaling.

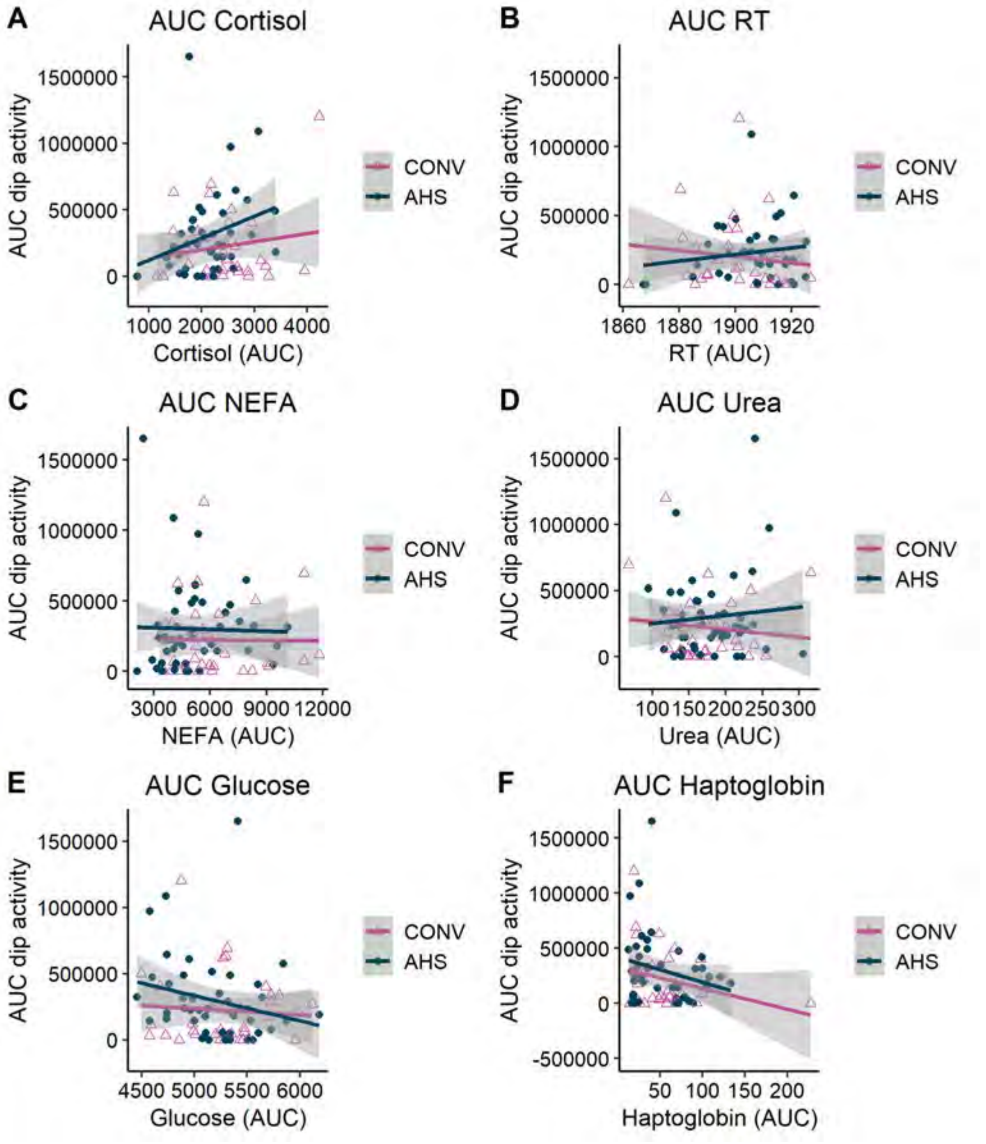


Figure 6.2 – Area under the curve (AUC) of the dip in activity in relation with AUC cortisol (A), Rectal temperature (RT) (B), AUC Non-Esterified Fatty Acids (NEFA) (C), AUC urea (D), AUC glucose (E), AUC haptoglobin (F) for both pigs housed in a conventional (CONV, purple open triangles) and alternative (AHS, dark blue solid circles) housing system. Please note, different Y-axis scaling.

AHS pigs (-0.004 ± 0.02, P = 0.80). There was no effect (P = 0.67) of the error during baseline activity on RT.

6.3.3 Post-injection activity

The duration of the dip in activity tended to be affected by AUC cortisol (P = 0.05). AUC glucose tended (P=0.06) to affect the duration of the dip in activity, with a lower activity dip with a higher AUC glucose, i.e. a smaller depletion (Supplementary **Figure S6.2**). The AUC of the dip in activity was affected by AUC cortisol (P = 0.04) and AUC glucose (P = 0.04), where higher AUC cortisol and lower AUC glucose led to a higher AUC of the dip in activity (**Figure 6.2**). None of the physiological variables affected the ratio of AUC:duration, but the ratio was affected by housing system, where AHS had a higher ratio than CONV (P = 0.03). AUC glucose (P = 0.09) and AUC haptoglobin (P = 0.08) tended to affect the AUC:duration ratio, with a lower AUC glucose and haptoglobin relating to a higher ratio (Supplementary **Figure S6.3**).

The maximal deviation in activity following LPS injection, expressed in a percentual decrease, was affected by AUC cortisol (P = 0.04), AUC urea (P = 0.05) and AUC glucose (P = 0.01) (**Figure 6.3**). A higher AUC cortisol was associated with a larger decrease, whereas a higher AUC urea and AUC glucose were related to a smaller maximal decrease in activity. The time to reach the maximal deviation in activity was affected by AUC urea (P = 0.02) and AUC haptoglobin (P = 0.04) (Supplementary **Figure S6.4**). No other effects of the physiological variables on activity variables following LPS injection were found.

6.3.4 Factor analysis

Four factors were retained from the factor analysis conducted on the physiological parameters (**Table 6.1**). The first factor had high positive loadings for basal cortisol and haptoglobin concentrations and haptoglobin level 24h after the challenge. The second factor had high positive loadings for cortisol AUC and peak level. The third factor had high positive loadings for urea concentrations at baseline and at 24h after the challenge, and the fourth factor had a high positive loading for minimum glucose level and somewhat weaker negative loading for peak NEFA concentrations. Basal NEFA and basal glucose

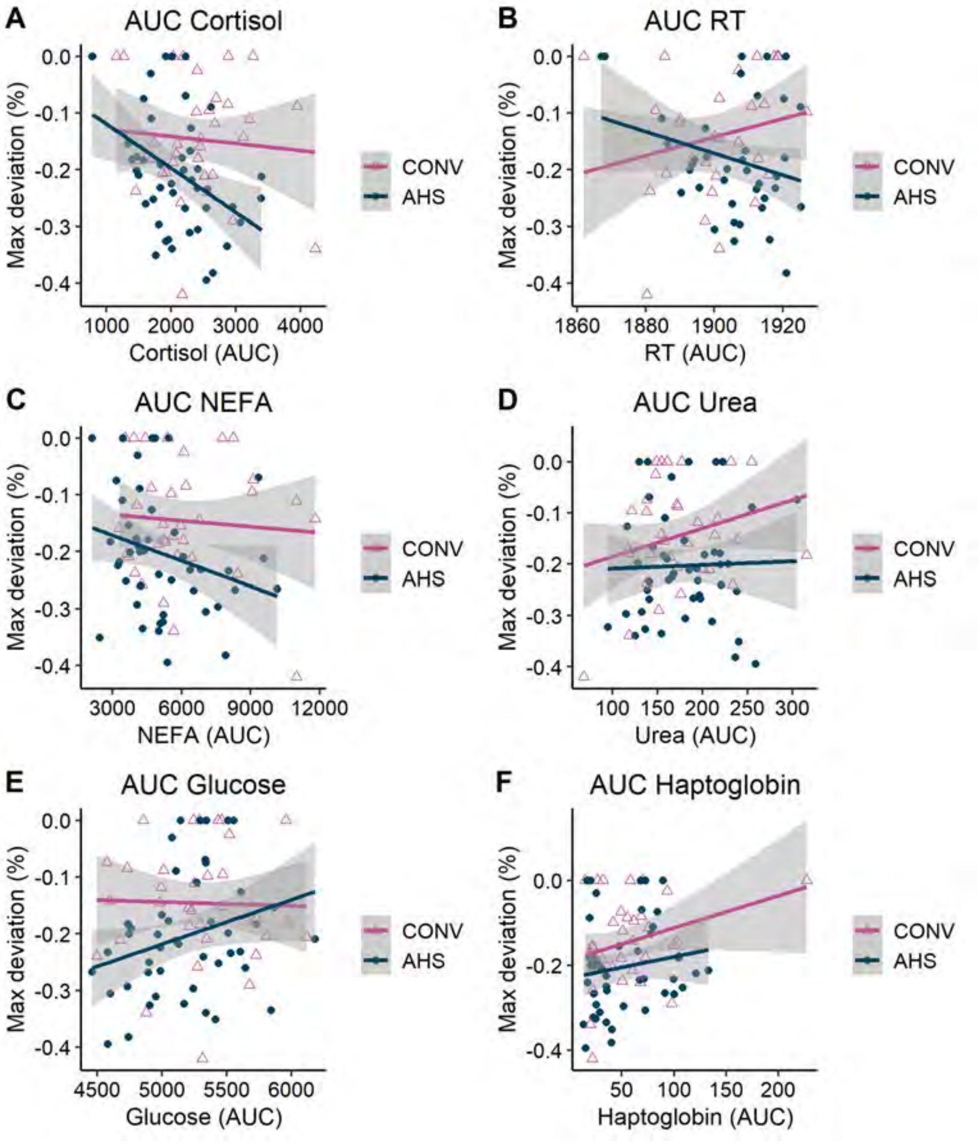


Figure 6.3 - Maximum deviation of the dip in activity in relation with AUC cortisol (A), Rectal temperature (RT) (B), AUC Non-Esterified Fatty Acids (NEFA) (C), AUC urea (D), AUC glucose (E), AUC haptoglobin (F) for both pigs housed in a conventional (CONV, purple open triangles) and alternative (AHS, dark blue solid circles) housing system. Please note, different X and Y-axis scaling.

levels did not load strongly on any of the factors, possibly because these fluctuate over the day, depending on the feeding pattern of the pig.

Table 6.3 shows Spearman correlations between these factors and activity parameters. Factor 1 was significantly negatively correlated with the AUC (r=-0.30; P = 0.01) and duration (r=-0.29; P = 0.02) of the dip in activity following LPS injection, indicating that high baseline cortisol and haptoglobin levels, and high levels of haptoglobin at 24 h post injection were associated with a relatively low AUC and duration of the activity dip. Pigs with a low mean baseline activity tended (P = 0.07) to have lower scores for Factor 1 (basal cortisol, basal haptoglobin and haptoglobin at +24h). The same trend held for Factor 4 (P = 0.06), meaning that pigs with a low mean baseline activity tended to have a lower minimum in glucose level (i.e., stronger response) following LPS injection. Factor 3 was negatively correlated with the error of the baseline (P = 0.01), i.e., high levels of urea at baseline and 24 h after the challenge seemed to be associated with a low maximum deviation observed from the mean activity during the three days before the LPS challenge. Factor 2 (reflecting the cortisol response to the LPS challenge) was not correlated to any of the activity variables.

Table 6.3 – Spearman correlations between factor scores and activity parameters.

	Factor 1	Factor 2	Factor 3	Factor 4
Mean baseline activity	*-0.21*	-0.01	-0.18	*-0.21*
Error baseline activity	-0.17	0.07	**-0.28**	-0.18
AUC activity dip	**-0.30**	0.00	-0.02	-0.07
Duration activity dip	**-0.29**	-0.02	0.1	0.08
AUC:duration ratio	-0.15	0.06	-0.1	-0.18
Maximum deviation	0.15	-0.13	0.1	0.11
Time to max deviation	-0.19	0.08	0	0.13

bold p < 0.05, italic p < 0.1

6.4 Discussion

In this study, the relationships between behavioural changes to LPS injection and a set of physiological changes measured (cortisol, glucose, NEFA, urea, haptoglobin and rectal temperature) were explored. It was expected that the observed activity changes would be related to the physiological response to LPS, as these are both part of the sickness response. Strong correlations between physiological and behavioural responses would imply that the resilience of pigs to a sickness challenge could be captured by non-invasive behavioural observations. Additionally, we explored whether baseline activity and the variation therein, expressed as the maximum deviation from the mean, is related to the physiological response to LPS.

6.4.1 Baseline activity and physiological response to LPS

Surprisingly, activity during the three-days baseline period before the LPS challenge affected the AUC of glucose and haptoglobin, and the AUC of NEFA and urea in a housing-dependent manner. A higher baseline activity was associated with a larger dip in glucose (small AUC), and a lower AUC in haptoglobin following LPS injection. The positive association with NEFA AUC and negative with urea AUC was stronger for the CONV pigs than for the AHS pigs. In addition, a high maximum deviation from the mean baseline activity was associated with a higher AUC of cortisol, and, particularly in CONV pigs, a high AUC of NEFA, and lower glucose and urea AUCs. The latter association is in line with weak to moderate correlations between Factor 3 (basal and peak urea concentration) and deviation in mean activity.

Parois et al. (2022a) showed a housing effect on the cortisol response to LPS in the pigs used in this study, where AHS pigs had lower cortisol levels and AUC than CONV pigs. Results from this study show that adding the mean baseline activity pre-injection to the model did not influence this result. However, adding the maximum error of baseline activity resulted in a non-significant effect of housing on the AUC of cortisol. Possibly the housing effect is confounded with the variation in baseline activity, as the AHS pigs had a higher variation in activity than the CONV pigs. Still, adding baseline activity to the original model of Parois et al. (2022a) improved the fit of the model (data not shown).

We can only speculate why baseline activity and deviations from the mean activity are related to the physiological response of pigs to LPS. Possibly, more active animals have an inherently different metabolic state that affects their sickness response. It has been hypothesized that activity may attenuate inflammatory responses and thereby reduce the sickness response, but it was found that voluntary wheel running in mice did not affect LPS-induced sickness behaviour (Martin et al., 2013). Only within the CONV, there was a clear relationship between the AUC of cortisol following LPS injection and variation in activity pre-challenge, with a more pronounced cortisol response in pigs with higher maximum deviation from the mean baseline activity. It could be speculated that particularly in barren housed pigs, deviations in baseline activity, i.e., extreme minimum and maximum levels, may reflect poor welfare. Peaks of activity could for instance represent restlessness due to tail biting outbreaks, which occur more often in barren housing (e.g., Ursinus et al., 2014), or aggressive outbursts (e.g., when competing for resources like lying space). At the other extreme, boredom or negative affective states in barren housed pigs, induced by limited possibilities to show important behaviours, may cause lethargic behaviour (see Bolhuis et al., 2005; Fureix and Meagher, 2015; Luo et al., 2020b). Van der Zande et al., (2022) indeed showed lower activity of barren housed pigs as compared with those in enriched housing. The stress, restlessness or negative affective states that potentially underly the high variation in activity in barren housed pigs, might then exacerbate the cortisol response to LPS. In enriched housed pigs, on the other hand, activity bursts might be more often related to play behaviour or exploration, which are observed more in enriched conditions (e.g., Luo et al., 2020) and considered to reflect good welfare (Lawrence et al., 2018). More detailed observations on the specific behavioural activities in barren and enriched housed pigs are needed, however, to support this hypothetical consideration.

Alternatively, the activity level and maximum deviation from the mean might somehow reflect the personality of the pigs, which is associated with inherent physiological and immunological differences (see O'Malley et al., 2019 for review). Bolhuis et al. (2005) showed that pigs with a reactive coping style, which generally respond more passively under acute stress, tended to be more active than proactive pigs under stable conditions. Burdick Sanchez et al. (2014) showed that temperament of Brahman bulls, as assessed by their reactivity to a human observer, affected the NEFA, urea and glucose response to an LPS challenge. It was suggested that this is the result of inherent, pre-challenge, differences in metabolism associated with the bulls' temperament. A possible influence of the basal physiological state of animals on their response to a LPS challenge is also illustrated in the negative correlation that we found between Factor 1, on which both basal cortisol and basal haptoglobin loaded, and the duration and AUC of the activity dip post-injection.

Further research is needed to elucidate how and why activity levels and deviations therein on the days prior to a sickness challenge are related with the response of several physiological variable to this challenge. A more detailed analysis of pre-challenge behaviours and of the personality of the pigs could possibly give more insight in the differences between low vs. high active animals in both barren and enriched housing.

6.4.2 Physiological responses to the LPS challenge and the change in activity

Some of the physiological responses to LPS explained variation in characteristics of the activity dip. For instance, a high AUC of cortisol was related to a longer duration of the dip in activity, a larger AUC of the dip in activity, and a stronger percentual decrease. Also a stronger glucose depletion (smaller AUC) was associated with a longer duration and larger AUC of the activity dip, and a higher percentual decrease. A higher AUC of urea, which is released when amino acids are oxidised as an alternative source of energy, was associated with a stronger maximum percentual decrease in activity and a shorter time to reach this maximum. Taken together, some of the physiological changes that depict the metabolic response to the LPS challenge, seem to affect aspects of the decrease in activity, with generally stronger physiological responses being associated with stronger or prolonged changes in activity.

A stronger glucose depletion was associated with a longer and stronger dip in activity. The glucose and activity dip might together reflect the extent to which pigs are affected by the LPS challenge. Activation of the immune system and development of the fever response require energy. Reduced activity is considered as a strategy to conserve the energy needed for these processes, of which fever is particularly energy demanding (Aubert, 1999). This study showed, however, no direct link between rectal temperature and the estimated activity proxies. Glucose is the first available energy source, and when glucose is depreciated, NEFA are produced as an alternative source of energy (Huntley et al., 2018; Kvidera et al., 2017). However, the NEFA response to LPS was not associated with characteristics of the activity dip.

Baseline cortisol, baseline concentration of the acute phase protein haptoglobin and haptoglobin levels at 24h after LPS injection loaded on Factor 1. Factor 1 scores were negatively correlated with the AUC and duration of the dip in activity, i.e., pigs with high basal levels of cortisol and haptoglobin showed a less severe sickness-related activity dip (e.g., Reimert et al., 2014). Basal haptoglobin levels are affected by the sanitary conditions under which pigs are kept (e.g., Kampman-van de Hoek et al., 2016; van der Meer et al., 2017), reflecting immune activation, but also by stress and housing, with lower levels in enriched systems compared to barren housing systems (Reimert et al., 2014a; Scott et al., 2006). It should be noted that in pigs from the current study such an effect of housing was not found around the LPS challenge, but barren housed pigs did show higher basal levels of haptoglobin around the transport challenge to which they were exposed three weeks before LPS injection. Also increased levels of baseline cortisol have been associated with chronic stress and barren housing (Reimert et al., 2014b; van de Weerd and Day, 2009), and, in line with this, the barren pigs from this study showed a higher accumulation of cortisol in their hairs, pointing to a chronically heightened activation of the hypothalamic pituitary adrenal axis (Parois et al. 2022a). Factor 1 also tended to be related to a low pre-challenge, baseline, activity. It could be hypothesised that this low activity is, in line with the increased basal levels of haptoglobin and cortisol, another indication of chronic stress or heightened immune activation, both of which may lead to (mild) sickness behaviour including lethargy (e.g., Nordgreen et al., 2018). It is not fully clear why animals with higher Factor 1 scores, representing high basal cortisol and haptoglobin levels, showed a smaller AUC and duration of the dip in activity following LPS injection, but this might be related to their low baseline activity level (van der Zande et al., 2022). This baseline activity level of the pigs was considered as their original state and used to calculate duration and AUC of the activity dip. If the activity level is already low at baseline, whether reflecting an original state with impaired health or welfare or not, this means that it cannot go much lower following the LPS challenge. More research on the status of animals with a low activity and high baseline cortisol and haptoglobin levels and the subsequent consequences for responding to a sickness challenge is needed.

Importantly, the effects of physiological responses found on activity parameters should be taken with caution, as many relationships were tested, and relationships were weak to moderate. The sickness response is characterized by a wide range of metabolic, physiological and immunological changes, part of which occur in the brain, with each a different onset and time course. A limited number of these changes was assessed in our pigs to characterize their resilience to a LPS challenge (Parois et al., 2022a). Our study reveals only limited relationships between these physiological changes and parameters reflecting the activity dip that is part of the sickness behaviour response to LPS injection.

Hence, changes in activity patterns following LPS injection that reflect sickness-induced depression of activity cannot simply be used as reflection of physiological data. This does not imply that the activity decrease in itself is not an important aspect of the sickness response which may have consequences for animal health. In fact, our previous paper in which accelerometer data were used to assess the activity patterns of pigs following a PRRSV infection demonstrated that a larger decrease in activity post-injection was associated with an increased probability of clinical signs (van der Zande et al., 2020). Further research should elucidate whether and how infection-induced changes in activity patterns as estimated by computer vision link to clinical signs of disease and/or long-term health consequences.

