Author/s:
Lucas, Megan Elinor

Title:
Positive Human-Animal Interactions, Early Life Experiences and Stress Resilience in Pigs

Date:
2022

Persistent Link:
https://hdl.handle.net/11343/315704

Positive Human-Animal Interactions, Early Life Experiences and Stress Resilience in Pigs

Megan Lucas (née Hayes)

ORCID ID: 0000-0002-5730-7535

Submitted in total fulfilment for the degree of Doctor of Philosophy

May 2022

Animal Welfare Science Centre
Faculty of Veterinary and Agricultural Sciences
The University of Melbourne

Abstract

Pigs in intensive production systems routinely encounter several challenging situations including abrupt weaning, painful husbandry procedures, intense contact with stockpeople, and exposure to novel social and physical environments. The ability of pigs to cope with these routine stressors has implications for their welfare and productivity and may be affected by their previous experiences with humans. Furthermore, experiences that occur early in life, including interactions with the dam, the physical environment as well as early experiences with humans, may have a profound effect on the development of stress coping mechanisms that impacts how pigs cope with stress throughout their lives. The aim of this thesis was to examine the effects of positive interactions with humans and early life human and housing experiences on stress resilience in pigs. Stress resilience was measured based on: 1. behavioural and physiological responses to stressors such as routine husbandry and management practices, isolation, novelty, and humans; 2. basal behavioural and physiological measurements that reflect how pigs cope with their general environment; and 3. measurements of biological fitness.

Overall, positive interactions with humans, including patting, stroking and scratching pigs, reduced pigs' fear of humans and their stress responses to routine husbandry and management practices imposed by stockpeople. Sows that received 2 min of daily positive human contact during gestation showed less avoidance of and more interaction with stockpeople imposing pregnancy testing and vaccination in the home pens, and piglets that received 3 or 5 minutes of regular positive human contact showed less escape behaviour and vocalisations during husbandry procedures in the lactation period. Providing opportunities for positive human interaction from 0-4 wk of age was extremely effective in reducing fear of humans, based on positively handled piglets showing increased approach and interaction with an unfamiliar human at 2 and 3 wk of age. Although the effects of handling on fear of humans appeared to weaken over time, there was evidence of early positive human contact resulting in pigs showing less fearful responses to a human at 6, 9 and 14 wk of age, indicating that some reduction in fear of humans lasted well beyond the period when the handling treatment was imposed. In addition to reducing fear of humans, there was evidence that early positive human contact conferred broader effects on stress resilience. Early positive handling reduced pigs' fear of a novel object at 3 wk of age, escape behaviour after weaning, and cortisol concentrations after weaning and after isolation at 7 wk of age. Furthermore, early positive handling reduced pigs' injuries and increased serum concentrations of brain-derived neurotrophic factor (BDNF) at 4 wk of age, with limited evidence indicating similar effects on these measurements much later in life at 17 wk of age.

The early housing environment had a considerable effect on the human-animal relationship, with pigs reared in farrowing crates showing less fear of humans at 2, 3, 4 and 6 wk of age compared to pigs reared in loose farrowing and lactation pens with more space, physical complexity, and opportunity for interaction with the sow. During the lactation period, piglets in loose pens showed more play behaviour and less repetitive nosing of pen mates and had higher concentrations of serum BDNF compared to piglets in farrowing crates, but surprisingly, they were less able to cope with several stressors before and after weaning. During the lactation period, loose pen piglets showed more avoidance of a novel object, a greater intensity of escape behaviour during capture by a stockperson, and more injuries at 2 wk of age. After weaning, loose pen pigs showed more escape behaviour, less inactivity and had higher cortisol concentrations. At 7 wk of age, loose pen pigs showed more vocalising in a novel area and had higher cortisol concentrations after isolation, and at 21 wk of age, they showed more baulking when being moved out of the home pen by a stockperson.