In conclusion, our explorative study reveals a limited number of weak to moderate correlations between characteristics of the change in activity and the physiological variables measured following LPS injection. There were links between pre-challenge activity levels and several physiological responses to the LPS sickness challenge, however, which merit further research.

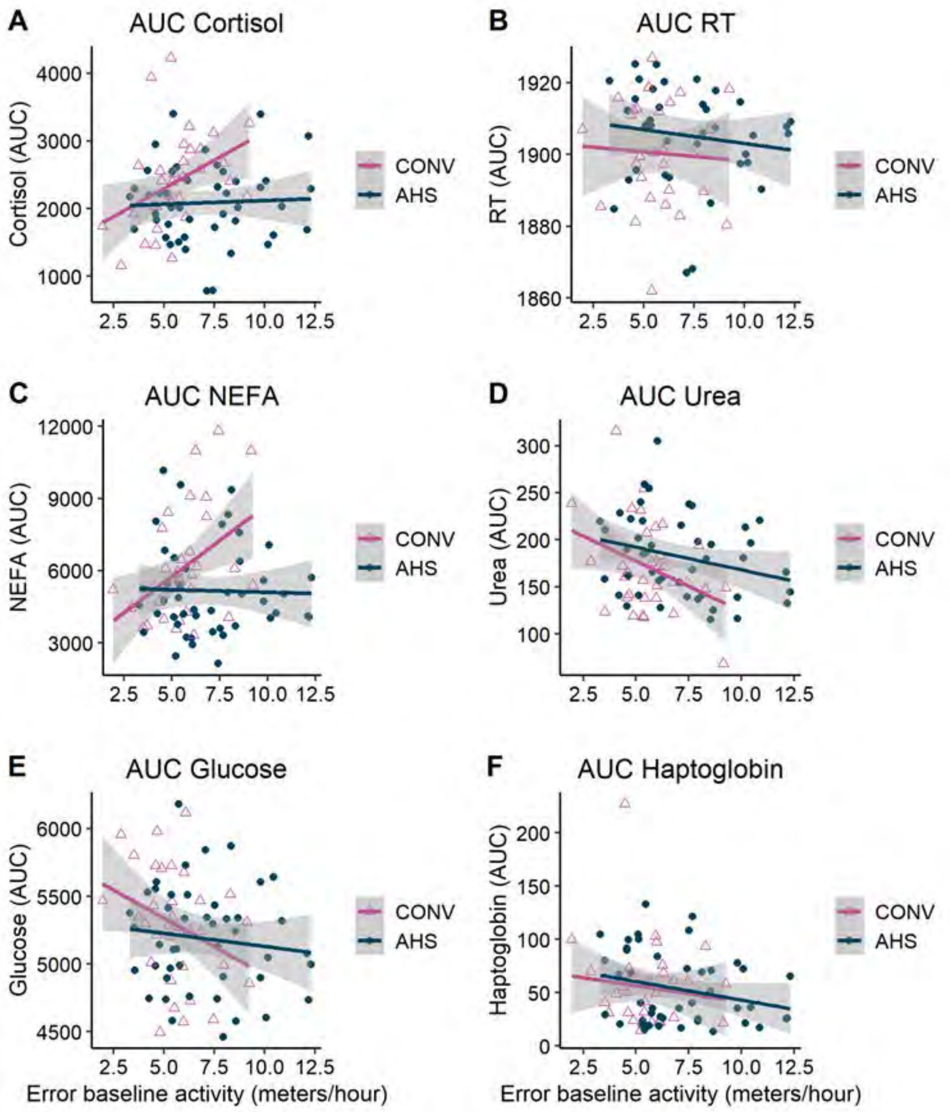
6.5 Conclusion

Baseline activity influenced the response of glucose, haptoglobin, NEFA and urea following LPS injection. In addition, elevated levels of cortisol and depletion of glucose were associated with a higher duration and AUC of the dip in activity. Correlations between physiological and activity parameters were limited and weak to moderate, which implies that non-invasive behavioural observations capture another aspect of the sickness response than physiological parameters. This explorative study contributes, however, to understanding the relation between behavioural and physiological sickness response and further research is needed to confirm relationships between different resilience parameters.

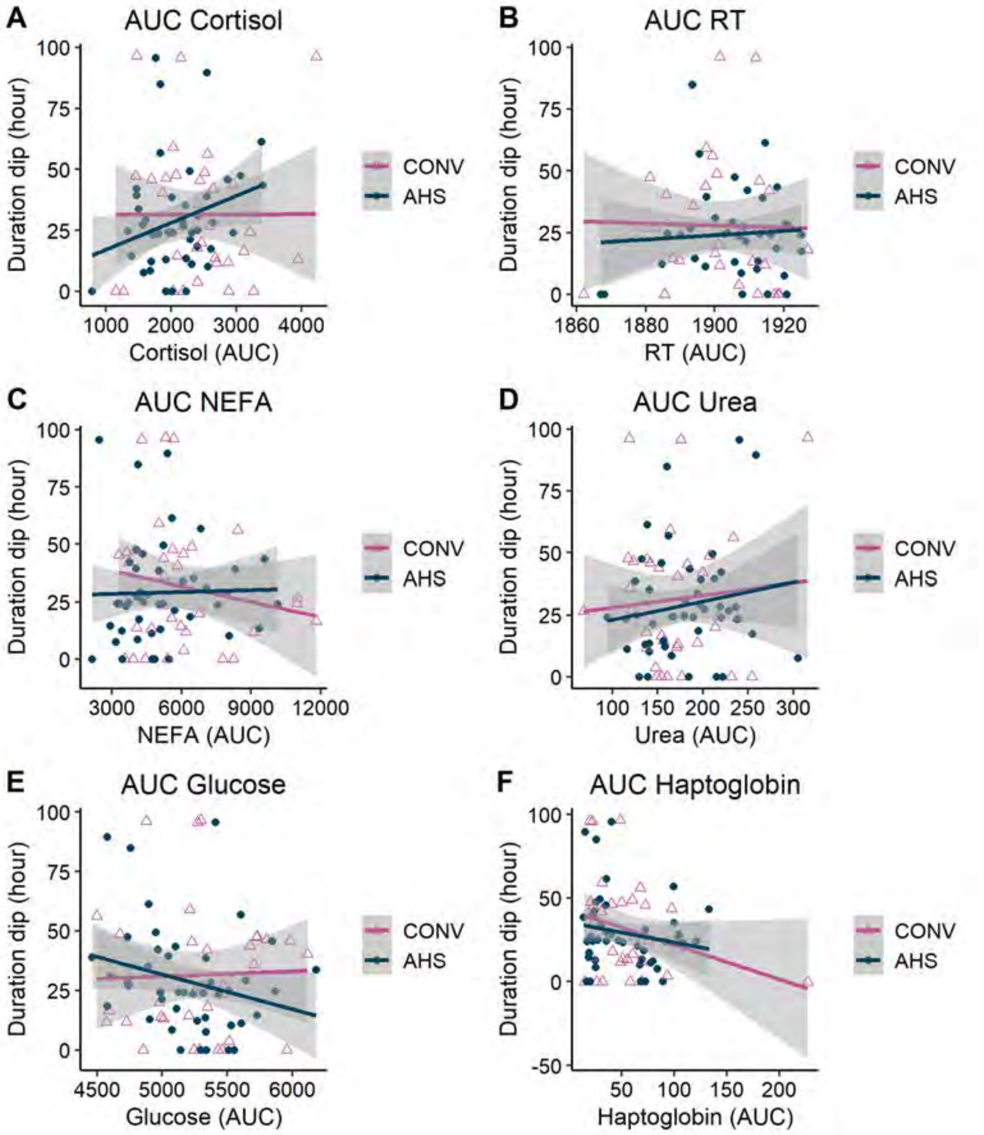
6.6 Acknowledgements

The authors would like to thank the farm staff at the Swine Innovation Centre (Sterksel, the Netherlands) and at Carus research facilities (Wageningen, the Netherlands) for daily animal care and their help in the data collection. They would also like to address a special thanks to the technicians and laboratory persons from the Adaptation Physiology group: Joop Arts, Rudie Koopmanschap, Bjorge Laurenssen, Manon van Marwijk and Monique Ooms. They are also grateful for the invaluable help of the students involved in the project: Marith Booijen, Tao Cui, Jori Noordenbos, Marloes van Beurden, Sandra van Iwaarden and Shiv Vasa.

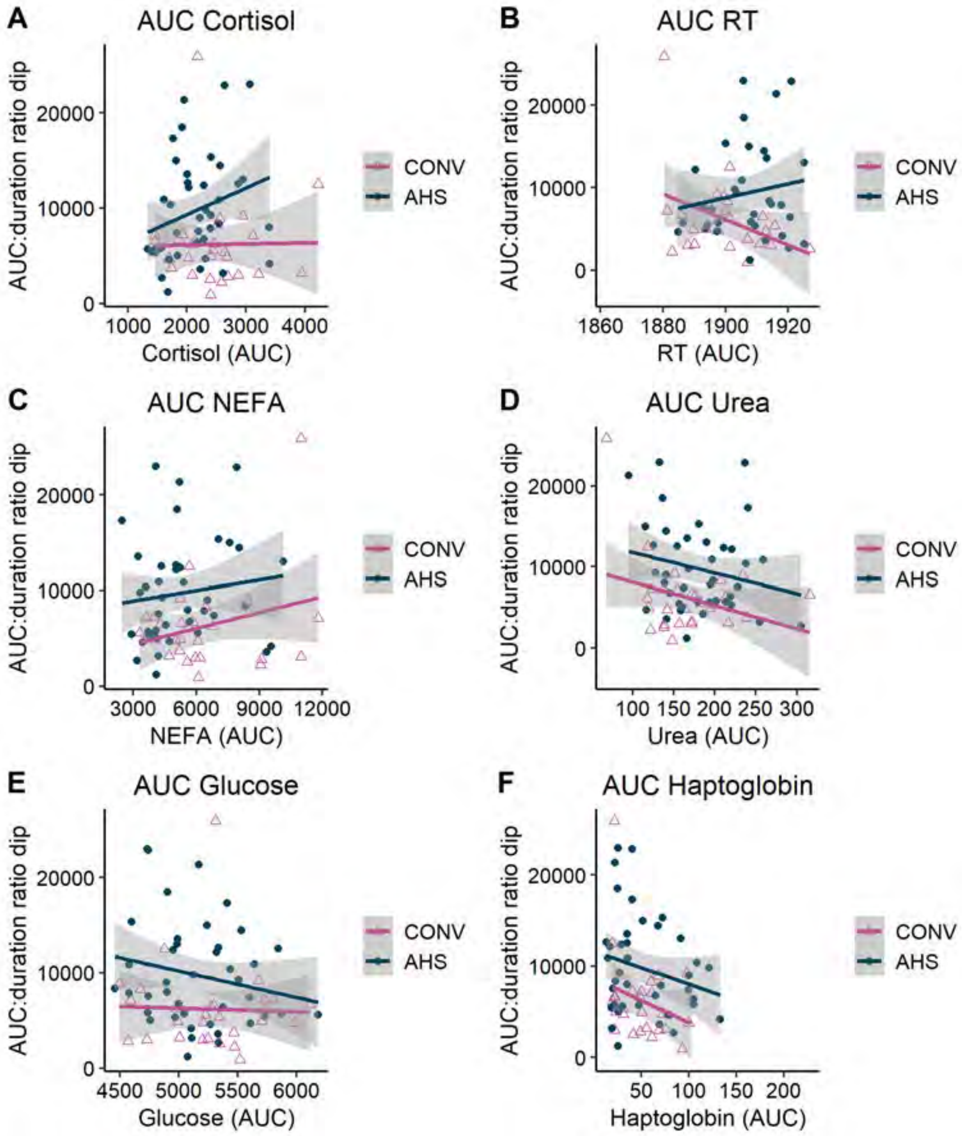
6.7 Supplementary figures



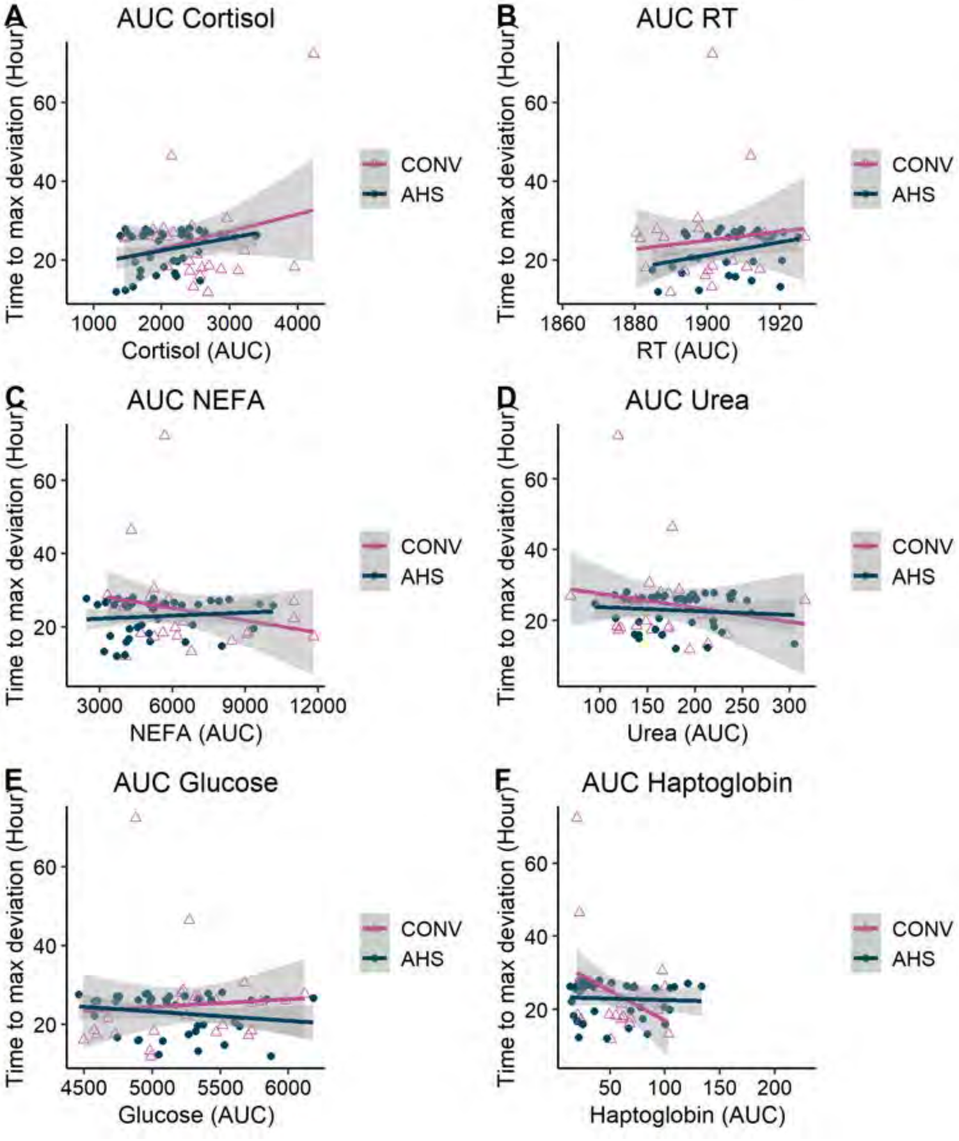
Supplementary Figure S6.1 – Area under the curve (AUC) cortisol (A), Rectal temperature (RT) (B), AUC Non-Esterified Fatty Acids (NEFA) (C), AUC urea (D), AUC glucose (E), AUC haptoglobin (F) for both pigs housed in a conventional (CONV, purple open triangles) and alternative (AHS, dark blue solid circles) housing system related to error baseline activity (maximum deviation observed from the mean baseline activity). Please note, different Y-axis scaling.



Supplementary Figure S6.2 – Duration of the dip in activity in relation with AUC cortisol (A), Rectal temperature (RT) (B), AUC Non-Esterified Fatty Acids (NEFA) (C), AUC urea (D), AUC glucose (E), AUC haptoglobin (F) for both pigs housed in a conventional (CONV, purple open triangles) and alternative (AHS, dark blue solid circles) housing system. Please note, different Y-axis scaling.



Supplementary Figure S6.3 – Area under the curve (AUC):duration of the dip in activity ratio in relation with AUC cortisol (A), Rectal temperature (RT) (B), AUC Non-Esterified Fatty Acids (NEFA) (C), AUC urea (D), AUC glucose (E), AUC haptoglobin (F) for both pigs housed in a conventional (CONV, purple open triangles) and alternative (AHS, dark blue solid circles) housing system. Please note, different Y-axis scaling.



Supplementary Figure S6.4 – Time to maximum deviation of the dip in activity in relation with AUC cortisol (A), Rectal temperature (RT) (B), AUC Non-Esterified Fatty Acids (NEFA) (C), AUC urea (D), AUC glucose (E), AUC haptoglobin (F) for both pigs housed in a conventional (CONV, purple open triangles) and alternative (AHS, dark blue solid circles) housing system.

Chapter 7

General discussion

7.1 Introduction

Resilience is referred to as the capacity to be minimally affected by and swiftly recover from a perturbation (Colditz and Hine, 2016). Resilience is a highly relevant trait, since it might reflect an animal's adaptive capacity and therefore influence its welfare. To measure recovery from challenges as a reflection of resilience regular, or even continuous, monitoring of the animal is necessary. Detailed physiological and behavioural responses may be difficult to monitor frequently or continuously. Instead, proxy traits could be used. Proxy traits are traits related to the trait of interest, but are often easier to measure (for example tail damage score as a proxy for receiving tail biting behaviour). A potentially relevant proxy measure for resilience is the deviation in general activity of individual animals in response to a challenge. The main goal of this thesis was to explore technologies to measure activity changes as a proxy for resilience, and to apply them in a relevant context with variation in resilience.

The first objective was to find suitable technology to measure activity in individual pigs, focusing on accelerometers and computer vision. In Chapter 2, the use of accelerometers was tested, and it was shown that accelerometers can be used to quantify activity. Activity changes as assessed by accelerometer data tended to be associated with morbidity and mortality following a Porcine Reproductive and Respiratory Syndrome Virus (PRRSV) infection. However, the use of accelerometers is a black box approach, could be invasive as sensors need to be attached to the animal, and spatial information is not recorded. Therefore, Chapter 3 addressed the use of computer vision, combining open-sourced algorithms to create an individual pig tracking algorithm. The algorithm could accurately detect and follow individual pigs for almost one hour on average. This algorithm was used in further studies in Chapter 5 and 6. The second objective was to explore potential resilience indicators. Chapter 4 focused on using physiological parameters to measure resilience. A contrast in housing systems was applied and it was found that pigs housed in an enriched environment from birth onwards (with group housing during lactation, delayed weaning, enrichment material and more space) showed a faster physiological recovery from several challenges than conventionally housed pigs. The individual pig tracking algorithm was applied to the same dataset to measure the resilience to a lipopolysaccharide (LPS) induced sickness challenge using activity changes (Chapter 5). The sickness challenge caused a dip in activity and several characteristics of the dip could be defined. Pigs from the enriched housing system were more active before the challenge than conventionally housed pigs, but there were limited effects of housing on characteristics of the activity dip. How the physiological parameters measured in Chapter 4 and activity parameters measured in Chapter 5 were related to each other was explored in Chapter 6. Weak relationships between physiological and activity changes in response to the sickness challenge were found, so these measures seem to reflect different aspects of the sickness response and to complement each other.

In this final chapter, I will discuss and bring together the main findings of the thesis and place them in a broader context. First, I will discuss the advantages and limitations of measuring activity with accelerometers and computer vision to quantify resilience. In addition, the use of activity-related potential proxy measures of resilience in livestock will be discussed. Next, I will elaborate on how to enhance resilience. Finally, I will discuss the implementation of technology, housing improvements and genetics to enhance resilience in pig husbandry.

7.2 Technology to measure activity as a potential proxy of resilience

In this thesis, both accelerometers and computer vision were used to measure activity from which different activity parameters could be derived as potential indicators of resilience. Both technologies have their own advantages and limitations, so the choice of technology depends on the question one would like to answer.

7.2.1 Advantages and limitations of accelerometers and computer vision to measure activity

Accelerometers and computer vision are very different technologies. Accelerometers are attachable and a unique identity (ID) is incorporated within the hardware, whereas the computer vision approach mainly consists of software with no physical ID attributed to the animal. In Chapter 2 activity was measured for 65 days using accelerometers. From 232 animals, 47 were removed from the study as the sensors failed for more than one day. In Chapter 3 a developed computer vision algorithm was presented which enabled tracking of individual pigs in an enriched environment for up to one hour, without losing the ID. Chapter 5 implemented this tracking algorithm to assess activity changes in response to a sickness challenge and the activity of pigs was estimated for eight days. To do so, still human intervention was needed to correct ID switches. Both technologies should, once fully developed, aid human observations and ultimately record more data with less effort compared to human observation. **Box 7.1** shows a comparison of time spent observing by a human observer compared with the computer vision tracking algorithm in its current state as developed in Chapter 3. Time needed to observe and score pig activity is not mentioned in literature, so **Box 7.1** is a limited perspective on how much time could potentially be saved, based on the work conducted for Chapter 5. However, it still provides an indication that the use of computer vision is twice as fast as human observations, even when human intervention is needed to correct ID numbers. Reduction of ID switches or losses with further improvement of the algorithm and/or the use of Radio Frequency Identification (RFID) for re-identification of individuals, will increase time saving even more. From personal experience, the accelerometers used in Chapter 2 could estimate activity for more days than the computer vision algorithm as developed in Chapter 3 (65 vs. 8 days), with approximately the same time investment from data collection to a complete dataset. So also activity measurement based on accelerometer technology is much less time consuming than human observation of activity. Often, to reduce time needed for observations, instantaneous scan sampling is used, i.e., behaviour of individual pigs is scored at a limited number of time points, with regular intervals (e.g., every 10 minutes). This has immediate consequences for the density of the collected data, and information on activity between scan sampling intervals is lost. Continuous sampling would be ideal, even more so for behaviours with a short duration, which could be missed using scan sampling.

Table 7.1 provides an overview of the advantages of both technologies to track activity in pigs, i.e., accelerometers and computer vision. Accelerometers have a unique identity fixed in the hardware, whereas computer vision has no identity identification or confirmation on its own. To overcome this limitation, one can combine the use of computer vision with another type of hardware (e.g., RFID) to confirm identities of individual pigs. Accelerometers are not bound to a

Box 7.1 Time advantage of using computer vision as developed in Chapter 3 and applied in Chapter 5 compared with human observation

For many years, technology has helped to save time for humans. The same holds for behavioural research. Behaviour was initially scored using pen and paper, and characteristics of behaviours like frequencies, durations and sequences needed to be assessed manually. Later, handheld computers were developed and software could be used for scoring behaviour and generating frequencies and durations of behaviours. When multiple groups of animals need to be observed over a long period of time, it is sometimes chosen to record the animals and score behaviour from videos. These videos could potentially be automatically analysed using technology.

To illustrate the time saving of computer vision applied to measure activity in pigs, the time investment using the algorithm as developed in Chapter 3 was compared with the estimated time investment of human observations. In Chapter 5, recordings of 21 pens with six individuals each, containing 178 hours of video per pen, were analysed. This includes the nights, when pigs are mostly sleeping. When a human observer would analyse the video recordings per pen, replaying video in real time, the total observation time would be 3,738 hours, assuming that it is feasible to observe all six pigs per pen at the same time. One observer working 40 hours per week would thus need almost 2 years to complete the observations. When videos could be played at a higher speed when observing, the observation time could be reduced. Watching 12 hours of footage taken during the day with active pigs in the pen at double speed, and 12 hours of footage recorded at night at 10x speed would still take the observer 1,121 hours, or 28 working weeks to complete the observations. To minimize these extreme observation times, scan sampling is often used, but this leads to less accurate activity measurement. Computer vision would enable continuous collection of data, with less effort.

The tracking algorithm described in Chapter 3 and applied in Chapter 5 included different steps. The first step from video recording to data, was the analysis of the videos by the tracking algorithm. To analyse all 3,738 hours of video, took the PC* approximately 1,000 hours, which is 3.7 times faster than human observation in real time. Another benefit is that a computer can run 24 hours a day and 7 days a week, so all videos were analysed within 50 days. At this stage, the tracking algorithm generated individual tracks with a virtual ID coding, hence it was unknown which track belonged to which individual pig. This was corrected using human labour. This part was the most time-consuming part of obtaining the data for Chapter 5. In total 164,418 virtual IDs were created for 126 individual pigs. All of them needed to be traced back to either a false positive detection or to the corresponding colour marking of the pig. This reviewing process took in total approximately 350 hours, or almost 9 working weeks to complete. To conclude, a fast human observer, when videos are sped up, would need 32 weeks to complete the behaviour observation, while the current set-up needed 7 weeks of computer time and 9 weeks of human labour (Figure B7.1.1). The current set-up is already tremendously faster with regards to human labour. When IDs could be updated with for example RFID information, reviewing of virtual IDs by humans would be further reduced (Guzhva et al., 2018). In addition, the tracking algorithm now only detects location, but it is expected that more complex behaviours could be observed by computer vision. Complex observations take more time for the human observer.

*Specifications of the PC; GPU: NVIDIA Geforce 2080 Super; CPU: AMD Ryzen 5 6-Core Processor; memory: 32GB RAM

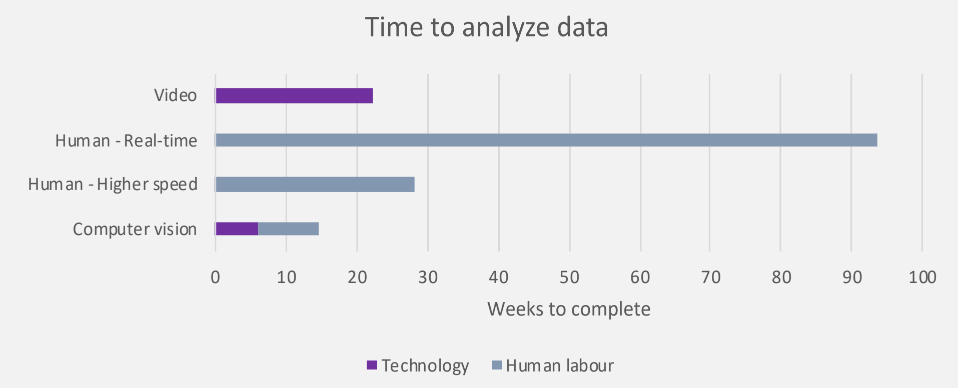


Figure B7.1.1 – Overview of weeks to complete analysis of the videos used in Chapter 5 as an illustration of the potential of computer vision to save labour. Video represents the total duration of the videos to analyse. Human – Real-time represents the number of weeks it would take for a human observer to replay and analyse all videos in real-time speed, assuming all six pigs per pen can be observed simultaneously. Human – Higher speeds represents the number of weeks to analyse the videos when half of the 24-h cycle (night) is sped up 10 times and the other half (day) 2 times. Computer vision has a technology part which is automated, and a human labour part, where human input is required to identify and correct the IDs of the pigs.

physical space to operate, where computer vision is limited to the field of view of the camera. In addition, accelerometers are robust. They are minimally affected by dust, water, ammonia and other influences of the environment. Computer vision is dependent on the view of a camera, and dust, flies and other environmental influences might hamper the view of the camera (Ardö et al., 2018).

Once trained, computer vision algorithms can visually confirm the behaviour of interest, where accelerometers work as a black box approach, and only videos to train machine learning models to recognize behaviours of interest would be stored. To illustrate this, Chapter 2 classified 5-second windows of accelerations as active or non-active. In the data of Chapter 5, we spotted in one batch higher activity three days post-challenge - mostly in enriched pens. After reviewing the videos, it turned out that the bedding material in the enriched pens was completely replenished, which caused extra activity in almost every pen in this batch. However, when computer vision is applied on a larger scale, it would be costly to store all videos and the algorithm would be directly applied to the video stream, deleting videos or storing them only for a short period of time, which will result in a similar black box approach. Computer vision is a non-invasive method, as there is only a camera recording the pigs. Where accelerometers contain a battery, which limits running time, cameras are connected to the power net. The biggest advantage of computer vision over accelerometers is the possibility to observe more complex behaviours. Computer vision algorithms could be trained to recognize certain complex behaviours, where accelerations have more noise due to random motion of the sensor, making it harder to recognize such behaviours (Hounslow et al., 2019). Especially behaviours with a short duration, such as aggressive or tail biting interactions, would be easier for computer vision to recognize than for accelerometers. In addition, computer vision algorithms can estimate the spatial information (i.e., individual location) within the field of view.

Table 7.1 – Overview of advantages of accelerometers and computer vision to assess individual activity of pigs.

Accelerometers	Computer vision
Identity fixed in hardware	Visual confirmation of behaviour
Not bound to a narrow field of view of a camera	Non-invasive to the animal
Robust	Not dependent on a battery
	More complex behaviours possible
	Spatial information

Unfortunately, both technologies still have limitations that hamper implementation on a larger scale. I will discuss some of the main issues for both accelerometers and computer vision. Although this does not cover all limitations, it provides perspective on the direction of future development.

The main limitation of accelerometers is that animals could lose their sensor or that the sensor stops working. De Mol et al. (2013) showed the performance of accelerometers attached to the fetlock joint of the rear leg of a cow. Where 100 sensors were available for this study, on the worst day, less than 10 sensors provided valid measurements. After approximately 700 days and renewal of all sensors three times during the experimental period, all problems with the sensors were solved and nearly all sensors provided valid acceleration measurements. In addition, the resolution (i.e., sampling rate) of the accelerometer affects the level of detail that is measured, but high-resolution increases costs, data storage, and computing requirements, while reducing battery life (O'Leary et al., 2020). Finally, the placement of the sensors for pigs is challenging. Accelerometers could be attached to the neck, ear, back or legs of the pig using a collar, adhesive tape, ear tags or velcro (Cornou et al., 2011; Oczak et al., 2015; Ringgenberg et al., 2010; Thompson et al., 2019). Applications of sensors in pigs are always at risk of being pulled off or damaged by pen mates, given the explorative nature of pigs. The functionality of accelerometers is dependent on the placement of the sensor, as damaged or lost sensors do not provide reliable data.

The main challenge for application of computer vision is the ability to recognize and reidentify individual pigs. Pigs have a similar appearance and due to their social nature and often small housing, pigs tend to be close to each other causing occlusions. Pigs often have ear tags with unique numbers that link to, for example, a management system. No computer vision algorithms are available to recognize numbers on standard ear tags. Psota et al. (2020) designed a set of ear tags with a variety of different colours and alphanumeric characters that could be recognized from various viewing positions. These ear tags make it possible for a computer vision system to recognize animals and the video data can be directly linked to other data collected on the animal. Another possibility to overcome identity issues is the use of other sensors. RFID chips can be incorporated in regular ear tags. An antenna reads the RFID tag, and this read-out could be linked to a computer vision algorithm to update the identity of animals. Another limitation of computer vision might be the overestimation of movement. In contrast to the inaccuracy of accelerometers, this inaccuracy is not caused by micro movements of the sensor, but caused by the algorithm itself. The algorithm still has room for improvement and could overcome the overestimation, whereas accelerometers record micro movements such as flapping of the ear. Chapter 5 showed a 34.1 fold overestimation of distance moved by pigs in enriched pens. This overestimation of distance moved was mainly caused by micro movements of the bounding box when pigs were inactive. Ideally, only frames with movement should be used to estimated distance moved. **Box 7.2** shows the implementation of a threshold to move to the next frame (variable frame rate to calculate movement) to prevent the overestimation observed in Chapter 3. Different thresholds to move to the next frame were tested. Only when distance moved exceeded the threshold, distance moved was calculated between the frames, otherwise the frame was skipped. As shown in **Box 7.2**, a threshold of 2.4 cm (2 pixels) is already enough the reduce the overestimation to a minimum.

7.2.2 Traits measured by accelerometers and computer vision beyond activity

This thesis focussed on measuring activity using accelerometers and computer vision. However, more traits could be observed or deduced from the activity data gathered using these technologies. Since the trait of interest influences the choice of technology used, I will discuss per technology which traits could be measured.

Most accelerometers, just as in Chapter 2, are used to measure activity (Cornou et al., 2011; Cornou and Lundbye-Christensen, 2010; Escalante et al., 2013; Oczak et al., 2016). However, activity can include multiple behaviours, such as walking, rooting, feeding, standing and nest building, and these were not distinguished in the studies mentioned in Chapter 2. Certain active behaviours could potentially be detected separately. For instance, supervised machine learning could be used to recognize labelled behaviours from acceleration patterns (Escalante et al., 2013). Feeding could be recognized based on larger acceleration variations. Escalante et al. (2013) reported that 75% of all feeding observations were classified correctly when the accelerometer was attached to a neck collar. Thompson et al. (2019) attached two accelerometers to the back of the pigs and found an accuracy of 60% for feeding behaviour when the two sensors were combined. Feeding was most often confused with rooting (25%), which involves similar movements directed at the floor of the pen (Escalante et al., 2013). Other studies also classified lying laterally and lying sternally from acceleration data. Lying could be used as an indicator for the onset of farrowing, because sows spend less time lying down just before the onset of farrowing. The accuracy of both lying laterally and ventrally was above 95%,

Box 7.2 Variable frame rate to prevent overestimation

Chapter 3 showed an overestimation of activity, especially in periods with low activity. I propose to only use the frames to calculate distance moved that exceed a certain threshold. The gold standard used in Chapter 3 was used to test this solution for overestimation. **Table B7.2.1** shows the distance moved for the annotated gold standard, the tracking algorithm without a threshold (i.e., original distance moved based on 0.5 fps), and thresholds of 1, 2, or 3 pixels (1 pixel is 1.2 cm). When the pig moved less than the threshold between frame 1 and 2, the distance between frame 1 and 3 was calculated. If the pig moved less than the threshold between frame 1 and 3, the distance between frame 1 and 4 was calculated, and so on, until the threshold was exceeded. The table shows a stable distance moved, irrespective of threshold, close to the gold standard for high activity periods. Without threshold, the low activity periods had an average overestimation of distance moved of 18 times the original gold standard. By introducing the threshold of only a few pixels, this overestimation in low activity periods is reduced, with an optimum at 2 pixels. The threshold set at 3 pixels already underestimates distance moved.

Table B7.2.1 – Overview of distance moved in meters using the annotated gold standard, or based on the original tracking algorithm using 0.5 fps with no threshold (Chapter 3), or using a threshold of 1, 2 or 3 pixels (px) for movement. In bold the average overestimation compared to the gold standard expressed in a ratio (algorithm:gold standard). Three videos (1, 2, 3) of barren and enriched housing with high and low activity (high, low) were annotated for 3 minutes (n=12).

	Distance moved (m)				
Video	**Gold standard**	**No threshold**	**1 px threshold**	**2 px threshold**	**3 px threshold**
Barren – high 1	14.94	14.87	14.83	14.67	14.55
Barren – high 2	3.62	3.90	3.66	3.33	2.99
Barren – high 3	7.82	11.71	11.69	11.61	11.29
Enriched – high 1	10.36	11.52	11.46	11.15	10.81
Enriched – high 2	15.25	15.19	15.11	14.86	14.66
Enriched – high 3	23.65	23.75	23.58	23.35	22.99
High activity ratio	**1.00**	**1.11**	**1.10**	**1.07**	**1.04**
Barren – low 1	0.04	0.61	0.17	0.06	0.01
Barren – low 2	0.05	0.77	0.32	0.10	0.10
Barren – low 3	0.07	0.83	0.37	0.01	0.01
Enriched – low 1	3.32	3.55	3.33	2.94	2.62
Enriched – low 2	0.09	0.77	0.38	0.18	0.09
Enriched – low 3	0.01	0.72	0.26	0.01	0.01
Low activity ratio	**1.00**	**18.34**	**7.07**	**1.21**	**0.84**

when the accelerometer was attached to a neck collar (Cornou and Lundbye-Christensen, 2010). The extent to which accelerometers correctly detected the posture for both lying types was over 90% when an accelerometer was attached to the back and hind leg (Ringgenberg et al., 2010). Oczak et al. (2015) studied nest building behaviour using accelerometers. They attached accelerometers to the ears of the sows and showed a sensitivity of 87% for nest building behaviour. So, it is possible to accurately detect some specific behaviours in pigs using accelerometers attached to different body parts.

Chapter 3 showed the calculation of distance moved based on locations of individual pigs based on computer vision. However, location could be used for other traits, such as proximity to other pigs and presence in a certain zone. Distance moved was calculated based on the distance between two different frames of the same pig. Proximity could be calculated as the distance between individual pigs within a single frame. Proximity could be a useful indicator for social behaviours or social network analysis (Goumon et al., 2020). In addition to proximity, detection of the location could be utilized, for example the presence of a pig in a dunging area or around the feeding trough could be detected (Alameer et al., 2020b). Kashiha et al. (2013) equipped pens with cameras to monitor water usage on a pen level by recording drinking nipple visits. Results showed that half-hourly water usage could be estimated with an accuracy of 92%. Besides presence at a certain location, other traits could be recorded by computer vision technology. Weight could be estimated used 3D cameras. Depth information from the top could be used to estimate the shape and height of the pig (Kongsro, 2014). Several studies show a strong correlation between estimated weight based on camera observation and recorded weight from a weighing scale (Cang et al., 2019; Kongsro, 2014; Pezzuolo et al., 2018). Similarly to accelerometers, computer vision can also be used to detect postures like lying and standing. Shao et al. (2021) used a deep convolutional neural network to detect different postures. They classified different postures with an accuracy of 92%. Another study detected standing, lying laterally and lying sternally with an average precision of over 90% (Nasirahmadi et al., 2019). There is even a computer vision algorithm developed to predict tail biting outbreaks. D'Eath et al. (2018) used 3D cameras to detect tail postures, where lowered tail postures could indicate an upcoming tail biting outbreak. They found an accuracy of 74% in detecting low tails. A more recent study showed 89%

accuracy of direct detection of tail biting behaviour using computer vision (Liu et al., 2020). However, this method is applicable to tail biting interactions with escaping and chasing. Early signs of tail biting do not necessarily invoke a reaction of the victim pig, which is not detected by this method.

This exemplifies that based on data obtained from both accelerometers and computer vision, more behavioural traits besides activity can be measured. As these technologies develop rapidly, it is expected that more and more types of behaviours can be detected in the future.

7.2.3 Activity measurements at individual or group level

In this thesis, activity as a potential indicator of resilience was assessed on an individual level. However, resilience can also be a characteristic of a group of animals, referred to as herd resilience. Herd resilience depends on the adaptive capacity of the individuals in the herd (i.e., depends on individual resilience), together with social interactions and management decisions (Blanc et al., 2013). For example, in the case of disease pressure, an infected individual could contribute to the spread of the disease, and potentially affect the health and performance of susceptible animals. Hence, herd resilience as a whole is more than just the sum of individual resilience of each animal in the herd. The main challenge of computer vision is maintaining individual identities. If (loss of) herd resilience is reflected in changes in activity, it would be sufficient to measure activity at the group level to detect loss of resilience. As, in that case, pig identities do not need to be detected or maintained, herd resilience seems therefore easier to measure with technology than individual resilience (Doeschl-Wilson et al., 2021). Measurement of activity at a group level can be advantageous to detect upcoming problems, such as tail biting outbreaks (Ursinus et al., 2014).

However, there are two reasons to still invest in measurements of individual activity as an indicator of resilience. The first reason is that individual data are useful as a management tool. Technology could assist the farmer when for example resilience is impaired. Technology providing alerts at an individual level could save time for the farmer as it pinpoints exactly which animal needs extra care. This could aid reduction of the usage of antibiotics and other medicines, as individual animals could then be treated instead of the whole group (Alameer et al., 2020a). In addition, with an early warning system, treatment duration might be shorter. Another reason to invest in detection of activity changes at the individual level as potential proxy for resilience is genetic improvement of resilience traits (read more in Chapter 7.3.2). Resilience traits are found to be partially heritable (Berghof et al., 2019b; Knap and Doeschl-Wilson, 2020), meaning that it is possible to breed into a desired direction. In order to do so, individual data are necessary.

7.3 Resilience in pig husbandry

Resilience could enhance pig welfare, as fast-recovering, i.e., resilient pigs will have a shorter negative experience from a perturbation and, following the definition of Colditz and Hine (2016) (see below), a less intense response to it. Given its implications for pig welfare, resilience is highly relevant to assess and improve, but it is not as directly or easily measurable in pigs as, for instance, growth. Proxies, which are indirect measures of a related trait, are needed to measure resilience. This section will put several proxies that were measured in Chapters 2 and 4 to 6 as potential indicators of resilience into perspective. Thereafter, possible ways to improve resilience in pigs will be discussed.