This research showed that positive interactions with humans can ameliorate the stress associated with routine husbandry practices involving stockpeople. When imposed early in life, positive handling had an extended effect on reducing pigs' fear of humans and appeared to foster stress resilience in a general capacity, based on fewer injuries, higher BDNF concentrations and reduced responses to challenges such as novelty, weaning and isolation in positively handled pigs. In addition to reducing fear of humans through habituation and conditioning, brief and regular close human contact early in life may provide a minor challenge to overcome, that improves the competence of pigs to cope with other types of challenges faced in the future. This research also demonstrated considerable differences in the stress resilience of pigs reared in the two housing treatments studied. While piglets in the loose system showed more play behaviour and had higher BDNF concentrations during the lactation period (both of which are linked to stress resilience) they showed far less flexibility in response to stressors such as routine husbandry practices, weaning, isolation, and exposure to novelty and humans. Piglets in the loose system were reared in a more isolated environment with less contact with people and other pigs and less visual stimulation in general. This may have resulted in loose pen piglets having fewer opportunities to learn to cope with small challenges such as frequent and/or close exposure to stockpeople and other pigs, thus increasing their vulnerability to stress. In addition, heightened maternal responses of loose pen sows towards stockpeople may have increased loose pen piglets' fear of humans. More research is needed to determine the specific features of the early housing environment that affect both immediate and long-term fear and stress responses of pigs. Overall, this research: 1. showed that the early human and housing environment for pigs can have both immediate and longer-term consequences on stress resilience; and 2. contributed to a growing body of work showing that humans are a key determining factor in the welfare of pigs.

Declaration

This is to certify that:

I. This thesis comprises only my original work towards the degree of Doctor of Philosophy except where indicated in the preface.
II. Due acknowledgement has been made in the text to all other material used.
III. This thesis is fewer than 100 000 words in length, exclusive of tables and references.

Megan Lucas (née Hayes)

May 2022

Preface

The experiments presented in this thesis were conducted at the research and innovation unit of a commercial piggery in NSW, Australia. All animal procedures were conducted with prior institutional ethical approval under the requirements of the New South Wales Prevention of Cruelty to Animals Act 1985 in accordance with the National Health and Medical Research Council/Commonwealth Scientific and Industrial Research Organization/Australian Animal Commission Code of Practice for the Care and Use of Animals for Scientific Purposes.

Chapter 2 is presented as the author-accepted manuscript of a peer-reviewed article:

Hayes, M. E., Hemsworth, L. M., Morrison, R. S., Butler, K. L., Rice, M., Rault, J.-L., & Hemsworth, P. H. (2021). Effects of positive human contact during gestation on the behaviour, physiology and reproductive performance of sows. *Animals, 11*(1), 214.

The following contributions were made by authors: Conceptualisation and methodology, P.H.H., L.M.H., R.S.M., J.L.R., K.L.B. and M.R.; investigation, M.E.H., L.M.H. and M.R.; formal analysis, M.E.H. and K.L.B.; supervision, P.H.H., L.M.H., and R.S.M.; writing—original draft preparation, M.E.H.; writing—review and editing, M.E.H., P.H.H., L.M.H., R.S.M., J.-L.R., K.L.B and M.R.

The research presented in Chapter 2 was funded by the Australian Pork Co-operative Research Centre (1C-120).

Chapter 3 is presented as the author-accepted manuscript of a peer-reviewed article:

Hayes, M. E., Hemsworth, L. M., Morrison, R. S., Tilbrook, A. J., & Hemsworth, P. H. (2021). Positive human contact and housing systems impact the responses of piglets to various stressors. *Animals, 11*(6), 1619.

The following contributions were made by authors: Conceptualisation and methodology, M.E.H., P.H.H., L.M.H., R.S.M., and A.J.T.; investigation, M.E.H., and L.M.H.; formal analysis, M.E.H.; supervision, P.H.H., L.M.H., R.S.M., and A.J.T; writing—original draft preparation, M.E.H.; writing—review and editing, M.E.H., P.H.H., L.M.H., R.S.M., and A.J.T.

The research presented in Chapter 3 was funded by an Australian Pork Limited postgraduate study award (2017/003) and Rivalea Australia (19B012C).

Chapter 4 presents research that has not been submitted for publication at the time of submission of this thesis.

The following authors contributed to the research presented in Chapter 4:

Lucas, M. E., Hemsworth, L. M., Butler, K. L., Morrison, R. S., Tilbrook, A. J., Rault, J.-L., Marchant, J. N., Galea, R.Y., Hemsworth, P. H.