7.3.1 Proxies of resilience

Resilience is according to Colditz & Hine (2016) defined as “the capacity of the animal to be minimally affected by a disturbance or to rapidly return to the physiological, behavioural, cognitive, health, affective and production states that pertained before exposure to a disturbance”. Scheffer et al. (2018) defined resilience as “the capacity to bounce back to normal functioning after a perturbation”. Both definitions emphasise the return to the original state after a perturbation or a challenge. This could be seen as the recovery speed of an animal, where an animal that returns faster to the original state is more resilient than an animal that returns slower to the original state. Colditz & Hine (2016) also include “to be minimally affected by a disturbance”. This could be reflected in the strength of the response of the animal to the disturbance. Thus, using the resilience definition of Colditz & Hine (2016), both the characteristics of an animal’s response to a challenge and the recovery from that challenge can be used to get information about the resilience of that individual. This approach has been followed in Chapter 4, in which both the dynamics of several physiological responses to different challenges, as well as the strength of the responses were characterized. For this purpose, blood samples were taken at regular intervals, and, for the LPS challenge only, the rectal temperature response was assessed as well. For several physiological responses (e.g., cortisol, Non-Esterified Fatty Acids (NEFA) and rectal temperature), a clear return to baseline was seen, whereas haptoglobin and urea levels were still rising at the final sampling point, so in hindsight ideally more samples should have been taken to assess the recovery of these latter variables. The chosen sampling time points influence the recovery curve. This underlines that the dynamics of the response of a particular variable need to be known to fully assess the recovery of that variable. Also in Chapter 5, in which changes in activity were assessed following an LPS challenge, variables were used to both reflect the strength of the response (e.g., the maximum deviation from the mean activity) and the recovery (e.g., the duration of the changed activity). In Chapter 2, the dynamic patterns in activity level after PRRSV infection were measured as a reflection of the strength of the response to the virus. The change in the level of activity was predictive of the probability of morbidity, i.e., showing clinical signs of disease, suggesting that activity changes in response to the infection reflect resilience (Chapter 2).

The response of the physiological variables to the diverse challenges in Chapter 4 and the activity changes following LPS injection in Chapter 5 were also used to calculate the Area Under the Curve (AUC). The AUC fits the definition of resilience well (Colditz and Hine, 2016), as it reflects both the strength of the response and the recovery curve. Traditionally, as in Chapter 4, AUC is calculated for the area above or below the x-axis, i.e., above or below the zero level. However, the definition of resilience refers to 'recovery to the original state', i.e., the baseline. The difference between the original state, i.e., the baseline level, might be of influence on the calculation of the AUC. When two animals show the same response to a challenge, but with a different baseline, the one with a lower baseline will have a smaller AUC, while both animals have the same recovery curve. **Box 7.3** shows a comparison of the AUC of the cortisol response to the LPS challenge calculated using the x-axis, i.e., zero level, or the animal's baseline as minimum using the data from Chapter 4. The correlation between both types of AUC was 0.6. The traditional AUC was more strongly correlated to the baseline concentration of cortisol than the alternative AUC. However, both AUCs were correlated with the recovery time of cortisol, i.e., the

Box 7.3 – Different calculations of the Area Under the Curve

The Area Under the Curve is traditionally calculated for the area above or below the x-axis (**Figure B7.3.1**). The definition of resilience includes the ability of an animal to rapidly return to the original state, i.e., the state before the disturbance. For physiological parameters, the original state could be measured by the baseline level of the given parameter, which is usually not zero. The difference between baseline and the x-axis, i.e., the baseline level, might potentially bias the resilience status based on AUC, as two animals with the same recovery curve but a different baseline will have different AUCs. Using data of the cortisol response to LPS from Chapter 4, correlations between the traditional AUC to the x-axis (here referred to as AUC x-axis) and an alternative AUC to the baseline (referred to as AUC baseline), the baseline and peak levels were calculated (**Table B7.3.1**). In addition, the recovery time was calculated as the duration for cortisol levels to return to the baseline level using the slope between measurements.

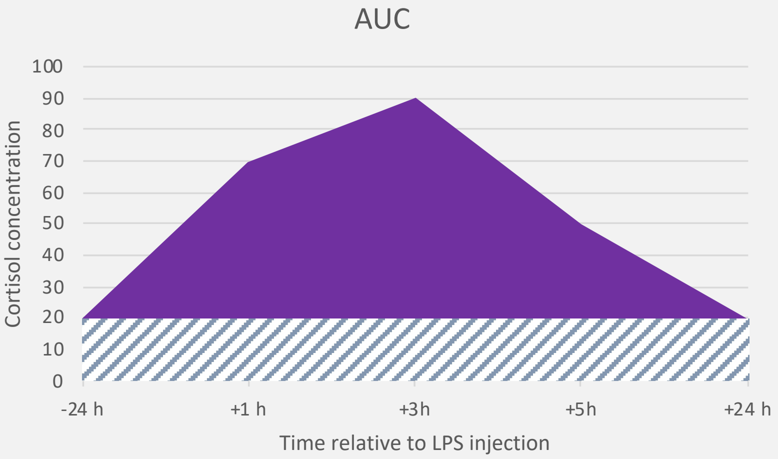


Figure B7.3.1 – Graphical example of the Area Under the Curve (AUC), where AUC baseline consist of only the solid purple area and AUC x-axis combines the solid purple area and slatted grey area.

The two different methods to calculate AUC of cortisol in response to the LPS challenge were not perfectly correlated, indicating indeed a difference between the two AUCs. Remarkable is the different correlation between the two AUCs with the baseline level. AUC x-axis and baseline levels showed a positive correlation of 0.53 and the correlation between AUC baseline and baseline levels was -0.35. If the AUC baseline would only represent the recovery curve, irrespective of the baseline level, it would be expected that the correlation would be zero. The negative correlation found, could be due to the small number of animals used for this correlation matrix (n = 80). Larger datasets might give a better representation of the population and correlations could become weaker. On the other hand, animals with a higher baseline concentration cortisol level could already be in a different state (e.g., more stressed), potentially influencing their response. Both AUCs were strongly correlated with recovery time and peak level cortisol.

Based on these correlations, it can be concluded that the influence of the baseline level of cortisol on AUC x-axis was not as strong as expected, but was still present. The peak of cortisol was almost five times as high as the baseline level (Chapter 4), which probably causes a stronger correlation of the AUCs with the peak level of cortisol than with the baseline level of cortisol. The recovery time (estimated time between injection of LPS and return to baseline) was strongly correlated with AUC x-axis. Therefore, both AUCs seem to provide a suitable representation of the recovery curve and the strength of the response (i.e., peak level) in this small dataset. These results should be verified in larger datasets and with additional physiological parameters.

Table B7.3.1 – Phenotypic correlations (R) of different characteristics of the cortisol response to LPS.

	AUC x-axis	AUC baseline	Baseline level	Peak level	Recovery time
AUC x-axis	1				
AUC baseline	0.60	1			
Baseline level	0.53	-0.35	1		
Peak level	0.77	0.61	0.25	1	
Recovery time	0.77	0.57	0.29	0.37	1

time needed to return to baseline level. Therefore, in this case of the cortisol response to LPS, both AUCs seemed to represent the speed of recovery. When a large variation in the baseline of a variable is observed, it is advised to use the AUC calculated from the pre-challenge baseline to quantify the resilience to a challenge. Therefore, in Chapter 5, in which characteristics of the activity dip following LPS injection were assessed, first the baseline activity of each individual was calculated (based on a three-day pre-challenge average), as activity was highly variable between individuals and, moreover, was influenced by housing. The importance of measuring baseline activity is further stressed by the results of Chapter 2, in which not the activity level per se, but the change in activity patterns following PRRSV as compared to baseline levels tended to be predictive of morbidity and mortality to PRRSV.

Apart from changes in the level of relevant biological variables per se, deviations in longitudinal patterns of repeatedly or continuously measured variables may be related to resilience (Gijzel, 2020; Scheffer et al., 2018). Parameters that capture deviations in such longitudinal patterns, such as variance, skewness, variation and autocorrelation of deviations, are then used as dynamical indicators of resilience (DIORs). These DIORs could give an impression of a system's or an animal's capacity to recover (Gijzel, 2020). Multiple variables could be used to calculate DIORs, however, it is important that the variable is based on measurements capturing system dynamics, i.e., changes over time, and acts as an indicator of a (non) resilient outcome (Gijzel, 2020). Examples of such variables in livestock are daily milk yield in dairy cattle (Poppe et al., 2020), body weight development in chickens (Berghof et al., 2019a), or feed intake patterns in pigs (Putz et al., 2019). Poppe et al. (2020) used lactation curve deviations to estimate variation in daily milk yield in dairy cows, as milk production responds to various kinds of disturbances, such as compromised udder health and ketosis. They found that a lower variance in milk yield was genetically associated with better udder health, longevity and fertility, as well as a smaller risk of ketosis, and a higher body condition score. In a follow-up study, low variance in milk yield was also genetically associated with a weak response in milk yield to a heat wave (Poppe et al., 2021). Additionally, low autocorrelation in milk yield was genetically correlated with reduced perturbation length and quick recovery after the heat wave (Poppe et al., 2021). Berghof et al. (2019) calculated DIORs based on body weight deviations in laying hens. They found a weak relationship between immunity and disease resistance. Putz et al. (2019) used root mean square error (RMSE) to measure the variation in daily feed intake in finisher pigs and identified a positive relationship between variation in feed intake, and mortality and number of medical treatments.

In Chapter 2, apart from the change in activity following PRRSV infection per se, skewness, autocorrelation, and RMSE based on continuous activity monitoring around a PRRSV infection were calculated. It was found that a higher RMSE post-infection and an increase in RMSE from pre- to post-infection tended to increase the risk of morbidity. In addition, a higher skewness in activity post-infection and a larger change in skewness from pre- to post-challenge tended to be and were, respectively, associated with a higher risk of mortality. This suggests that, also in pigs, dynamic patterns in a continuously measured variable, i.e., activity, could be used to assess resilience. Another indication that deviations in patterns might reflect resilience in pigs is the lower variance in weight gain in enriched-housed pigs, which generally showed improved resilience to challenges, found in Chapter 4. It should be noted, though, that not all potential DIORs used in Chapter 2 were strongly related to morbidity and mortality. Moreover, autocorrelation based on activity was not related to resilience to PRRSV at all. Where this thesis only found relationships between skewness and RMSE and morbidity and mortality to PRRSV, others found relationships between particular DIORs and resilience (Berghof et al., 2019a; Poppe et al., 2021, 2020; Putz et al., 2019) that were not found in Chapter 2. Each potential DIOR may capture different aspects of resilience (for details see Berghof et al., 2019a). Moreover, the main difference between our results and those of others is the longitudinally measured variable used to calculate DIORs. In Chapter 2, DIORs were calculated based on activity, whereas others used performance traits like daily milk yield or growth. Both performance traits and activity will deviate from their original state following a perturbation, however, physiological traits often seem to fluctuate within a boundary and have a 'natural' homeostasis, which does not hold for activity (Gijzel, 2020). In addition, activity can easily be influenced by environmental factors, for instance forced movement may occur in pigs when bedding material is replenished, which would lead to deviations in activity likely unrelated to changes in resilience. Nevertheless, Chapter 2 did demonstrate a relationship between some DIORs based on activity and mortality and/or morbidity following a PRRSV infection. Moreover, the change in activity per se (delta from baseline) in Chapter 2 tended to predict morbidity. Chapter 5, additionally, demonstrated that characteristics of the activity dip following LPS injection can be of added value to characterize the sickness response in pigs. Taken together, this indicates that activity patterns and deviations therein are, supplementary to physiological variables, promising indicators of (loss of) resilience in pigs.

7.3.2 Improvement of resilience

The phenotype of an animal is established by its environment and its genotype. Therefore, there are two ways to enhance the resilience of pigs. First, by improving their (early life) environment (as investigated in Chapter 4), or by genetic selection of pigs that are better able to cope with disturbances.

Chapter 4 demonstrated that an alternative housing system, encompassing group housing during lactation, delayed weaning, more space and provision of enrichment, reduced the degree to which animals were affected by various challenges. Pigs from the alternative system, here referred to as enriched housed pigs, showed a faster recovery and/or a lower response than conventionally housed pigs, referred to as barren housed pigs, in several physiological indicators following a 2h transport, a heat stress challenge and an LPS challenge. Here we found that enriched housed pigs showed lower O_2 consumption and higher lower CH_4 and CO_2 production in a heat stress challenge and lower NEFA in a transport challenge compared to barren housed pigs. Further, enriched housed animals had a lower cortisol and NEFA response following LPS injection compared to barren housed animals. Enriched housed animals also had lower cortisol accumulation in hairs, reflecting lower accumulative stress, than barren housed animals. This enhanced resilience in pigs from the alternative housing system would be in line with suggestions from other studies that raising animals in housing conditions that promote the satisfaction of their essential behavioural needs may improve resilience (Colditz and Hine, 2016; Fraser, 1988; Ko et al., 2020; van Dixhoorn et al., 2016). There could be several reasons for improved resilience of pigs raised in more enriched conditions. First, a more stimulating early life environment could facilitate the development of skills that are beneficial for coping with perturbations. Examples of these are social skills promoted by mingling of piglets to reduce the impact of mixing (Camerlink et al., 2018; Ko et al., 2020; van Nieuwamerongen et al., 2014) or improved development of foraging and feeding behaviour through eating with adult sows and provision of rooting materials to reduce weaning stress (Oostindjer et al., 2014b, 2011a), both of which of were applied in the alternative system. In addition, also enrichment later in life may improve the resilience of pigs as compared with pigs kept in more barren conditions (e.g., Luo et al., 2022, 2020). It has been shown that hampering the execution of important behaviours in barren housing causes chronic stress (Beattie et al., 2000; de Jong et al., 2000b). The ‘wear and tear’ on the body imposed by chronic stress may diminish the pigs’ resilience to future challenges. Finally, enriched pigs could utilize features from their environment to reduce stress arising from a disturbance, for instance, root in the bedding materials applied when feeding is frustrated (Mkwanazi et al., 2019).

Besides through the improvement of the environment, resilience could potentially be enhanced when incorporated in the breeding program. The potential to enhance resilience through breeding and the effect of resilience traits in a selection index depends on the heritability of the traits. Heritability is the proportion of a phenotype that is explained by genetics. The higher the heritability, the larger the effect will be in the breeding program. Heritability of resilience proxies has been investigated in a number of studies in other species. Variance in daily milk yield had the highest heritability among various DIORs in dairy cows, ranging from 0.20 to 0.24 (Poppe et al., 2020). In laying hens, the heritability of variance in body weight was estimated to be 0.10 (Berghof et al., 2019a). Heritabilities of autocorrelation and skewness in daily milk yield of dairy cows and body weight of laying hens were low (Berghof et al., 2019a; Poppe et al., 2020).

Berghof et al. (2019) showed the potential to improve resilience of livestock through inclusion of a simulated resilience indicator in the breeding goal. In a simulation of a simplified breeding program, the responses in the breeding goal were shown. When resilience and growth would have an unfavourable genetic correlation, with only growth rate in the selection index, no progress could be made to obtain more resilient animals. Balanced breeding, with the inclusion of a resilience indicator in the breeding goal, could result in obtaining more resilient animals in the next generations, while still improving performance. A positive genetic correlation between resilience and performance could already improve resilience when only performance is in the selection index. However, the exact genetic correlation between resilience and performance in pigs is unknown. Further research is needed to estimate genetic correlations between resilience and existing traits in the selection index.

The pigs used in Chapter 4 were all genotyped and therefore an exploratory genetic analysis on the cortisol response to LPS, which was one of the physiological traits that may reflect resilience, could be performed. Results from **Box 7.4** show that cortisol baseline concentrations, as well as the AUC, baseline, and peak level following LPS injection are heritable. The heritability reported in **Box 7.4** (0.22-0.37) is relatively low, as in literature, heritability of cortisol response to LPS ranged from 0.36 to 0.68 (Larzul et al., 2015). These heritabilities indicate that approximately 50% of the cortisol response is explained by genetics and the remaining 50% is explained by other factors. **Box 7.4** also showed a weak phenotypic correlation between baseline level and AUC to the x-axis of cortisol. However, the genetic correlation is close to one. If

Box 7.4 – Estimation of genetic parameters of cortisol response to LPS

In Chapter 4, 92 animals were exposed to an LPS challenge. This number is too low to properly estimate genetic parameters. Therefore, data of the study of Luo et al. (2022) were included in which the same protocol was used. This added 384 animals to the dataset to a total of 518 animals. All animals were genotyped. Using a bivariate animal model in ASReml with the different studies as fixed effect and the animal as random effect, genetic parameters were estimated of the traditional x-axis AUC, baseline and peak level of cortisol in the LPS challenge. **Table B7.4.1** shows the heritability, phenotypic and genetic correlations of the AUC of the cortisol response to an LPS challenge, the baseline and peak level.

Heritability of the cortisol baseline concentrations as measured in an LPS challenge was calculated to be 0.22 ± 0.09. The genetic correlation between AUC and both baseline and peak level of cortisol was close to 1. In practice, this indicates that when selecting for lower baseline or peak levels, the AUC of the cortisol response to LPS would also decrease.

Table B7.4.1 – Heritability (on diagonal), phenotypic correlations (above diagonal) and genetic correlations (below diagonal) of area under the curve (AUC), baseline concentration and peak level of cortisol as measured around the LPS challenge in Chapter 4 and Luo et al. (2022).

	AUC	**Baseline level**	**Peak level**
AUC	0.23 ± 0.09	0.20 ± 0.05	0.68 ± 0.03
Baseline level	0.96 ± 0.28	0.22 ± 0.09	0.23 ± 0.05
Peak level	0.94 ± 0.09	0.72 ± 0.23	0.37 ± 0.1

baseline levels of cortisol and the cortisol response are indeed strongly genetically correlated, and if they represent a meaningful aspect of resilience, baseline levels might be used for genetic selection of resilience. However, these results are based on a small population, and on the response of one physiological variable to a single challenge, and they should be validated in a larger and broader study. Moreover, further research should elucidate the consequences of selection based on the response of a single physiological variable to a single challenge for resilience of pigs.

7.4 Implementation of enhanced resilience into society

The animal welfare legislation sets minimum welfare standards for pork production in the EU. Despite welfare regulations within the EU, the pig industry still faces major animal welfare challenges (Pedersen, 2018). Enhanced resilience could potentially aid improvement of pig welfare, as resilient pigs will be less affected by challenges and recover more quickly. Chapter 4 showed that the other way around is also possible, i.e., housing pigs in a system that better meets their behavioural needs was found to enhance resilience. This section will elaborate on what is needed in society to further improve resilience and thereby animal welfare.

This thesis adds to the abundance of evidence that technology will have an important role in future pig production. Technology could aid the farmer in managing their animals. For instance, it might help to prevent outbreaks of diseases or of damaging behaviours like tail biting, monitor farrowing and record water usage. As shown in this thesis, technology might also be used to record indicators of resilience, and therewith help in detection of poor resilience and in the evaluation of the effects of management and housing conditions on resilience. However, technology could also record unforeseen information, for example animal abuse or missed feeding time by an automatic feeder (Giersberg and Meijboom, 2022). Implementation of technology in livestock production could thus result in dilemmas regarding ethics, privacy and integrity. It is essential to create a culture of openness for discussion and reflection on these dilemmas to ensure safe and ethical use of technology (Giersberg and Meijboom, 2022).

Chapter 4 described an alternative housing system that provides enrichment from birth onwards. This system is considerably different in various aspects compared to conventional housing systems. Sows were not constrained in farrowing crates for the entire lactation period, piglets could mingle with other litters during lactation, more space was available, enrichment was provided, and piglets were weaned at a later age. It is not realistic to implement all these aspects in current housing systems. However, when new barns are built, the design of the housing system could be changed to take the biological needs of the pigs into account, for example the opportunity to root and build a nest. Enrichment and space are essential features of the environment for pigs to perform their natural behaviour. Proper environmental enrichment allows pig to actively interact with the materials by rooting and chewing, and more space provides them with a freedom of choice where to perform certain behaviours, and with more opportunities for locomotion and play behaviour. Animal welfare could also be enhanced in current housing systems. When for example the space availability cannot be increased, fewer pigs per pen could be kept, increasing the space per pig within the pen. Due to liquid slurry systems used to handle manure, pigs are rarely provided with straw bedding or only a limited amount of bedding material is given (Pedersen, 2018). So development of slurry systems that can handle more organic material is needed to allow housing systems to use more bedding material without blockage of the slurry system. Still, in existing systems, smart choices could be made regarding the selection of 'toys' and enrichment materials other than bedding, as, from the perspective of pigs, there are large differences in the quality of these materials and the extent to which they satisfy the needs of pigs to root and chew (van de Weerd et al., 2003; van de Weerd and Day, 2009). Delayed weaning up till nine weeks of age as implemented in the alternative system will be hard to apply in current systems, but there are options to allow for co-mingling of piglets during lactation by removing partitions between pens. This positively affects social behavioural development of piglets, reduces aggression, and improves coping with the weaning transition (e.g., Kutzer et al., 2009; Salazar et al., 2018; van de Weerd et al., 2003; van de Weerd and Day, 2009; van Nieuwamerongen et al., 2014). Another aspect of the alternative system applied in Chapter 4 is the opportunity of piglets to learn from the sow how and what to eat, which has beneficial effects on coping with weaning stress (see Oostindjer et al. (2014) for review). Promoting this sow-piglet information transfer is also possible in conventional systems, e.g., by lowering the sow's feeder to allow piglets to eat together with the sow. There are strong indications that application of only some of the features of the alternative system can promote resilience (e.g., Luo et al., 2022, 2020b; van Dixhoorn et al., 2018), albeit the effects may be less strong.

Besides providing environmental enrichment, resilience could also be improved through genetics. Breeding for enhanced resilience should go hand in hand with improvement of the environment. It should not be a strategy to select for pigs that better cope with suboptimal management and housing systems, as adapting animals to conditions with poor welfare standards does not equal good animal welfare. Ideally, first an ideal housing system should be designed that fits the needs of pigs, and then a breeding program should be used to enhance the resilience in that system. In this way, one breeds for enhanced resilience in an animal welfare friendly environment. Welfare friendly systems that meet the natural behavioural needs of pigs can still be challenging, e.g., due to a more complex social environment, and, even in those systems, pigs may be exposed to challenges that are hard to avoid, such as heat stress or disease. Therefore, breeding for enhanced resilience may have an added value to improve pig welfare.

7.5 Conclusions

In this thesis, accelerometers and computer vision were used to measure activity as a putative indicator for resilience. Changes in accelerometer-based activity patterns were related with morbidity and mortality following PRRSV infection. Computer vision-based measurement of activity showed the potential to automatically observe sickness behaviour following an LPS challenge. Characteristics of the sickness-induced activity decrease were weakly related to physiological response, so these seem to highlight different aspects of resilience. In addition, it was demonstrated that rearing in an alternative system with group farrowing, delayed weaning and an enriched environment enhanced resilience to several challenges, and reduced signs of chronic stress. This indicates that more welfare-friendly conditions may enhance general resilience of pigs. Taken together, the findings of this thesis indicate that automated activity measurements may be valuable to pinpoint resilience. However, more research is needed on the contribution of continuous behavioural monitoring to monitor resilience. Overall, the results of this thesis contribute to the understanding of resilience and future enhancement of animal welfare.

References

References

Aarnink, A.J.A., van den Berg, A.J., Keen, A., Hoeksma, P., Verstegen, M.W.A., 1996. Ef fect of Slatted Floor Area on Ammonia Emission and on the Excretory and Lying Behaviour of Growing Pigs, J. agric. Engng Res.

Ahrendt, P., Gregersen, T., Karstoft, H., 2011. Development of a real-time computer vision system for tracking loose-housed pigs. Comput Electron Agric 76, 169–174. https://doi.org/10.1016/j.compag.2011.01.011

Alameer, A., Kyriazakis, I., Bacardit, J., 2020a. Automated recognition of postures and drinking behaviour for the detection of compromised health in pigs. Sci Rep 10. https://doi.org/10.1038/s41598-020-70688-6

Alameer, A., Kyriazakis, I., Dalton, H.A., Miller, A.L., Bacardit, J., 2020b. Automatic recognition of feeding and foraging behaviour in pigs using deep learning. Biosyst Eng 197, 91–104. https://doi.org/10.1016/j.biosystemseng.2020.06.013

Almeida, M.N., Zimmerman, J.J., Wang, C., Linhares, D.C.L., 2018. Assessment of abattoir based monitoring of PRRSV using oral fluids. Prev Vet Med 158, 137–145. https://doi.org/10.1016/j.prevetmed.2018.08.002

Ardö, H., Guzhva, O., Nilsson, M., Herlin, A.H., 2018. Convolutional neural network-based cow interaction watchdog. IET Computer Vision 12, 171–177. https://doi.org/10.1049/iet-cvi.2017.0077

Aubert, A., 1999. Sickness and behaviour in animals: a motivational perspective. Neuroscience and Biobehavioral Review 23, 1029–1036.

Baes, C., Mattei, S., Luther, H., Ampuero, S., Sidler, X., Bee, G., Spring, P., Hofer, A., 2013. A performance test for boar taint compounds in live boars. Animal 7, 714–720. https://doi.org/10.1017/S1751731112002273

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, 443–450. https://doi.org/10.1017/S1357729800051791

Beattie, V.E., Walker, N., Sneddon, I.A., 1995. Effects of environmental enrichment on behaviour and productivity of growing pigs. Animal Welfare 4, 207–220.

Benjdira, B., Khursheed, T., Koubaa, A., Ammar, A., Ouni, K., 2018. Car Detection using Unmanned Aerial Vehicles: Comparison between Faster R-CNN and YOLOv3, in: 2019 1st International Conference on Unmanned Vehicle Systems-Oman (UVS). pp. 1–6.

Berghof, T.V.L., Bovenhuis, H., Mulder, H.A., 2019a. Body Weight Deviations as Indicator for Resilience in Layer Chickens. Front Genet 10. https://doi.org/10.3389/fgene.2019.01216

Berghof, T.V.L., Poppe, M., Mulder, H.A., 2019b. Opportunities to improve resilience in animal breeding programs. Front Genet 10. https://doi.org/10.3389/fgene.2018.00692

Best, A., White, A., Boots, M., 2008. Maintenance of host variation in tolerance to pathogens and parasites. Proceedings of the National Academy of Sciences 105, 20786–20791.

Bewley, A., Ge, Z., Ott, L., Ramos, F., Upcroft, B., 2016. Simple Online and Realtime Tracking, in: 2016 IEEE International Conference on Image Processing (ICIP). pp. 3464–3468. https://doi.org/10.1109/ICIP.2016.7533003

Blanc, F., Ollion, E., Piullet, L., Delaby, L., Ingrand, S., Tichit, M., Friggens, N.C., 2013. Principles for a quantitative evaluation of robustness at the animal and herd levels. Proceedings of the 20th Rencontres Autour des Recherches sur les Ruminants 20, 265–272.

Bolhuis, J.E., Schouten, W.G.P., Schrama, J.W., Wiegant, V.M., 2005. Behavioural development of pigs with different coping characteristics in barren and substrate-enriched housing conditions. Appl Anim Behav Sci 93, 213–228. https://doi.org/10.1016/j.applanim.2005.01.006

Bracke, M.B.M., Rodenburg, T.B., Vermeer, H.M., van Niekerk, T.G.C.M., 2018. Towards a common conceptual framework and illustrative model for feather pecking in poultry and tail biting in pigs-Connecting science to solutions Reading guide.

Bracke, M.B.M., Zonderland, J.J., Lenskens, P., Schouten, W.G.P., Vermeer, H., Spoolder, H.A.M., Hendriks, H.J.M., Hopster, H., 2006. Formalised review of environmental enrichment for pigs in relation to political decision making. Appl Anim Behav Sci. https://doi.org/10.1016/j.applanim.2005.08.021

Brunberg, E.I., Bas Rodenburg, T., Rydhmer, L., Kjaer, J.B., Jensen, P., Keeling, L.J., 2016. Omnivores going astray: A review and new synthesis of abnormal behavior in pigs and laying hens. Front Vet Sci. https://doi.org/10.3389/fvets.2016.00057

Buijs, S., Muns, R., 2019. A review of the effects of non-straw enrichment on tail biting in pigs. Animals. https://doi.org/10.3390/ani9100824

Bünger, B., Schrader, L., Schrade, H., Zacharias, B., 2015. Agonistic behaviour, skin lesions and activity pattern of entire male, female and castrated male finishing pigs. Appl Anim Behav Sci 171, 64–68. https://doi.org/10.1016/j.applanim.2015.08.024

Burdick Sanchez, N.C., Carroll, J.A., Randel, R.D., Vann, R.C., Welsh, T.H., 2014. Associations between endotoxin-induced metabolic changes and temperament in Brahman bulls,. J Anim Physiol Anim Nutr (Berl) 98, 178–190. https://doi.org/10.1111/jpn.12074

Camerlink, I., Farish, M., D'eath, R.B., Arnott, G., Turner, S.P., 2018. Long term benefits on social behaviour after early life socialization of piglets. Animals 8. https://doi.org/10.3390/ani8110192

Camerlink, I., Turner, S.P., Bijma, P., Bolhuis, J.E., 2013. Indirect Genetic Effects and Housing Conditions in Relation to Aggressive Behaviour in Pigs. PLoS One 8. https://doi.org/10.1371/journal.pone.0065136

Campbell, J.M., Crenshaw, J.D., Polo, J., 2013. The biological stress of early weaned piglets. ournal of Animal Science and Biotechnology 4.

Cang, Y., He, H., Qiao, Y., 2019. An Intelligent Pig Weights Estimate Method Based on Deep Learning in Sow Stall Environments. IEEE Access 7, 164867–164875. https://doi.org/10.1109/ACCESS.2019.2953099

Carpenter, S.R., Arrow, K.J., Barrett, S., Biggs, R., Brock, W.A., Crépin, A.S., Engström, G., Folke, C., Hughes, T.P., Kautsky, N., Li, C.Z., Mccarney, G., Meng, K., Mäler, K.G., Polasky, S., Scheffer, M., Shogren, J., Sterner, T., Vincent, J.R., Walker, B., Xepapadeas, A., de Zeeuw, A., 2012. General resilience to cope with extreme events. Sustainability 4, 3248–3259. https://doi.org/10.3390/su4123248

CBS, 2021. Varkensstapel, 1981-2020 [WWW Document]. URL https://www.cbs.nl/nl-nl/cijfers/detail/7373llb (accessed 3.16.22).

Chaloupková, H., Illmann, G., Bartoš, L., Špinka, M., 2007. The effect of pre-weaning housing on the play and agonistic behaviour of domestic pigs. Appl Anim Behav Sci 103, 25–34. https://doi.org/10.1016/j.applanim.2006.04.020

Chen, Y., Tibbs Cortes, L.E., Ashley, C., Putz, A.M., Lim, K.S., Dyck, M.K., Fortin, F., Plastow, G.S., Dekkers, J.C.M., Harding, J.C.S., 2020. The genetic basis of natural antibody titers of young healthy pigs and relationships with disease resilience. BMC Genomics 21. https://doi.org/10.1186/s12864-020-06994-0

Chung, Y., Kim, H., Lee, H., Park, D., Jeon, T., Chang, H.H., 2014. A cost-effective pigsty monitoring system based on a video sensor. KSII Transactions on Internet and Information Systems 8, 1481–1498. https://doi.org/10.3837/tiis.2014.04.018

Clouard, C., Souza, A.S., Gerrits, W.J.J., Hovenier, R., Lammers, A., Bolhuis, J.E., 2015. Maternal fish oil supplementation affects the social behavior, brain fatty acid profile, and sickness response of piglets. Journal of Nutrition 145, 2176–2184. https://doi.org/10.3945/jn.115.214650

Colditz, I.G., Hine, B.C., 2016. Resilience in farm animals: Biology, management, breeding and implications for animal welfare. Anim Prod Sci. https://doi.org/10.1071/AN15297

Cornou, C., Lundbye-Christensen, S., 2010. Classification of sows' activity types from acceleration patterns using univariate and multivariate models. Comput Electron Agric 72, 53–60. https://doi.org/10.1016/j.compag.2010.01.006

Cornou, C., Lundbye-Christensen, S., 2008. Classifying sows' activity types from acceleration patterns. An application of the Multi-Process Kalman Filter. Appl Anim Behav Sci 111, 262–273. https://doi.org/10.1016/j.applanim.2007.06.021

Cornou, C., Lundbye-Christensen, S., Kristensen, A.R., 2011. Modelling and monitoring sows' activity types in farrowing house using acceleration data. Comput Electron Agric 76, 316–324. https://doi.org/10.1016/j.compag.2011.02.010

Costa, A., Ismayilova, G., Borgonovo, F., Viazzi, S., Berckmans, D., Guarino, M., 2014. Image-processing technique to measure pig activity in response to climatic variation in a pig barn. Anim Prod Sci 54, 1075–1083. https://doi.org/10.1071/AN13031

Coutinho, A., Kazatchkine, M.D., Avrameas, S., 1995. Natural autoantibodies. Curr Opin Immunol 7, 812–818.

Cowton, J., Kyriazakis, I., Bacardit, J., 2019. Automated Individual Pig Localisation, Tracking and Behaviour Metric Extraction Using Deep Learning. IEEE Access 7, 108049–108060. https://doi.org/10.1109/ACCESS.2019.2933060

Cray, C., Zaias, J., Altman, N.H., 2009. Acute phase response in animals: a review. Comp Med 59, 517–526.

Day, J.E.L., van de Weerd, H.A., Edwards, S.A., 2008. The effect of varying lengths of straw bedding on the behaviour of growing pigs. Appl Anim Behav Sci 109, 249–260. https://doi.org/10.1016/j.applanim.2007.02.006

de Greeff, S., Mouton, J., 2017. NethMap 2017 Consumption of antimicrobial agents and antimicrobial resistance among medically important bacteria in the Netherlands Monitoring of Antimicrobial Resistance and Antibiotic Usage in Animals in the Netherlands in 2016.

de Greeff, S., Schoffelen, A., Verduin, C., 2020. NethMap 2020 Consumption of antimicrobial agents and antimicrobial resistance among medically important bacteria in the Netherlands. https://doi.org/10.21945/RIVM-2020-0065

de Groot, J., Kranendonk, G., Fillerup, M., Hopster, H., Boersma, W., Hodgson, D., van Reenen, K., Taverne, M., 2007. Response to LPS in female offspring from sows treated with cortisol during pregnancy. Physiol Behav 90, 612–618. https://doi.org/10.1016/j.physbeh.2006.11.013

de Jong, I.C., Lambooij, E., Korte, S.M., Blokhuis, H.J., Koolhaas, J.M., 1999. Mixing induces long-term hyperthermia in growing pigs. Animal Science 69, 601–605. https://doi.org/10.1017/s1357729800051456

de Jong, I.C., Prelle, I.T., van de Burgwal, J.A., Lambooij, E., Korte, S.M., Blokhuis, H.J., Koolhaas, J.M., 2000a. Effects of rearing conditions on behavioural and physiological responses of pigs to preslaughter handling and mixing at transport. Can J Anim Sci 80, 451–458.

de Jong, I.C., Prelle, I.T., van de Burgwal, J.A., Lambooij, E., Mechiel Korte, S., Blokhuis, H.J., Koolhaas, J.M., 2000b. Effects of environmental enrichment on behavioral responses to novelty, learning, and memory, and the circadian rhythm in cortisol in growing pigs, Physiology & Behavior.

de Mol, R.M., André, G., Bleumer, E.J.B., van der Werf, J.T.N., de Haas, Y., van Reenen, C.G., 2013. Applicability of day-to-day variation in behavior for the automated detection of lameness in dairy cows. J Dairy Sci 96, 3703–3712. https://doi.org/10.3168/jds.2012-6305

D'Eath, R.B., 2005. Socialising piglets before weaning improves social hierarchy formation when pigs are mixed post-weaning. Appl Anim Behav Sci 93, 199–211. https://doi.org/10.1016/j.applanim.2004.11.019

D'Eath, R.B., Foister, S., Jack, M., Bowers, N., Zhu, Q., Barclay, D., Baxter, E.M., 2021. Changes in tail posture detected by a 3D machine vision system are associated with injury from damaging behaviours and ill health on commercial pig farms. PLoS One 16. https://doi.org/10.1371/journal.pone.0258895

D'Eath, R.B., Jack, M., Futro, A., Talbot, D., Zhu, Q., Barclay, D., Baxter, E.M., 2018. Automatic early warning of tail biting in pigs: 3D cameras can detect lowered tail posture before an outbreak. PLoS One 13. https://doi.org/10.1371/journal.pone.0194524

DeBoer, S.P., Garner, J.P., McCain, R.R., Lay, D.C., Eicher, S.D., Marchant-Forde, J.N., 2015. An initial investigation into the effects of isolation and enrichment on the welfare of laboratory pigs housed in the PigTurn® system, assessed using tear staining, behaviour, physiology and haematology. Animal Welfare 24, 15–27. https://doi.org/10.7120/09627286.24.1.015

Dee, S., Guzman, J.E., Hanson, D., Garbes, N., Morrison, R., Amodie, D., Pantoja, L.G., 2018. A randomized controlled trial to evaluate performance of pigs raised in antibiotic-free or conventional production systems following challenge with porcine reproductive and respiratory syndrome virus. PLoS One 13. https://doi.org/10.1371/journal.pone.0208430

Docking, C.M., van de Weerd, H.A., Day, J.E.L., Edwards, S.A., 2008. The influence of age on the use of potential enrichment objects and synchronisation of behaviour of pigs. Appl Anim Behav Sci 110, 244–257. https://doi.org/10.1016/j.applanim.2007.05.004

Doeschl-Wilson, A., Knap, P.W., Opriessnig, T., More, S.J., 2021. Review: Livestock disease resilience: from individual to herd level. Animal. https://doi.org/10.1016/j.animal.2021.100286

Döring, T.F., Vieweger, A., Pautasso, M., Vaarst, M., Finckh, M.R., Wolfe, M.S., 2014. Resilience as a universal criterion of health. J Sci Food Agric 95, 455–465. https://doi.org/10.1002/jsfa.6539

Douglas, C., Bateson, M., Walsh, C., Bédué, A., Edwards, S.A., 2012. Environmental enrichment induces optimistic cognitive biases in pigs. Appl Anim Behav Sci 139, 65–73. https://doi.org/10.1016/j.applanim.2012.02.018

Elenkov, I.J., Chrousos, G.P., 2002. Stress Hormones, Proinflammatory and Antiinflammatory Cytokines, and Autoimmunity, Ann. N.Y. Acad. Sci.

Ellen, E.D., van der Sluis, M., Siegford, J., Guzhva, O., Toscano, M.J., Bennewitz, J., van der Zande, L.E., van der Eijk, J.A.J., de Haas, E.N., Norton, T., Piette, D., Tetens, J., de Klerk, B., Visser, B., Bas Rodenburg, T., 2019. Review of sensor technologies in animal breeding: Phenotyping behaviors of laying hens to select against feather pecking. Animals. https://doi.org/10.3390/ani9030108

Ernst, K., Tuchscherer, M., Kanitz, E., Puppe, B., Manteuffel, G., 2006. Effects of attention and rewarded activity on immune parameters and wound healing in pigs. Physiol Behav 89, 448–456. https://doi.org/10.1016/j.physbeh.2006.07.001

Escalante, H.J., Rodriguez, S. v., Cordero, J., Kristensen, A.R., Cornou, C., 2013. Sow-activity classification from acceleration patterns: A machine learning approach. Comput Electron Agric 93, 17–26. https://doi.org/10.1016/j.compag.2013.01.003

Escobar, J., van Alstine, W.G., Baker, D.H., Johnson, R.W., 2007. Behaviour of pigs with viral and bacterial pneumonia. Appl Anim Behav Sci 105, 42–50. https://doi.org/10.1016/j.applanim.2006.06.005

Etim, N., Offiong, E., Etim, N.N., Williams, M.E., Evans, E.I., Offiong, E.E.A., 2013. Physiological and Behavioural Responses of Farm Animals to Stress: Implications to Animal Productivity. AMERICAN JOURNAL OF ADVANCED AGRICULTURAL RESEARCH 1, 53–61.

Fallon, I.P., Tanner, M.K., Greenwood, B.N., Baratta, M. v., 2020. Sex differences in resilience: Experiential factors and their mechanisms. European Journal of Neuroscience. https://doi.org/10.1111/ejn.14639

FAOSTAT, 2022. Crops and livestock products [WWW Document]. URL https://www.fao.org/faostat/en/#data/QCL (accessed 3.16.22).

Farm Animal Welfare Council, 1979. Farm Animal Welfare Council - Press Statement. Press Statement.

Ferguson, D.M., 2014. Key features of “environmental fit” that promote good animal welfare in different husbandry systems, Rev. sci. tech. Off. int. Epiz.

Fraser, A.F., 1988. Behavioural Needs in Relation to Livestock Maintenance. Appl Anim Behav Sci 368–376.

Fraser, D., 1985. Selection of bedded and unbedded areas by pigs in relation to environmental temperature and behaviour. Appl Anim Behav Sci 14, 117–126.