The following contributions were made by authors: Conceptualisation and methodology, M.E.L., P.H.H., A.J.T. L.M.H., R.S.M., K.L.B., J.N.M., and J.-L.R.; investigation, M.E.L., R.Y.G., J.N.M., and L.M.H.; formal analysis, M.E.L., and K.L.B.; supervision, P.H.H., L.M.H., R.S.M., and A.J.T; writing—original draft preparation, M.E.H.; writing—review and editing, M.E.L., P.H.H., L.M.H., A.J.T., K.L.B. and J.N.M.

The research presented in Chapter 4 was funded by an Australian Research Council Linkage project grant (LP180100218) in collaboration with the Australasian Pork Research Institute Ltd.

My PhD candidature was supported by an Australian Government Research Training Program Scholarship and a postgraduate study award from Australian Pork Limited.

Acknowledgements

First and foremost, I would like to acknowledge my primary supervisor, Prof. Paul Hemsworth, and my co-supervisors, Dr. Lauren Hemsworth, Prof. Alan Tilbrook, and Dr. Rebecca Morrison. Together these four people truly do make a dream team of supervisors for a PhD student. Being given the opportunity to learn about research under the guidance of Paul Hemsworth is a privilege. Paul has constantly challenged me with his critical thinking and never failed to provide me with the most valuable and constructive feedback on drafts of abstracts/manuscripts/presentations, even when I didn't provide them to him in a timely manner… Lauren Hemsworth has been a source of unwavering support in so many ways throughout my PhD. Lauren has continuously sought out opportunities for me outside of my PhD that have allowed me to build my skills as a researcher. Rebecca Morrison taught me so much about pig production and pig research, and she is surely one of the only supervisors in the world who would keep you company spinning blood samples in the lab on a Friday night instead of going home to their family. Alan Tilbrook has always been available to answer my questions and share his knowledge of stress physiology. Alan diligently attended every one of my supervisor meetings, patiently listening to Paul, Lauren and I discuss the logistics of working with GoPro cameras for hours on end. I feel so grateful to have been able to learn from each of these talented people and I look forward to contributing to more pig welfare research with them in the future.

This research would not have been possible without the help of many research assistants and students from the Animal Welfare Science Centre. A huge thank you to everyone that worked on our experiments by assisting with on farm data collection, video observations, assays, admin and more. The support of the postgraduate students at the AWSC has been immeasurable. A special thanks to those who have kept me company on Zoom in endless virtual workspace sessions since 2020.

I'm so grateful for the pigs, stockpeople and the entire technical team at Rivalea that facilitated this research, welcomed me, adapted their schedules to work around me, and took to the time to teach me about the pig industry.

Lastly, I must acknowledge my two biggest supporters, my husband Jack, and our friend Nacho. Jack, upon reflection it was a little psychotic to organise a wedding and submit my thesis in the same few months. But it made for the best year of my life so thanks for marrying me while I was still a "Uni student". Most of all, thanks for your patience and tolerance of me during this journey.

Table of Contents

List of Tables

Chapter 2

Chapter 3

Chapter 4

List of Figures

Chapter 1

Chapter 2

Chapter 3

Chapter 4

Chapter 1

Literature Review

1.1 Introduction

Pigs in modern indoor production systems are regularly challenged with stressors such as abrupt weaning, painful husbandry procedures, intense contact with stockpeople and exposure to novel social and physical environments. A lack of competency of pigs to cope with these routine stressors poses a risk to their welfare and to farm productivity. Coping ability, or stress resilience, can be affected by several factors in the pig's environment. Given that close interactions between pigs and stockpeople occur frequently in intensive production systems, the human-animal relationship is one such factor. A considerable body of research has shown that regular and/or intense negative experiences with humans induce high levels of stress in pigs (Hemsworth & Coleman, 2011; Waiblinger et al., 2006). However, with increasing societal concern for animal welfare, research has shifted to focus on providing animals with positive affective experiences rather than only alleviating negative ones, and as such there is growing interest in understanding how positive experiences with humans affect animals (Mellor et al., 2020; Rault et al., 2020).