Fraser, D., Weary, D.M., Pajor, E.A., Milligan, B.N., 1997. A Scientific Conception of Animal Welfare that Reflects Ethical Concerns.

Fureix, C., Meagher, R.K., 2015. What can inactivity (in its various forms) reveal about affective states in non-human animals? A review. Appl Anim Behav Sci. https://doi.org/10.1016/j.applanim.2015.08.036

Gentz, M., Meckbach, C., Zeidler, S., Loges, V., Brünger, J., Koch, R., Traulsen, I., 2020. Activity behaviour of pigs: comparison of manual and automated video analyses. Dissertation.

Geverink, N.A., de Jong, I.C., Lambooij, E., Blokhuis, H.J., Wiegant, V.M., 1999. Influence of housing conditions on responses of pigs to preslaughter treatment and consequences for meat quality.

Geverink, N.A., Heetkamp, M.J.W., Schouten, W.G.P., Wiegant, V.M., Schrama, J.W., 2004. Backtest type and housing condition of pigs influence energy metabolism 1, J. Anim. Sci.

Giammanco, M., Tabacchi, G., Giammanco, S., di Majo, D., la Guardia, M., 2005. Testosterone and aggressiveness. Med Sci Monit 11, 136–145.

Giersberg, M.F., Meijboom, F.L.B., 2022. Caught on Camera: On the Need of Responsible Use of Video Observation for Animal Behavior and Welfare Research. Front Vet Sci 9. https://doi.org/10.3389/fvets.2022.864677

Gijzel, S., 2020. Bouncing back: Using a complex dynamical systems approach to measure physical resilience in older adults (Dissertation). Radboudumc, Nijmegen.

Gijzel, S.M.W., van de Leemput, I.A., Scheffer, M., van Bon, G.E.A., Weerdesteyn, V., Eijsvogels, T.M.H., Hopman, M.T.E., Olde Rikkert, M.G.M., Melis, R.J.F., 2019. Dynamical Indicators of Resilience in Postural Balance Time Series Are Related to Successful Aging in High-Functioning Older Adults. Journals of

Gerontology - Series A Biological Sciences and Medical Sciences 74, 1119–1126. https://doi.org/10.1093/gerona/gly170

Godyń, D., Nowicki, J., Herbut, P., 2019. Effects of environmental enrichment on pig welfare—A review. Animals. https://doi.org/10.3390/ani9060383

Goumon, S., Illmann, G., Leszkowová, I., Dostalová, A., Cantor, M., 2020. Dyadic affiliative preferences in a stable group of domestic pigs. Appl Anim Behav Sci 230. https://doi.org/10.1016/j.applanim.2020.105045

Grégoire, J., Bergeron, R., D'Allaire, S., Meunier-Salaün, M.C., Devillers, N., 2013. Assessment of lameness in sows using gait, footprints, postural behaviour and foot lesion analysis. Animal 7, 1163–1173. https://doi.org/10.1017/S1751731113000098

Gronskyte, R., Clemmensen, L.H., Hviid, M.S., Kulahci, M., 2016. Monitoring pig movement at the slaughterhouse using optical flow and modified angular histograms. Biosyst Eng 141, 19–30. https://doi.org/10.1016/j.biosystemseng.2015.10.002

Guo, Y. zheng, Zhu, W. xing, Jiao, P. peng, Ma, C. hua, Yang, J. jun, 2015. Multi-object extraction from topview group-housed pig images based on adaptive partitioning and multilevel thresholding segmentation. Biosyst Eng 135, 54–60. https://doi.org/10.1016/j.biosystemseng.2015.05.001

Guy, S.Z.Y., Thomson, P.C., Hermesch, S., 2012. Selection of pigs for improved coping with health and environmental challenges: Breeding for resistance or tolerance? Front Genet. https://doi.org/10.3389/fgene.2012.00281

Guzhva, O., Ardö, H., Nilsson, M., Herlin, A., Tufvesson, L., 2018. Now you see me: Convolutional neural network based tracker for dairy cows. Frontiers Robotics AI 5. https://doi.org/10.3389/frobt.2018.00107

Harlizius, B., Mathur, P., Knol, E.F., 2020. Breeding for resilience: New opportunities in a modern pig breeding program, in: Journal of Animal Science. Oxford University Press, pp. S150–S154. https://doi.org/10.1093/JAS/SKAA141

Hart, B.L., 1988. Biological Basis of the Behavior of Sick Animals, Neuroscience & Biobehavioral Reviews. ~ Pergamon Press plc.

Hart, B.L., Hart, L.A., 2019. Sickness behavior in animals: implications for health and wellness. Encyclopedia of animal behavior 1.

Heimbürge, S., Kanitz, E., Tuchscherer, A., Otten, W., 2020. Is it getting in the hair? – Cortisol concentrations in native, regrown and segmented hairs of cattle and pigs after repeated ACTH administrations. Gen Comp Endocrinol 295. https://doi.org/10.1016/j.ygcen.2020.113534

Hennig-Pauka, I., Menzel, A., Boehme, T.R., Schierbaum, H., Ganter, M., Schulz, J., 2019. Haptoglobin and C-reactive protein-non-specific markers for nursery conditions in swine. Front Vet Sci 6. https://doi.org/10.3389/fvets.2019.00092

Hermesch, S., Li, L., Doeschl-Wilson, A.B., Gilbert, Hélène, Gilbert, H, 2015. Selection for productivity and robustness traits in pigs. Anim Prod Sci 55. https://doi.org/10.1071/AN15275ï

Hermesch, S., Luxford, B.G., 2018. Genetic parameters for white blood cells, haemoglobin and growth in weaner pigsfor genetic improvement of disease resilience. Proceedings of the World Congress on Genetics Applied to Livestock Production 11–16.

Hess, A.S., Islam, Z., Hess, M.K., Rowland, R.R.R., Lunney, J.K., Doeschl-Wilson, A., Plastow, G.S., Dekkers, J.C.M., 2016. Comparison of host genetic factors influencing pig response to infection with two North American isolates of porcine reproductive and respiratory syndrome virus. Genetics Selection Evolution 48. https://doi.org/10.1186/s12711-016-0222-0

Hessing, M.J., Geudeke, M.J., Scheepens, C.J., Tielen, M.J., Schouten, W.G., Wiepkema, P.R., 1992. Mucosal lesions in the pars esophagus in swine: prevalence and the effect of stress. Tijdschr Diergeneeskd 117, 445–450.

Hodes, G.E., Epperson, C.N., 2019. Sex Differences in Vulnerability and Resilience to Stress Across the Life Span. Biol Psychiatry. https://doi.org/10.1016/j.biopsych.2019.04.028

Hodgson, D., McDonald, J.L., Hosken, D.J., 2015. What do you mean, "resilient"? Trends Ecol Evol. https://doi.org/10.1016/j.tree.2015.06.010

Holinger, M., Früh, B., Hillmann, E., 2015. Group composition for fattening entire male pigs under enriched housing conditions-Influences on behaviour, injuries and boar taint compounds. Appl Anim Behav Sci 165, 47–56. https://doi.org/10.1016/j.applanim.2015.01.016

Homma, C., Hirose, K., Ito, T., Kamikawa, M., Toma, S., Nikaido, S., Satoh, M., Uemoto, Y., 2021. Estimation of genetic parameter for feed efficiency and resilience traits in three pig breeds. Animal 15. https://doi.org/10.1016/j.animal.2021.100384

Hortet, P., Seegers, H., 1998. Loss in milk yield and related composition changes resulting from clinical mastitis in dairy cows. Prev Vet Med 37, 1–20.

Hounslow, J.L., Brewster, L.R., Lear, K.O., Guttridge, T.L., Daly, R., Whitney, N.M., Gleiss, A.C., 2019. Assessing the effects of sampling frequency on behavioural classification of accelerometer data. J Exp Mar Biol Ecol 512, 22–30. https://doi.org/10.1016/j.jembe.2018.12.003

Huang, W., Zhu, W., Ma, C., Guo, Y., Chen, C., 2018. Identification of group-housed pigs based on Gabor and Local Binary Pattern features. Biosyst Eng 166, 90–100. https://doi.org/10.1016/j.biosystemseng.2017.11.007

Huntley, N.F., Nyachoti, C.M., Patience, J.F., 2018. Lipopolysaccharide immune stimulation but not β-mannanase supplementation affects maintenance energy requirements in young weaned pigs. J Anim Sci Biotechnol 9. https://doi.org/10.1186/s40104-018-0264-y

Huynh, T.T.T., Aarnink, A.J.A., Gerrits, W.J.J., Heetkamp, M.J.H., Canh, T.T., Spoolder, H.A.M., Kemp, B., Verstegen, M.W.A., 2005. Thermal behaviour of growing pigs in response to high temperature and humidity. Appl Anim Behav Sci 91, 1–16. https://doi.org/10.1016/j.applanim.2004.10.020

Johnson, R.W., von Borell, R., 1994. Lipopolysaccharide-induced sickness behavior in pigs is inhibited by pretreatment with indomethacin. J Anim Sci 72, 309–314.

Kampman-van de Hoek, E., Jansman, A.J.M., van den Borne, J.J.G.C., van der Peet-Schwering, C.M.C., van Beers-Schreurs, H., Gerrits, W.J.J., 2016. Dietary amino acid deficiency reduces the utilization of amino acids for growth in growing pigs after a period of poor health. Journal of Nutrition 146, 51–58. https://doi.org/10.3945/jn.115.216044

Kashiha, M., Bahr, C., Haredasht, S.A., Ott, S., Moons, C.P.H., Niewold, T.A., Ödberg, F.O., Berckmans, D., 2013a. The automatic monitoring of pigs water use by cameras. Comput Electron Agric 90, 164–169. https://doi.org/10.1016/j.compag.2012.09.015

Kashiha, M., Bahr, C., Ott, S., Moons, C.P.H., Niewold, T.A., Ödberg, F.O., Berckmans, D., 2013b. Automatic identification of marked pigs in a pen using image pattern recognition. Comput Electron Agric 93, 111–120. https://doi.org/10.1016/j.compag.2013.01.013

Knap, P.W., Doeschl-Wilson, A., 2020. Why breed disease-resilient livestock, and how? Genetics Selection Evolution. https://doi.org/10.1186/s12711-020-00580-4

Ko, H.L., Chong, Q., Escribano, D., Camerlink, I., Manteca, X., Llonch, P., 2020. Pre-weaning socialization and environmental enrichment affect life-long response to regrouping in commercially-reared pigs. Appl Anim Behav Sci 229. https://doi.org/10.1016/j.applanim.2020.105044

Kongsro, J., 2014. Estimation of pig weight using a Microsoft Kinect prototype imaging system. Comput Electron Agric 109, 32–35. https://doi.org/10.1016/j.compag.2014.08.008

Kutzer, T., Bünger, B., Kjaer, J.B., Schrader, L., 2009. Effects of early contact between non-littermate piglets and of the complexity of farrowing conditions on social behaviour and weight gain. Appl Anim Behav Sci 121, 16–24. https://doi.org/10.1016/j.applanim.2009.08.004

Kvidera, S.K., Horst, E.A., Mayorga, E.J., Sanz-Fernandez, M. v., Abuajamieh, M., Baumgard, L.H., 2017. Estimating glucose requirements of an activated immune system in growing pigs. J Anim Sci 95, 5020–5029. https://doi.org/10.2527/jas2017.1830

Larsen, M.L.V., Wang, M., Norton, T., 2021. Information technologies for welfare monitoring in pigs and their relation to welfare quality®. Sustainability (Switzerland). https://doi.org/10.3390/su13020692

Larzul, C., Terenina, E., Foury, A., Billon, Y., Louveau, I., Merlot, E., Mormede, P., 2015. The cortisol response to ACTH in pigs, heritability and influence of corticosteroid-binding globulin. Animal 9, 1929–1934. https://doi.org/10.1017/S1751731115001767

Latham, N., Mason, G., 2010. Frustration and perseveration in stereotypic captive animals: Is a taste of enrichment worse than none at all? Behavioural Brain Research 211, 96–104. https://doi.org/10.1016/j.bbr.2010.03.018

Lawrence, A.B., Newberry, R.C., Špinka, M., 2018. Positive welfare: What does it add to the debate over pig welfare?, in: Advances in Pig Welfare. Elsevier Inc., pp. 415–444. https://doi.org/10.1016/B978-0-08-101012-9.00014-9

Lekagul, A., Tangcharoensathien, V., Yeung, S., 2019. Patterns of antibiotic use in global pig production: A systematic review. Vet Anim Sci. https://doi.org/10.1016/j.vas.2019.100058

Li, Y., Huang, C., Nevetia, R., 2009. Computer Vision and Pattern Recognition, 2009, CVPR 2009, IEEE Conference on : dates: 20-25 June 2009., in: 2009 IEEE Conference on Computer Vision and Pattern Recognition . IEEE, pp. 2953–2960.

Lind, N.M., Vinther, M., Hemmingsen, R.P., Hansen, A.K., 2005. Validation of a digital video tracking system for recording pig locomotor behaviour. J Neurosci Methods 143, 123–132. https://doi.org/10.1016/j.jneumeth.2004.09.019

Liu, D., Oczak, M., Maschat, K., Baumgartner, J., Pletzer, B., He, D., Norton, T., 2020. A computer vision-based method for spatial-temporal action recognition of tail-biting behaviour in group-housed pigs. Biosyst Eng 195, 27–41. https://doi.org/10.1016/j.biosystemseng.2020.04.007

Llamas Moya, S., Boyle, L., Lynch, P.B., Arkins, S., 2006. Pro-inflammatory cytokine and acute phase protein responses to low-dose lipopolysaccharide (LPS) challenge in pigs. Animal Science 82, 527–534. https://doi.org/10.1079/ASC200665

Llamas Moya, S., Boyle, L.A., Lynch, P.B., Arkins, S., 2008. Surgical castration of pigs affects the behavioural response to a low-dose lipopolysaccharide (LPS) challenge after weaning. Appl Anim Behav Sci 112, 40–57. https://doi.org/10.1016/j.applanim.2007.07.001

Lopez, O.J., Osorio, F.A., 2004. Role of neutralizing antibodies in PRRSV protective immunity. Vet Immunol Immunopathol. https://doi.org/10.1016/j.vetimm.2004.09.005

Lorschy, M.L., 2005. Definitions of Ambient Temperature Requirements for Pigs: a Review. Prairie Swine Inc.

Luo, L., Jansen, C.A., Bolhuis, J.E., Arts, J.A.J., Kemp, B., Parmentier, H.K., 2020a. Early and later life environmental enrichment affect specific antibody responses and blood leukocyte subpopulations in pigs. Physiol Behav 217. https://doi.org/10.1016/j.physbeh.2020.112799

Luo, L., Reimert, I., Graat, E.A.M., Smeets, S., Kemp, B., Bolhuis, J.E., 2020b. Effects of early life and current housing on sensitivity to reward loss in a successive negative contrast test in pigs. Anim Cogn 23, 121–130. https://doi.org/10.1007/s10071-019-01322-w

Luo, L., van der Zande, L.E., van Marwijk, M.A., Knol, E.F., Rodenburg, T.B., Bolhuis, J.E., Parois, S.P., 2022. Impact of Enrichment and Repeated Mixing on Resilience in Pigs. Front Vet Sci 9. https://doi.org/10.3389/fvets.2022.829060

Luo, L., van Dixhoorn, I.D.E., Reimert, I., Kemp, B., Bolhuis, J.E., Parmentier, H.K., 2017. Effect of enriched housing on levels of natural (auto-)antibodies in pigs co-infected with porcine reproductive and respiratory syndrome virus (PRRSV) and Actinobacillus pleuropneumoniae. Vet Res 48. https://doi.org/10.1186/s13567-017-0481-y

Marchant-Forde, J.N., Marchant-Forde, R.M., 2005. Minimizing inter-pig aggression during mixing. Pig News and Information 26, 63N-71N.

Marco-Ramell, A., Pato, R., Peña, R., Saco, Y., Manteca, X., Ruiz de la Torre, J.L., Bassols, A., 2011. Identification of serum stress biomarkers in pigs housed at different stocking densities. Veterinary Journal 190. https://doi.org/10.1016/j.tvjl.2011.01.003

Martin, S.A., Pence, B.D., Greene, R.M., Johnson, S.J., Dantzer, R., Kelley, K.W., Woods, J.A., 2013. Effects of voluntary wheel running on LPS-induced sickness behavior in aged mice. Brain Behav Immun 29, 113–123. https://doi.org/10.1016/j.bbi.2012.12.014

Martínez-Miró, S., Tecles, F., Ramón, M., Escribano, D., Hernández, F., Madrid, J., Orengo, J., Martínez-Subiela, S., Manteca, X., Cerón, J.J., 2016. Causes, consequences and biomarkers of stress in swine: An update. BMC Vet Res. https://doi.org/10.1186/s12917-016-0791-8

Maselyne, J., Saeys, W., de Ketelaere, B., Briene, P., S. Millet, Tuyttens, F., van Nuffel, A., 2014. How do fattening pigs spend their day?, in: Proceedings of the 6th International Conference on the Assessment of Animal Welfare at Farm and Group Level. p. 157.

Matthews, S.G., Miller, A.L., Clapp, J., Plötz, T., Kyriazakis, I., 2016. Early detection of health and welfare compromises through automated detection of behavioural changes in pigs. Veterinary Journal. https://doi.org/10.1016/j.tvjl.2016.09.005

Matthews, S.G., Miller, A.L., Plötz, T., Kyriazakis, I., 2017. Automated tracking to measure behavioural changes in pigs for health and welfare monitoring. Sci Rep 7. https://doi.org/10.1038/s41598-017-17451-6

Matuszewich, L., Yamamoto, B.K., 2003. Long-lasting effects of chronic stress on doi-induced hyperthermia in male rats. Psychopharmacology (Berl) 169, 169–175. https://doi.org/10.1007/s00213-003-1498-7

Mcglone, J J, Salak, J.L., Lumpkin, E A, Nicholson, R I, Gibson, M, Norman, R L, Mcglone, John J, Sal&, J.L., Lumpkin, Ellen A, Nicholson, Rhonda I, Gibson, Matt, Norman, Reid L, 1993. Shipping Stress and Social Status Effects on Pig Performance, Plasma Cortisol, Natural Killer Cell Activity, and Leukocyte Numbers'. J Anim Sci 71, 888–896.

Mellor, D.J., 2016. Updating animal welfare thinking: moving beyond the "Five Freedoms" towards "a Life Worth Living. Animals 6. https://doi.org/10.3390/ani6030021

Melotti, L., Oostindjer, M., Bolhuis, J.E., Held, S., Mendl, M., 2011. Coping personality type and environmental enrichment affect aggression at weaning in pigs. Appl Anim Behav Sci 133, 144–153. https://doi.org/10.1016/j.applanim.2011.05.018

Merlot, E., Thomas, F., Prunier, A., 2013. Comparison of immune and health markers in intact and neonatally castrated male pigs. Veterinary Record 173, 317. https://doi.org/10.1136/vr.101667

Mkwanazi, M.V., Ncobela, C.N., Kanengoni, A.T., Chimonyo, M., 2019. Effects of environmental enrichment on behaviour, physiology and performance of pigs - A review. Asian-Australas J Anim Sci. https://doi.org/10.5713/ajas.17.0138

Munsterhjelm, C., Nordgreen, J., Aae, F., Heinonen, M., Valros, A., Janczak, A.M., 2019. Sick and grumpy: Changes in social behaviour after a controlled immune stimulation in group-housed gilts. Physiol Behav 198, 76–83. https://doi.org/10.1016/j.physbeh.2018.09.018

Nasirahmadi, A., Edwards, S., Richter, U., Sturm, B., 2015. Automatic detection of changes in pig group lying behaviour using image analysis. 2015 ASABE Annual International Meeting.

Nasirahmadi, A., Hensel, O., Edwards, S.A., Sturm, B., 2016. Automatic detection of mounting behaviours among pigs using image analysis. Comput Electron Agric 124, 295–302. https://doi.org/10.1016/j.compag.2016.04.022

Nasirahmadi, A., Sturm, B., Edwards, S., Jeppsson, K.H., Olsson, A.C., Müller, S., Hensel, O., 2019. Deep learning and machine vision approaches for posture detection of individual pigs. Sensors (Switzerland) 19. https://doi.org/10.3390/s19173738

Nathues, H., Alarcon, P., Rushton, J., Jolie, R., Fiebig, K., Jimenez, M., Geurts, V., Nathues, C., 2017. Cost of porcine reproductive and respiratory syndrome virus at individual farm level – An economic disease model. Prev Vet Med 142, 16–29. https://doi.org/10.1016/j.prevetmed.2017.04.006

Newberry, R.C., 1995. Environmental enrichment: Increasing the biological relevance of captive environments, Applied Animal Behaviour Science.

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. Physiol Behav 195, 98–111. https://doi.org/10.1016/j.physbeh.2018.07.013

Oczak, M., Maschat, K., Berckmans, D., Vranken, E., Baumgartner, J., 2016. Can an automated labelling method based on accelerometer data replace a human labeller? - Postural profile of farrowing sows. Comput Electron Agric 127, 168–175. https://doi.org/10.1016/j.compag.2016.06.013

Oczak, M., Maschat, K., Berckmans, D., Vranken, E., Baumgartner, J., 2015. Classification of nest-building behaviour in non-crated farrowing sows on the basis of accelerometer data. Biosyst Eng 140, 48–58. https://doi.org/10.1016/j.biosystemseng.2015.09.007

Oczak, M., Viazzi, S., Ismayilova, G., Sonoda, L.T., Roulston, N., Fels, M., Bahr, C., Hartung, J., Guarino, M., Berckmans, D., Vranken, E., 2014. Classification of aggressive behaviour in pigs by activity index and multilayer feed forward neural network. Biosyst Eng 119, 89–97. https://doi.org/10.1016/j.biosystemseng.2014.01.005

OECD/FAO, 2021. OECD-FAO Agricultural Outlook 2021-2030. https://doi.org/10.1787/agr-outl-data

Ohl, F., van der Staay, F.J., 2012. Animal welfare: At the interface between science and society. Veterinary Journal. https://doi.org/10.1016/j.tvjl.2011.05.019

O'Leary, N.W., Byrne, D.T., O'Connor, A.H., Shalloo, L., 2020. Invited review: Cattle lameness detection with accelerometers. J Dairy Sci. https://doi.org/10.3168/jds.2019-17123

O'Malley, C.I., Turner, S.P., D'Eath, R.B., Steibel, J.P., Bates, R.O., Ernst, C.W., Siegford, J.M., 2019. Animal personality in the management and welfare of pigs. Appl Anim Behav Sci. https://doi.org/10.1016/j.applanim.2019.06.002

Oostindjer, M., Bolhuis, J.E., Mendl, M., Held, S., van den Brand, H., Kemp, B., 2011a. Learning how to eat like a pig: Effectiveness of mechanisms for vertical social learning in piglets. Anim Behav 82, 503–511. https://doi.org/10.1016/j.anbehav.2011.05.031

Oostindjer, M., Kemp, B., van den Brand, H., Bolhuis, J.E., 2014a. Facilitating "learning from mom how to eat like a pig" to improve welfare of piglets around weaning. Appl Anim Behav Sci 160, 19–30. https://doi.org/10.1016/j.applanim.2014.09.006

Oostindjer, M., Kemp, B., van den Brand, H., Bolhuis, J.E., 2014b. Facilitating "learning from mom how to eat like a pig" to improve welfare of piglets around weaning. Appl Anim Behav Sci 160, 19–30. https://doi.org/10.1016/j.applanim.2014.09.006

Oostindjer, M., van den Brand, H., Kemp, B., Bolhuis, J.E., 2011b. Effects of environmental enrichment and loose housing of lactating sows on piglet behaviour before and after weaning. Appl Anim Behav Sci 134, 31–41. https://doi.org/10.1016/j.applanim.2011.06.011

Ott, S., Moons, C.P.H., Kashiha, M.A., Bahr, C., Tuyttens, F.A.M., Berckmans, D., Niewold, T.A., 2014. Automated video analysis of pig activity at pen level highly correlates to human observations of behavioural activities. Livest Sci 160, 132–137. https://doi.org/10.1016/j.livsci.2013.12.011

Pantoja, L.G., Kuhn, M., Hoover, T., Amodie, D., Weigel, D., Dice, C., Moeller, T.B., Farrand, E., Pantoja, G.L., 2013. Impact of a Husbandry Education Program on nursery pig mortality, productivity, and treatment cost. Journal of Swine Health and Production 21, 188–194.

Parois, S.P., van der Zande, L.E., Knol, E.F., Kemp, B., Rodenburg, T.B., Bolhuis, J.E., 2022a. A multi-suckling system combined with an enriched housing environment during the growing period promotes resilience to various challenges in pigs. Sci Rep 12, 1–16.

Parois, S.P., van der Zande, L.E., Knol, E.F., Kemp, B., Rodenburg, T.B., Bolhuis, J.E., 2022b. Effects of a Multi-Suckling System Combined With Enriched Housing Post-Weaning on Response and Cognitive Resilience to Isolation. Front Vet Sci 9. https://doi.org/10.3389/fvets.2022.868149

Pastorelli, H., van Milgen, J., Lovatto, P., Montagne, L., 2012. Meta-analysis of feed intake and growth responses of growing pigs after a sanitary challenge. Animal. https://doi.org/10.1017/S175173111100228X

Pearce, S.C., Mani, V., Weber, T.E., Rhoads, R.P., Patience, J.F., Baumgard, L.H., Gabler, N.K., 2013. Heat stress and reduced plane of nutrition decreases intestinal integrity and function in pigs 1. J. Anim. Sci 91, 5183–5193. https://doi.org/10.2527/jas2013-6759

Pecchi, E., Dallaporta, M., Jean, A., Thirion, S., Troadec, J.D., 2009. Prostaglandins and sickness behavior: Old story, new insights. Physiol Behav. https://doi.org/10.1016/j.physbeh.2009.02.040

Peden, R.S.E., Turner, S.P., Boyle, L.A., Camerlink, I., 2018. The translation of animal welfare research into practice: The case of mixing aggression between pigs. Appl Anim Behav Sci. https://doi.org/10.1016/j.applanim.2018.03.003

Pedersen, L.J., 2018. Overview of commercial pig production systems and their main welfare challenges, in: Advances in Pig Welfare. Elsevier Inc., pp. 3–25. https://doi.org/10.1016/B978-0-08-101012-9.00001-0

Pedersen, S., Nonnenmann, M., Rautiainen, R., Demmers, T.G.M., Banhazi, T., Lyngbye, M., 2000. Dust in pig buildings. J Agric Saf Health 6, 261.

Pezzuolo, A., Guarino, M., Sartori, L., González, L.A., Marinello, F., 2018. On-barn pig weight estimation based on body measurements by a Kinect v1 depth camera. Comput Electron Agric 148, 29–36. https://doi.org/10.1016/j.compag.2018.03.003

Pimm, S.L., 1984. The complexity and stability of ecosystems. Nature 307, 321–326.

Pitts, A.D., Weary, D.M., Pajor, E.A., Fraser, D., 2000. Mixing at young ages reduces fighting in unacquainted domestic pigs, Applied Animal Behaviour Science.

Place, S.E., 2017. Animal welfare and environmental issues, in: Advances in Agricultural Animal Welfare: Science and Practice. Elsevier, pp. 69–89. https://doi.org/10.1016/B978-0-08-101215-4.00004-3

Poppe, M., Mulder, H.A., Veerkamp, R.F., 2021. Validation of resilience indicators by estimating genetic correlations among daughter groups and with yield responses to a heat wave and disturbances at herd level. J Dairy Sci 104, 8094–8106. https://doi.org/10.3168/jds.2020-19817

Poppe, M., Veerkamp, R.F., van Pelt, M.L., Mulder, H.A., 2020. Exploration of variance, autocorrelation, and skewness of deviations from lactation curves as resilience indicators for breeding. J Dairy Sci 103, 1667–1684. https://doi.org/10.3168/jds.2019-17290

Proudfoot, K., Habing, G., 2015. Social stress as a cause of diseases in farm animals: Current knowledge and future directions. Veterinary Journal. https://doi.org/10.1016/j.tvjl.2015.05.024

Psota, E.T., Schmidt, T., Mote, B., C. Pérez, L., 2020a. Long-Term Tracking of Group-Housed Livestock Using Keypoint Detection and MAP Estimation for Individual Animal Identification. Sensors 20, 3670. https://doi.org/10.3390/s20133670

Psota, E.T., Schmidt, T., Mote, B., Pérez, L.C., 2020b. Long-term tracking of group-housed livestock using keypoint detection and map estimation for individual animal identification. Sensors (Switzerland) 20, 1–25. https://doi.org/10.3390/s20133670

Putz, A.M., Harding, J.C.S., Dyck, M.K., Fortin, F., Plastow, G.S., Dekkers, J.C.M., 2019. Novel resilience phenotypes using feed intake data from a natural disease challenge model in wean-to-finish pigs. Front Genet 10. https://doi.org/10.3389/fgene.2018.00660

R Core team, 2013. R: A Language and Environment for Statistical Computing.

Rault, J.L., Lawrence, A.J., Ralph, C.R., 2018. Brain-derived neurotrophic factor in serum as an animal welfare indicator of environmental enrichment in pigs. Domest Anim Endocrinol 65, 67–70. https://doi.org/10.1016/j.domaniend.2018.05.007

Rauw, W.M., Mayorga, E.J., Lei, S.M., Dekkers, J.C.M., Patience, J.F., Gabler, N.K., Lonergan, S.M., Baumgard, L.H., 2017. Effects of diet and genetics on growth performance of pigs in response to repeated exposure to heat stress. Front Genet 8. https://doi.org/10.3389/fgene.2017.00155

Redmon, J., Farhadi, A., 2018. YOLOv3: An Incremental Improvement.

Reimert, I., Bolhuis, J.E., Kemp, B., Rodenburg, T.B., 2015. Emotions on the loose: emotional contagion and the role of oxytocin in pigs. Anim Cogn 18, 517–532. https://doi.org/10.1007/s10071-014-0820-6

Reimert, I., Bolhuis, J.E., Kemp, B., Rodenburg, T.B., 2013. Indicators of positive and negative emotions and emotional contagion in pigs. Physiol Behav 109, 42–50. https://doi.org/10.1016/j.physbeh.2012.11.002

Reimert, I., Fong, S., Rodenburg, T.B., Bolhuis, J.E., 2017. Emotional states and emotional contagion in pigs after exposure to a positive and negative treatment. Appl Anim Behav Sci 193, 37–42. https://doi.org/10.1016/j.applanim.2017.03.009

Reimert, I., Rodenburg, T.B., Ursinus, W.W., Kemp, B., Bolhuis, J.E., 2014a. Selection based on indirect genetic effects for growth, environmental enrichment and coping style affect the immune status of pigs. PLoS One 9. https://doi.org/10.1371/journal.pone.0108700

Reimert, I., Rodenburg, T.B., Ursinus, W.W., Kemp, B., Bolhuis, J.E., 2014b. Responses to novel situations of female and castrated male pigs with divergent social breeding values and different backtest classifications in barren and straw-enriched housing. Appl Anim Behav Sci 151, 24–35. https://doi.org/10.1016/j.applanim.2013.11.015

Reiner, G., Hübner, K., Hepp, S., 2009. Suffering in diseased pigs as expressed by behavioural, clinical and clinical-chemical traits, in a well defined parasite model. Appl Anim Behav Sci 118, 222–231. https://doi.org/10.1016/j.applanim.2009.02.010

Ringgenberg, N., Bergeron, R., Devillers, N., 2010. Validation of accelerometers to automatically record sow postures and stepping behaviour. Appl Anim Behav Sci 128, 37–44. https://doi.org/10.1016/j.applanim.2010.09.018

Rioja-Lang, F.C., Brown, J.A., Brockhoff, E.J., Faucitano, L., 2019. A review of swine transportation research on priority welfare issues: A canadian perspective. Front Vet Sci. https://doi.org/10.3389/fvets.2019.00036

Rostagno, M.H., Eicher, S.D., Lay, D.C., 2011. Immunological, Physiological, and Behavioral Effects of Salmonella enterica Carriage and Shedding in Experimentally Infected Finishing Pigs. Foodborne Pathog Dis 8, 623–630. https://doi.org/10.1089/fpd.2010.0735

Salak-Johnson, J.L., Anderson, D.L., McGlone, J.J., 2004. Differential dose effects of central CRF and effects of CRF astressin on pig behavior. Physiol Behav 83, 143–150. https://doi.org/10.1016/j.physbeh.2004.08.005

Salamano, G., Mellia, E., Candiani, D., Ingravalle, F., Bruno, R., Ru, G., Doglione, L., 2008. Changes in haptoglobin, C-reactive protein and pig-MAP during a housing period following long distance transport in swine. Veterinary Journal 177, 110–115. https://doi.org/10.1016/j.tvjl.2007.03.015

Salazar, L.C., Ko, H.L., Yang, C.H., Llonch, L., Manteca, X., Camerlink, I., Llonch, P., 2018. Early socialisation as a strategy to increase piglets' social skills in intensive farming conditions. Appl Anim Behav Sci 206, 25–31. https://doi.org/10.1016/j.applanim.2018.05.033

Sali, V., Veit, C., Valros, A., Junnikkala, S., Heinonen, M., Nordgreen, J., 2021. Dynamics of Salivary Adenosine Deaminase, Haptoglobin, and Cortisol in Lipopolysaccharide-Challenged Growing Pigs. Front Vet Sci 8. https://doi.org/10.3389/fvets.2021.698628

Sanz Fernandez, M.V., Johnson, J.S., Abuajamieh, M., Stoakes, S.K., Seibert, J.T., Cox, L., Kahl, S., Elsasser, T.H., Ross, J.W., Clay Isom, S., Rhoads, R.P., Baumgard, L.H., 2015. Effects of heat stress on carbohydrate and lipid metabolism in growing pigs. Physiol Rep 3. https://doi.org/10.14814/phy2.12315

Scheffer, M., Bascompte, J., Brock, W.A., Brovkin, V., Carpenter, S.R., Dakos, V., Held, H., van Nes, E.H., Rietkerk, M., Sugihara, G., 2009. Early-warning signals for critical transitions. Nature. https://doi.org/10.1038/nature08227

Scheffer, M., Bolhuis, J.E., Borsboom, D., Buchman, T.G., Gijzel, S.M., Goulson, D., Kammenga, J.E., Kemp, B., van de Leemput, I.A., Levin, S., Martin, C.M., van Nes, E.H., Romero, L.M., Olde Rikkert, M.G., 2018. Quantifying resilience of humans and other animals. Proceedings of the National Academy of Sciences 115, 11883–11890. https://doi.org/10.1073/pnas.1810630115/-/DCSupplemental

Scheffer, M., Carpenter, S., Foley, J.A., Folke, C., Walkerk, B., 2001. Catastrophic shifts in ecosystems, NATURE.

Scheffer, M., Carpenter, S.R., Lenton, T.M., Bascompte, J., Brock, W., Dakos, V., van de Koppel, J., van de Leemput, I.A., Levin, S.A., van Nes, E.H., Pascual, M., Vandermeer, J., 2012. Anticipating critical transitions. Science (1979) 338, 344–348.

Schneider, C.A., Rasband, W.S., Eliceiri, K.W., 2012. NIH Image to ImageJ: 25 years of image analysis. Nat Methods. https://doi.org/10.1038/nmeth.2089

Schneider, D.S., Ayres, J.S., 2008. Two ways to survive infection: what resistance and tolerance can teach us about treating infectious diseases. Nat Rev Immunol 8, 889–895. https://doi.org/10.1038/nri2432

Scollo, A., di Martino, G., Bonfanti, L., Stefani, A.L., Schiavon, E., Marangon, S., Gottardo, F., 2013. Tail docking and the rearing of heavy pigs: The role played by gender and the presence of straw in the control of tail biting. Blood parameters, behaviour and skin lesions. Res Vet Sci 95, 825–830. https://doi.org/10.1016/j.rvsc.2013.06.019

Scott, K., Chennells, D.J., Campbell, F.M., Hunt, B., Armstrong, D., Taylor, L., Gill, B.P., Edwards, S.A., 2006. The welfare of finishing pigs in two contrasting housing systems: Fully-slatted versus straw-bedded accommodation. Livest Sci 103, 104–115. https://doi.org/10.1016/j.livsci.2006.01.008

Scott, P.R., 2013. The challenges to improve farm animal welfare in the united kingdom by reducing disease incidence with greater veterinary involvement on farm. Animals 3, 629–646. https://doi.org/10.3390/ani3030629

Sekachev, B., Nikita, M., Andrey, Z., 2019. Computer vision Annotation Tool: a universal approach to data annotation.

Shao, H., Pu, J., Mu, J., 2021. Pig-posture recognition based on computer vision: Dataset and exploration. Animals 11. https://doi.org/10.3390/ani11051295

Sharma, N., Singh, N.K., Bhadwal, M.S., 2011. Relationship of Somatic Cell Count and Mastitis: An Overview. Asian-Australas J Anim Sci 24, 429–438.

Simonsen, H.B., 1990. Behaviour and distribution of fattening pigs in the multi-activity pen. Appl Anim Behav Sci 27, 311–324.

Sloman, K.A., Motherwell, G., O'connor, K.I., Taylor, A.C., 2000. The effect of social stress on the Standard Metabolic Rate (SMR) of brown trout, Salmo trutta, Fish Physiology and Biochemistry.

Star, L., Frankena, K., Kemp, B., Nieuwland, M.G.B., Parmentier, H.K., 2007. IMMUNOLOGY, HEALTH, AND DISEASE Natural Humoral Immune Competence and Survival in Layers, Poultry Science.

Steinfeld, H., Gerber, P., Wassenaar, T.D., Castel, V., Rosales, M., Rosales, M., de Haan, C., 2006. Livestock's long shadow: environmental issues and options. Food & Agriculture Org.

Studnitz, M., Jensen, M.B., Pedersen, L.J., 2007. Why do pigs root and in what will they root?. A review on the exploratory behaviour of pigs in relation to environmental enrichment. Appl Anim Behav Sci. https://doi.org/10.1016/j.applanim.2006.11.013

Sun, Y., Parmentier, H.K., Frankena, K., van der Poel, J.J., 2011. Natural antibody isotypes as predictors of survival in laying hens. Poult Sci 90, 2263–2274. https://doi.org/10.3382/ps.2011-01613

Sutherland, M.A., Backus, B.L., McGlone, J.J., 2014. Effects of transport at weaning on the behavior, physiology and performance of pigs. Animals. https://doi.org/10.3390/ani4040657

Sutherland, M.A., Krebs, N., Smith, J.S., Dailey, J.W., Carroll, J.A., McGlone, J.J., 2009. The effect of three space allowances on the physiology and behavior of weaned pigs during transportation. Livest Sci 126, 183–188. https://doi.org/10.1016/j.livsci.2009.06.021

Telkänranta, H., Marchant-Forde, J.N., Valros, A., 2015. Tear staining in pigs: A potential tool for welfare assessment on commercial farms. Animal 10, 318–325. https://doi.org/10.1017/S175173111500172X

ten Thij, M., Bathina, K., Rutter, L.A., Lorenzo-Luaces, L., van de Leemput, I.A., Scheffer, M., Bollen, J., 2020. Depression alters the circadian pattern of online activity. Sci Rep 10. https://doi.org/10.1038/s41598-020-74314-3

Thompson, R.J., Matthews, S., Plötz, T., Kyriazakis, I., 2019. Freedom to lie: How farrowing environment affects sow lying behaviour assessment using inertial sensors. Comput Electron Agric 157, 549–557. https://doi.org/10.1016/j.compag.2019.01.035

Trevisan, C., Johansen, M.V., Mkupasi, E.M., Ngowi, H.A., Forkman, B., 2017. Disease behaviours of sows naturally infected with Taenia solium in Tanzania. Vet Parasitol 235, 69–74. https://doi.org/10.1016/j.vetpar.2017.01.008

Tzutalin, L., 2015. LabelImg; Git code.