Furthermore, in laboratory animals, early post-natal experiences appear to have a marked effect on the development of stress coping mechanisms, to the extent that certain early environmental experiences result in permanent changes to the responses of animals to stress (Lyons et al., 2009; Meaney, 2001; Parker & Maestripieri, 2011; Pryce & Feldon, 2003). While studied to a lesser extent in farm animals there is some indication of similar effects in pigs, whereby experiences early in life result in changes to behaviour, physiology and cognition later in life (Telkänranta & Edwards, 2018). Early life experiences with humans, as well as other experiences including those with the dam and the physical environment, may shape how pigs respond to routine stressors throughout their lives. If exposing pigs (or, not exposing them) to certain experiences during development can improve their competence in coping with stress in both an immediate and long-term capacity, then pig producers will be better able to prepare their animals to cope with routine husbandry and management challenges. Therefore, expanding our understanding of the effects of the human-animal relationship and early rearing experiences on stress resilience in pigs may provide opportunities to improve animal welfare and productivity.

1.2 Stress Vulnerability and Resilience in Animals

1.2.1 Definition and Development

The term ‘stress’ is most widely defined as *“the biological response elicited when an animal perceives a threat to its homeostasis”* (Moberg, 2000). This biological response involves several adaptive defenses to restore homeostasis, including changes to behaviour and activation of the autonomic nervous system, the neuroendocrine system and the immune system (Moberg, 2000). Activation of these defenses comes at the expense of biological resources being diverted away from other functions, and in cases where the stressor is intense or exposure is prolonged, the cost of coping with stress can impair animal welfare, growth, reproduction and immune competency (Moberg, 2000). Of course, not all stressful experiences result in these impairments. The degree to which stress negatively affects biological functioning is determined by the ability of the animal to cope with and recover from stress, which is referred to as stress resilience (Iacoviella & Charney, 2019). Stress resilience can be characterized both by resistance to adverse effects of stressors and fast recovery to normal functioning after stress exposure (Chen, 2019). Exposure to prolonged or intense stress can lead to stress vulnerability, while exposure to stress that is ‘challenging but not overwhelming’ fosters resilience by allowing animals to learn adaptive coping strategies for future stressful situations (Lyons et al., 2009; Lyons et al., 2010; Lyons & Schatzberg, 2019; Parker & Maestripieri, 2011). Early life experiences with stress can be particularly important in molding coping mechanisms as the brain is extremely plastic during early periods of development, which allows experiences, interacting with genetic factors, to have a marked effect on the behaviour and physiology of the animal as an adult (Knudsen, 2004). When learning to cope with stress takes place early in life, the effects on stress resilience can be sustained long-term (Lyons & Schatzberg, 2019).

Initial indications of the importance of early life experiences in shaping stress resilience came from an experiment by Levine and colleagues examining the long-term consequences of early trauma in rodents. Contrary to expectations, improved learning performance and greater behavioural flexibility as adults was observed in rats that had been exposed as neonates to electric shock and/or handling compared to undisturbed controls (Levine et al., 1956). These results indicated that early life stress improved adaptivity of the rats, which triggered a series of follow up experiments studying the effects of several different manipulations to the early environment on later behaviour and physiology. In most of these experiments, the focus was on the effects of early human handling while pups were separated from the dam. Results were consistent in demonstrating that brief exposure to these stressors early in life reduced the emotional response of rodents to stressors in adulthood. In comparison to non-handled controls, rats that were removed from the dam and handled outside the home cage for short periods during infancy showed increased activity, less defecation and lower corticosterone responses in an open field test as adults (Levine et al., 1967). Reduced basal corticosterone concentrations were also observed (Meaney et al., 1991; Meaney et al., 1988). These and several studies since (reviewed by (Meaney, 2001; Pryce & Feldon, 2003)) have provided strong evidence for moderate stressors such as handling and brief maternal separation altering activity of the hypothalamic-pituitary-adrenal (HPA) axis, a primary neuroendocrine axis responsible for regulating key biological functions affected by stress including behaviour, immune competency, metabolism and reproduction (Moberg, 2000). Moderate stress during the neonatal period increases the sensitivity of the hippocampus to glucocorticoids, which enhances negative feedback on HPA axis activity resulting in reduced behavioural and physiological responses to stressors (Meaney et al., 1988; Meaney et al., 1993).

One hypothesis for the effects of early handling on HPA axis programming is that the changes are mediated by differences in maternal behaviour induced by dam-pup separation for handling, rather than being a direct result of handling *per se*. After pups that have been handled are reunited with their dams, several maternal behaviours such as licking and grooming of pups increase in frequency (Smotherman et al., 1977). Increased occurrence of these maternal behaviours is correlated with a reduction in HPA axis activity in offspring, which suggests that the behaviour of the dam is responsible for programming the neuroendocrine stress response of her pups (Liu et al., 1997). Although, it has also been suggested that maternal behaviour and environmental stress (i.e., handling, maternal separation) exert independent effects on HPA axis activity (Macrì & Würbel, 2006). This is supported by research on maternal separation in non-human primates. Young squirrel monkeys exposed to brief separations from their social group either with or without their dam subsequently showed reduced cortisol responses to a novel environment compared to non-separated monkeys that remained with their groups (Parker et al., 2006). Changes in maternal behaviours in dams from the two separation treatments did not relate to differences in arousal regulation, suggesting that differences in HPA axis activity were more likely to be related directly to early stress exposure rather than to variations in maternal care.

Early environmental manipulations other than handling or maternal separation, without affecting maternal care, can also provide opportunities for animals to overcome challenge. Rearing in a physically or socially enriched environment reduces emotionality by way of providing behavioural options to cope with stressors and providing agency for animals to learn about aspects of their environment (Carlstead & Shepherdson, 2000). For example, a physically complex environment provides more chances for hiding in response to threats as well as more chances for learning spatial navigation skills, which fosters stress resilience by providing opportunities for animals to learn to cope with challenge (Crofton et al., 2015). In contrast, a lack of challenge early in life leads to stress vulnerability as there are too few opportunities to learn to cope with stress effectively (Parker & Maestripieri, 2011). Parker and Maestripieri (2011) suggest that exposure to early life stress, and the associated outcome on stress resilience, is best conceptualized as a quadratic function. In summary, brief intermittent stress exposure enhances HPA axis regulation and reduces emotionality, while the opposite effect is seen in response to chronic stress exposure or a severe lack of stress exposure altogether. While this model has primarily been studied in rodents and non-human primates, it is applicable to other species.

1.2.2 Measuring Stress Vulnerability and Resilience in Farm Animals

Livestock are exposed to several stressful challenges as part of routine management in animal production systems. Most of these stressors are acute and relatively brief in duration. Although, the cumulative effect of several acute stressors can lead to chronic stress, and both acute stress and particularly chronic stress can disrupt homeostasis and impact biological functions (Hemsworth & Coleman, 2011; Moberg, 2000). Stressors commonly encountered by livestock include exposure to abrupt weaning, transport, confinement, novel situations and environments, painful husbandry procedures and close contact with stockpeople. Livestock may also be required to live in large and dense groups in barren environments and may be restricted from performing highly motivated species-specific behaviours. Furthermore, livestock may be exposed to extreme temperatures, disease and restrictions in feed supply. Pigs in intensive production systems may experience all of these stressors. Understanding what makes pigs resilient or vulnerable to stress is of great interest as an impaired ability of pigs to cope with routine stressors is likely to negatively affect animal welfare and productivity. Therefore, there is considerable interest in understanding how best to measure stress in pigs and other farmed animals.

Measuring stress responses in animals involves examination of the physiological biomarkers implicated in responding to stress. By far, the HPA axis has received the greatest attention, with glucocorticoids being the most widely studied indicators of stress. Cortisol has been reported to increase in pigs in response to several challenges, including weaning (de Ruyter et al., 2017; Escribano et al., 2019; Turpin et al., 2017), individual housing (Marco-Ramell et al., 2016; Royo et al., 2005), mixing with other pigs (Escribano et al., 2015), reduced space allowance (Hemsworth et al., 2016; Hemsworth et al., 2013), transport (Dang & Kim, 2021), tail docking (Numberger et al., 2016; Sutherland et al., 2011), castration (Llamas Moya et al., 2008; Numberger et al., 2016; Sutherland et al., 2017), ear tagging (Numberger et al., 2016; Yonezawa et al., 2012) and restraint (De Jonge et al., 1996). However, the validity of using cortisol as a biomarker of stress has been questioned since the hormones released by frontline defense systems have several functions and are not solely synthesised in response to stress (Crofton et al., 2015; Moberg, 2000; Ralph & Tilbrook, 2016; Tilbrook & Ralph, 2018). Both positive and negative affective experiences can elicit synthesis of glucocorticoids (Buwalda et al., 2012), and thus elevations in cortisol need to be interpreted with caution.