Ursinus, W.W., van Reenen, C.G., Kemp, B., Bolhuis, J.E., 2014. Tail biting behaviour and tail damage in pigs and the relationship with general behaviour: Predicting the inevitable? Appl Anim Behav Sci 156, 22–36. https://doi.org/10.1016/j.applanim.2014.04.001

van de Weerd, H.A., Day, J.E.L., 2009. A review of environmental enrichment for pigs housed in intensive housing systems. Appl Anim Behav Sci. https://doi.org/10.1016/j.applanim.2008.08.001

van de Weerd, H.A., Docking, C.M., Day, J.E.L., Avery, P.J., Edwards, S.A., 2003. A systematic approach towards developing environmental enrichment for pigs. Appl Anim Behav Sci 84, 101–118. https://doi.org/10.1016/S0168-1591(03)00150-3

van de Weerd, H.A., Ison, S., 2019. Providing effective environmental enrichment to pigs: How far have we come? Animals 9. https://doi.org/10.3390/ani9050254

van der Meer, Y., Gerrits, W.J.J., Jansman, A.J.M., Kemp, B., Bolhuis, J.E., 2017. A link between damaging behaviour in pigs, sanitary conditions, and dietary protein and amino acid supply. PLoS One 12. https://doi.org/10.1371/journal.pone.0174688

van der Zande, L.E., Dunkelberger, J.R., Rodenburg, T.B., Bolhuis, J.E., Mathur, P.K., Cairns, W.J., Keyes, M.C., Eggert, J.M., Little, E.A., Dee, S.A., Knol, E.F., 2020. Quantifying Individual Response to PRRSV Using Dynamic Indicators of Resilience Based on Activity. Front Vet Sci 7. https://doi.org/10.3389/fvets.2020.00325 van der Zande, L.E., Guzhva, O., Parois, S., van de Leemput, I., Bolhuis, J., Rodenburg, T., 2022. Estimation of resilience parameters following LPS injection based on activity measured with computer vision. Frontiers in animal Science 3, 1–12.

van der Zande, L.E., Guzhva, O., Rodenburg, T.B., 2021. Individual Detection and Tracking of Group Housed Pigs in Their Home Pen Using Computer Vision. Frontiers in Animal Science 2. https://doi.org/10.3389/fanim.2021.669312

van Dixhoorn, I.D.E., de Mol, R.M., van der Werf, J.T.N., van Mourik, S., van Reenen, C.G., 2018. Indicators of resilience during the transition period in dairy cows: A case study. J Dairy Sci 101, 10271–10282. https://doi.org/10.3168/jds.2018-14779

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. https://doi.org/10.1371/journal.pone.0161832

van Nieuwamerongen, S.E., Bolhuis, J.E., van der Peet-Schwering, C.M.C., Soede, N.M., 2014. A review of sow and piglet behaviour and performance in group housing systems for lactating sows. Animal 8, 448–460. https://doi.org/10.1017/S1751731113002280

van Nieuwamerongen, S.E., Soede, N.M., van der Peet-Schwering, C.M.C., Kemp, B., Bolhuis, J.E., 2015. Development of piglets raised in a new multi-litter housing system vs. conventional single-litter housing until 9 weeks of age. J Anim Sci 93, 5442–5454.

van Ooijen, Q., 2021. 90 procent van varkensvleesvitrine Beter Leven.

Veit, C., Janczak, A.M., Ranheim, B., Vas, J., Valros, A., Sandercock, D.A., Piepponen, P., Dulgheriu, D., Nordgreen, J., 2021. The Effect of LPS and Ketoprofen on Cytokines, Brain Monoamines, and Social Behavior in Group-Housed Pigs. Front Vet Sci 7. https://doi.org/10.3389/fvets.2020.617634

Verdon, M., Morrison, R.S., Hemsworth, P.H., 2016. Rearing piglets in multi-litter group lactation systems: Effects on piglet aggression and injuries post-weaning. Appl Anim Behav Sci 183, 35–41. https://doi.org/10.1016/j.applanim.2016.05.008

Verdon, M., Morrison, R.S., Rault, J.L., 2019. Group lactation from 7 or 14 days of age reduces piglet aggression at weaning compared to farrowing crate housing. Animal 13, 2327–2335. https://doi.org/10.1017/S1751731119000478

Voss, A., Loeffen, F., Bakker, J., Klaassen, C., Wulf, M., 2005. Methicillin-resistant Staphylococcus aureus in Pig Farming. Emerg Infect Dis 11, 1965–1966.

Weary, D.M., Huzzey, J.M., von Keyserlingk, M.A.G., 2009. Board-invited Review: Using behavior to predict and identify ill health in animals. J Anim Sci 87, 770–777. https://doi.org/10.2527/jas.2008-1297

Webster, A.J.F., 2001. Farm Animal Welfare: The Five Freedoms and the Free Market. Veterinary Journal. https://doi.org/10.1053/tvjl.2000.0563

Wilson, R.L., Holyoake, P.K., Cronin, G.M., Doyle, R.E., 2016. A comparison of behavioural and clinical measures to assess recovery in pigs administered either meloxicam or ketoprofen.

Wondmeneh, E., van Arendonk, J.A.M., van der Waaij, E.H., Ducro, B.J., Parmentier, H.K., 2015. High natural antibody titers of indigenous chickens are related with increased hazard in confinement. Poult Sci 94, 1493–1498. https://doi.org/10.3382/ps/pev107

Wurtz, K., Camerlink, I., D'Eath, R.B., Fernández, A.P., Norton, T., Steibel, J., Siegford, J., 2019. Recording behaviour of indoor-housed farm animals automatically using machine vision technology: A systematic review. PLoS One 14. https://doi.org/10.1371/journal.pone.0226669

Yang, C.H., Ko, H.L., Salazar, L.C., Llonch, L., Manteca, X., Camerlink, I., Llonch, P., 2018. Pre-weaning environmental enrichment increases piglets' object play behaviour on a large scale commercial pig farm. Appl Anim Behav Sci 202, 7–12. https://doi.org/10.1016/j.applanim.2018.02.004

Yang, Q., Xiao, D., Lin, S., 2018. Feeding behavior recognition for group-housed pigs with the Faster R-CNN. Comput Electron Agric 155, 453–460. https://doi.org/10.1016/j.compag.2018.11.002

Zhang, L., Gray, H., Ye, X., Collins, L., Allinson, N., 2019. Automatic individual pig detection and tracking in pig farms. Sensors (Switzerland) 19. https://doi.org/10.3390/s19051188

Summary

As pork is the most consumed meat in the EU, large numbers of pigs are kept in Europe, predominantly in conventional pig husbandry systems. Attention for pig health and welfare is increasing in Europe, resulting in a demand for improved production systems. In any system, pigs will be exposed to more or less inevitable challenges. To reduce the impact of negative experiences on pig health and welfare, pigs should ideally be minimally affected by and swiftly recover from such challenges, i.e., be resilient. Resilience is, however, difficult to measure. To assess recovery from challenges as a reflection of resilience, regular, or even continuous, monitoring of the animal is necessary. Detailed physiological and behavioural responses may be difficult to monitor in a continuous manner. Instead, proxy traits could be used. Proxy traits are traits related to the trait of interest, but are often easier to measure. A potentially relevant proxy measure for resilience is the deviation in general activity of individual animals in response to a challenge. The main goal of this thesis was to explore technologies to measure activity changes as a proxy for resilience and to apply them in a relevant context with variation in resilience.

In Chapters 2 and 3, accelerometers and computer vision were implemented to monitor individual activity on a continuous basis. In Chapter 2 activity was monitored using accelerometers for 65 days around an infection with Porcine Reproductive and Respiratory Syndrome Virus (PRRSV). Using machine learning, each 5-second window of accelerations was classified as active or non-active and it was shown that accelerometers can be used to quantify activity. However, accelerometers need to be attached to the animal and spatial information is not recorded. Therefore, in Chapter 3, an individual tracking algorithm using computer vision was developed. Two videos of 7h each of pigs kept either in a barren or an enriched environment with more space and bedding material were used to test the tracking algorithm. The tracking algorithm performed better in the enriched environment than in the barren environment. Individual pigs were tracked on average for 22.3 min in the barren environment and for 57.8 min in the enriched environment, before individual identities were lost. To date, this is the most successful algorithm to track activity in individual pigs with a fixed identity.

Different indicators based on activity patterns and physiological variables as proxies to measure resilience were explored in Chapter 2, 4 and 5. It has been suggested that deviations in longitudinal patterns of repeatedly or continuously measured biological variables may be related to resilience of livestock. In Chapter 2 we investigated such dynamic indicators of resilience (DIORs) by calculating the root mean square error (RMSE), autocorrelation, and skewness of activity. Baseline, i.e., pre-challenge, DIORs were not predictive of morbidity or mortality caused by PRRSV. However, a higher RMSE within 3 days post-infection and a higher increase in RMSE from pre- to post-infection increased the probability of clinical signs of disease (morbidity) at 12 days post-infection. In addition, a higher skewness and larger increase in skewness of activity increased the risk of mortality. Moreover, a high change in activity from pre- to post-challenge tended to be associated with an increased risk of morbidity. In Chapter 4 physiological parameters were used to investigate the effect of housing environment on resilience. The recovery from a 2 h-transport challenge, a lipopolysaccharide (LPS) injection which induces a sickness response, 2 h-heat stress, and a biopsy wound was measured in conventionally housed pigs (CONV) and in pigs raised and kept in an alternative system comprising group farrowing, delayed weaning, more space and enrichment materials (AHS). AHS pigs showed a faster physiological recovery, i.e., quicker returns to baseline, after the transport and LPS challenge, a lower accumulation of cortisol in hairs and a lower variance in weight gain over the experimental period compared to CONV. Taken together, the AHS pigs, which were raised and kept in an environment that facilitates their natural behaviour and improves their welfare, appeared to be more resilient and experienced less chronic stress than CONV pigs. Chapter 5 explored activity data of the LPS challenge from Chapter 4. AHS pigs were more active than CONV pigs before the challenge. This could be a result from the availability of enrichment or the fact that they had twice as much space available to move around. As expected, LPS injected animals were more likely to show a dip in activity, which reflects sickness behaviour, than saline injected animals. The duration and area under the curve (AUC) of the activity dip, reflecting the combination of its strength and duration, were not affected by housing system. However, the AUC:duration ratio was higher in AHS pigs compared to CONV pigs, indicating a deeper dip in activity combined with a faster recovery.

Although there was a clear housing effect on resilience as based on physiological responses to the LPS challenge (Chapter 4), no housing effect was found on most of the activity parameters (Chapter 5). The relationship between activity and physiological parameters as measured around the LPS challenge was explored in Chapter 6. Only a limited number of weak to moderate relationships between characteristics of the change in activity and the physiological variables measured following LPS injection were found. Hence, changes in activity patterns following LPS injection as part of sickness behaviour do not simply reflect physiological responses. This does not imply that the activity decrease in itself is not an important aspect of the sickness response which may have consequences for animal health. Thus, characteristics of the activity dip following LPS injection can be of added value to characterize the sickness response in pigs. Taken together with the predictive value of activity-based DIORs for morbidity and mortality following a PRRSV infection (Chapter 2), this indicates that activity patterns and deviations therein are, supplementary to physiological variables, promising indicators of (loss of) resilience in pigs.

In Chapter 7, the general discussion, I brought together the results from the different chapters. I discussed the two technologies used in this thesis to measure activity in pigs: accelerometers and computer vision. I concluded that computer vision has more potential to be further developed and could measure more complex behaviours in the future. Next, I elaborated on resilience and the potential use of activity and deviations in activity as proxies for resilience. Calculation of the heritability of one of the physiological variables measured following LPS injection, the cortisol response, revealed that resilience might be partly genetically determined. Therefore, there are two ways to improve resilience: either by improving the environment in which pigs are kept, or through breeding. Breeding for enhanced resilience should not be a strategy to select for pigs that better cope with suboptimal management and housing systems, but go hand in hand with improvement of the pigs' environment. Overall, the results of this thesis contribute to the understanding of resilience and to the future enhancement of animal welfare.

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About the author

I, Lisette Elise van der Zande, was born the 17th of April 1993 in Pijnacker, the Netherlands. As a child, I was determined to persuade my parents to get us any kind of pet. The fascination with farm animals started when I enrolled in the BSc program Animal Sciences at Wageningen University in 2011. During my bachelor's program I got enthusiastic about genetics, leading to enrolment in the MSc program Animal Sciences at Wageningen University with a specialization in Animal Breeding and Genetics. After completing my thesis entitled: "Estimating genetic parameters of plumage condition in crossbred layers", I started my internship at Topigs Norsvin. During this internship I studied metabolites related to tail biting behaviour. After obtaining my MSc degree, I started as Junior Researcher at Topigs Norsvin. Here I started to be fascinated with the difficulty of measuring tail biting behaviour because of its complexity, and it was at that time that my interest in using technology to measure animal behaviour started. In 2018, I had the opportunity to start my PhD trajectory to study resilience of pigs using technology at the Adaptation Physiology (ADP) of Wageningen University. My PhD thesis focused on developing and testing different technologies to measure activity of pigs. These activity measurements could serve as a proxy for resilience, in addition to physiological measurements. Currently, I am appointed as Researcher at Topigs Norsvin, where I will work on the implementation of computer vision to study pig behaviour.

List of publications

Peer reviewed scientific publications

Ellen, E.D., van der Sluis, M., Siegford, J., Guzvha, O., Toscano, M.J., Bennewitz, J., van der Zande, L.E., van der Eijk, J.A.J., de Haas, E.N., Norton, T., Piette, D., Tetens, J., de Klerk, B., Visser, B. and Rodenburg, T.B. (2019) Review of sensor technologies in animal breeding: phenotyping behaviours of laying hens to select against feather pecking. Animals, 9, 108.

Canario, L., Bijma, P., David, I., Camerlink, I., Martin, A., Rauw, W.M., Flatres-Grall, L., van der Zande, L.E., Turner, S.P., Larzul C., Rydhmer, L. (2020) Prospects for the Analysis and Reduction of Damaging Behaviour in Group-Housed Livestock, With Application to Pig Breeding. Frontiers in Genetics, 11, 611073.

van der Zande, L.E., Dunkelberger, J.R., Rodenburg, T.B., Bolhuis, J.E., Mathur, P.K., Cairns, W.J., Keyes, M.C., Eggert, J.M., Little, E.A., Dee, S.A., & Knol, E.F. (2020) Quantifying Individual Response to PRRSV Using Dynamic Indicators of Resilience Based on Activity. Frontiers in Veterinary Science, 7, 325.

van der Zande, L.E., Guzhva, O., Rodenburg, T.B. (2021) Individual detection and tracking of group housed pigs in their home pen using computer vision. Frontiers in Animal Science, 2, 669312.

Dervishi, E., Reimert, I., van der Zande, L.E., Mathur, P.K., Knol, E.F., Plastow, G.S. (2021) Relationship between indirect genetic effects for growth, environmental enrichment, coping style and sex with the serum metabolome profile of pigs. Scientific reports, 11, 1.

Parois, S.P., van der Zande, L.E., Knol, E.F., Kemp, B., Rodenburg, T.B., Bolhuis, J.E. (2022) Effects of a Multi-Suckling System Combined With Enriched Housing Post-Weaning on Response and Cognitive Resilience to Isolation. Frontiers in Veterinary Science, 9, 868149.

Luo, L., van der Zande, L.E., van Marwijk, M., Knol, E.F., Rodenburg, T.B., Bolhuis, J.E., Parois, S. (2022). Impact of enrichment and repeated mixing on resilience in pigs. Frontiers in Veterinary Science, 9, 829060.

Parois, S.P., van der Zande, L.E., Knol, E.F., Kemp, B., Rodenburg, T.B., Bolhuis, J.E. (2022) A multi-suckling system combined with an enriched housing environment during the growing period promotes resilience to various challenges in pigs. Scientific reports, 12, 1.

van der Zande, L.E., Guzhva, O., Parois, S.P., van de Leemput, I., Bolhuis, J.E., Rodenburg, T.B. (2022) Estimation of resilience parameters following LPS injection based on activity measured with computer vision. Frontiers in Animal Science, 80 883940.

Contributions to scientific conferences

Rodenburg, T.B., van der Zande, LE., de Haas, E.N., Kostal, L., Pichova, K., Piette, D., Tetens, J., Visser, B., de Klerk, B., van der Sluis, M., Bennewitz, J., Siegford, J., Norton, T., Guzhva, O. and Ellen, E.D. (2019) Reduce damaging behaviour in laying hens and pigs by developing sensor technologies to inform breeding programs. 53rd International Congress of the International Society for Applied Ethology (ISAE). Bergen, Norway.

Rodenburg, T.B., Bennewitz, J., De Haas, E.N., Koštťál, L., Pichová, K., Piette, D., Tetens, J., Visser, B., De Klerk, B., van der Sluis, M., van der Zande, L.E., Siegford, J., Toscano, M., Norton, T., Guzhva, O. and Ellen, E.D. (2019). Developing sensor technologies to inform breeding approaches to reduce damaging behaviour in laying hens and pigs: The GroupHouseNet approach. 9th European Conference on Precision Livestock Farming (ECPLF). Cork, Ireland.

Dervishi, E., van der Zande, L.E., da Silva Valente, T., Reimert, I., Mathur, P.K., Lopes, M.S., Knol, E.F., Plastow, G.S. (2019). Combining metabolomics and genomics to elucidate physiological processes related to tail damage score in pigs. 37th International Society for Animal Genetics Conference (ISAG). Lleida, Spain.

van der Zande, L.E., Dunkelberger, J.R., Rodenburg, T.B., Mathur, P.K., Cairns, W.J., Keyes, M.C., Knol, E.F., Dee, S.A., & Little, E.A. (2019). Defining resilient pigs after a PRRS challenge using activity and feeding data from accelerometers. WIAS Science Day. Wageningen, Netherlands.

van der Zande, L.E., Dunkelberger, J.R., Rodenburg, T.B., Mathur, P.K., Cairns, W.J., Keyes, M.C., Eggert, J.M., Little, E.A., Dee, S.A., & Knol, E.F. (2019) Defining resilient pigs after a PRRS challenge using activity and feeding data from accelerometers. Precision Livestock Farming workshop. Copenhagen, Denmark.

van der Zande, L.E., Dunkelberger, J.R., Rodenburg, T.B., Mathur, P.K., Cairns, W.J., Keyes, M.C., Eggert, J.M., Little, E.A., Dee, S.A., & Knol, E.F. (2019). Defining resilient pigs after a Porcine Reproductive and Respiratory Syndrome Virus (PRRSV) challenge using activity and feeding data from accelerometers. 9th European Conference on Precision Livestock Farming (ECPLF). Cork, Ireland.

van der Zande, L.E., Guzhva, O., Rodenburg, T.B. (2021) Individual activity monitoring as a measure of the resilience of group-housed pigs using computer vision. 8th International Conference on the Assessment of Animal Welfare at Farm and Group level (WAFL). Online.

van der Zande, L.E., Guzhva, O., Rodenburg, T.B. (2021) Monitoring activity on an individual level of group-housed pigs using computer vision. 54th International Congress of the International Society for Applied Ethology (ISAE). Online.

van der Zande, L.E., Guzhva, O., Rodenburg, T.B. (2021) Individual detection and tracking of group-housed pigs in their home pen using computer vision. WIAS Annual Conference. Online.

van der Zande, L.E., Guzhva, O., Rodenburg, T.B. (2021) Detection and tracking of individual pigs housed in groups using computer vision. Precision Livestock Farming workshop. Online.

Parois, S., Rodenburg, T.B., van der Zande, L.E., Telkänranta, H., Kemp, B., Bolhuis, J.E. (2021) Measuring sickness recovery with thermal imaging. 72nd Annual meeting of the european federation of animal science (EAAP). Davos, Switzerland.

van der Zande, L.E., Guzhva, O., Parois, S.P., van de Leemput, I.A., van Nes, E.H., Bolhuis, J.E., Rodenburg, T.B. (2022) Estimation of resilience parameters based on activity measuredwith computer vision following LPS injection. 27th WIAS Annual Conference. Wageningen, Netherlands.

Education & Training certificate

The basic package (1.8 ECTS[1])

WIAS Introduction Day	2018
WGS course Scientific Integrity & Ethics and Animals Science	2018

Disciplinary Competences (9.6 ECTS)

Training school GroupHouseNet: Genetics of damaging behaviour	2018
Video and Image Analysis course	2021
Applied data science with Python	2022

Professional Competences (9.2 ECTS)

Writing literature survey	2018
Research Data Management	2018
Scientific Writing	2018
Scientific Publishing	2018
Writing propositions for your PhD	2021
Last Stretch of the PhD Programme	2021
The Final Touch	2022

Presentation Skills (maximum of 4 ECTS)

WIAS Science day, 18 March, Ede, poster	2019
PLF workshop, Copenhagen Denmark, 11-12 April, oral	2019
ECPLF, Cork Ireland, 26-29 August, oral	2019
ISAE, Bergen Norway, 5-9 August, oral	2019

WAFL, Online, 16-19 August, oral	2021
ISAE, Online, 2-6 August, oral	2021
PLF workshop, Online, 13-14 April, oral	2021
WIAS science day, Online, 28-29 April, oral	2021
WIAS science day, Lunteren Nederland, 11 February, oral	2022

Teaching competences (maximum of 6 ECTS)	
Supervising MSc Major	2019
Supervising MSc Minor	2020
Supervising project group Introduction to the Animal Sciences	2020
Co-develop course Sensor Data in Animal Sciences	2021
Lectures and practical in course Sensor Data in Animal Sciences	2021

Education and Training Total (30.6 ECTS)

[1] One ECTS credit equals a study load of approximately 28 hours

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