The use of stress biomarkers alternative to glucocorticoids is growing. Brain-derived neurotrophic factor (BDNF), a neurotrophin which plays an important role in brain plasticity, is thought to mediate stress resilience by regulating HPA axis activity at a young age (Taliaz et al., 2011). In pigs, serum concentrations of BDNF increase with the provision of a foraging block as enrichment (Rault et al., 2018), and hippocampal concentrations of BDNF decrease after exposure to high-stress transport conditions compared to low-stress transport conditions (Arroyo et al., 2019). Increased severity of tear staining, which refers to the porphyrin-pigmented secretion under the inner-corner of the eye of many vertebrates, has been used as an indicator of stress in rodents (Mason et al., 2004) and its usefulness is being explored in other species. In pigs, tear staining has been shown to increase in response to stressors such as social isolation and living in a barren environment (DeBoer et al., 2015). Additionally, tear staining has been shown to increase prior to tail damage (Larsen et al., 2019) and has been positively correlated with increased fear of novelty (Telkänranta et al., 2016). The mechanism and function of stress-induced tear staining is not well understood, although one hypothesis is that it is part of the immune response to stress (Payne, 1994).

Common indicators of the immune system response to stress include increases in the ratio of neutrophil to lymphocyte cells and increased production of immunoglobulins and acute phase proteins. The ratio of neutrophil to lymphocyte cells is known to increase after weaning in pigs (Puppe et al., 1997; Turpin et al., 2017; Turpin et al., 2016). Serum and plasma concentrations of the acute phase protein haptoglobin have also been seen to increase after weaning (De et al., 2017; Turpin et al., 2017; Turpin et al., 2016), as well as after other stressors such as isolation (Marco-Ramell et al., 2016) and transport (Piñeiro et al., 2007). Glucocorticoids, although not solely responsible, are thought to play a role in activating these aspects of the immune response to acute stress (Murata, 2007; Sapolsky et al., 2000). However, correlations between glucocorticoids and immune biomarkers are not always strong. For example, only a weak positive correlation was found between salivary cortisol and haptoglobin concentrations before and after pigs were exposed to stress induced by mixing and feed deprivation (Ott et al., 2014). This may be because the peak responses of different stress defense systems can occur at different times, which highlights the need to collect physiological measurements at multiple timepoints. Another consideration is that all the possible defense systems are not always utilized, as the stress response is non-specific. Different stressors can elicit different responses. Escribano and colleagues (2015) reported that salivary cortisol (neuroendocrine response) in pigs increased in response to social stress but not in response to isolation stress, while salivary concentrations of immunoglobulin A (immune response) increased in response to isolation but not social stress. Furthermore, while an initial release of glucocorticoids will activate the immune system, their chronic elevation has the opposite effect and suppresses immune activity which can increase the difficulty of interpreting immune responses to stress (Sapolsky, 2000; Sapolsky et al., 2000).

Changes in physiological systems should be interpreted alongside changes in behaviour when measuring stress in farm animals. Behavioural responses to stress can be assessed when animals are exposed to challenging situations, such as routine management practices or in response to psychological stressors in standardised emotionality tests. Measurements in such situations may include the magnitude and duration of startle responses, escape and avoidance behaviour, activity level, urination and defecation, vocalisations and the time taken to return to normal activity following stress exposure (Cook et al., 2000; Forkman et al., 2007). Emotionality tests used to study pigs most commonly assess behavioural responses to one or a combination of either a novel arena, a sudden auditory or visual stimulus, a novel object or an unfamiliar human that is stationary or approaching (Forkman et al., 2007). These tests are used to examine fear responses, and while fear and stress are not synonymous, it is recognised that fear contributes to stress and therefore high levels of fear can indicate stress vulnerability (Rushen et al., 1999).

Stress can emerge not just from stress-provoking stimuli but also from frustration and thwarting of highly motivated or goal-directed behaviours and a lack of variation in the environment. Stereotypic and other abnormal behaviours are known pathologies of such situations (Mason & Rushen, 2006). For example, feed-restricted sows perform oral stereotypies when foraging behaviour is thwarted (Lawrence & Terlouw, 1993). While the presence of stereotypic behaviour is frequently used as an indicator of stress, it has been debated that while such behaviours can appear to have no obvious function, they may actually reflect an adaptative response to the environment and as such may not be harmful to the animal or signify stress (Mason & Rushen, 2006; Price, 1985). Furthermore, the presence of stereotypies can reflect previous stress rather than current stress, since once developed, they can persist even in the absence of the original eliciting conditions (Mason & Rushen, 2006). Other abnormal behaviours that can develop under similar conditions as stereotypies are more obviously harmful, such as tail biting in pigs (Schrøder-Petersen & Simonsen, 2001) and feather pecking in poultry (Cronin & Glatz, 2021).

Deviations from normal behavioural patterns can be useful indicators of stress coping. Changes to pigs' feeding patterns and activity time budgets have been associated with and shown to precede clinical disease and tail biting (reviewed by: (Bus et al., 2021; Matthews et al., 2016)). The diversity of behaviours is also known to reduce when biological resources are being used to cope with stress (Cook et al., 2000). Behaviours associated with positive affective states may indicate an absence of stress since they are usually reduced under challenging conditions. For example, locomotor and social play behaviours reduce in response to stressors such as weaning (Donaldson et al., 2002), and also occur less frequently when pigs are housed in barren environments compared to environments enriched with straw (Chaloupková et al., 2007a). It's been suggested that one of the functions of play behaviour is to improve stress coping abilities by enabling animals to recover from unexpected events (Spinka et al., 2001). Therefore, play behaviour may both contribute to and indicate stress resilience.

In addition to examining behavioural and physiological responses to stress, it is important to look at the biological consequences of these responses on animal health and productivity to determine the extent to which the animal's attempt to cope with stress is successful or not (Moberg, 2000). Prolonged or repetitively high levels of fear towards humans can induce chronic stress in pigs and have been associated with several factors related to poor reproductive performance, including reduced pregnancy (Hemsworth et al., 1986a) and farrowing rate (Hemsworth et al., 1989), smaller litter sizes (Hemsworth et al., 1981b), longer durations of farrowing and longer inter-piglet birth intervals, (Janczak et al., 2003) and an increased incidence of piglets being stillborn (Hemsworth et al., 1999) crushed (Lensink et al., 2009) savaged (Marchant Forde, 2002), and dying without milk in their stomach (Janczak et al., 2003). Reduced growth in pigs has also been reported as a consequence of high fear of humans (Hemsworth et al., 1981a), as well as other stressors such as weaning (Weary et al., 2008), mixing with other pigs (Hyun et al., 1998) and high stocking densities (Hyun et al., 1998; Smit et al., 2021). It should be recognised that some stressors can pose a significant risk to animal welfare but may not necessarily result in impaired health or productivity. For example, sows in farrowing crates maintain high levels of reproductive performance despite the restrictions this housing system imposes on the ability of sows to move, interact with their piglets, and perform other highly motivated behaviours.

Animal stress is best measured by examining the behavioural and physiological responses that typically occur in response to challenging situations, and their subsequent effects on health and productivity. Ideally a suite of physiological biomarkers from the different systems involved in responding to stress should be examined at several timepoints following stress exposure and interpreted in tandem to changes in the animal's behaviour. The behaviour of the animal in its habitual environment can indicate how the animal is coping in general, while behavioural responses to challenging or fear-provoking stimuli or situations can indicate how the animal copes with additional stressors in its environment. Furthermore, the consequences of stress on the animal's health and productivity should be considered. There are clearly some obvious challenges in measuring stress, however, this approach is currently the best tool to determine factors that affect stress vulnerability and resilience in pigs and thus welfare.

1.3 Human-Animal Interactions

1.3.1 Development and Assessment of the Human-Animal Relationship

Pigs in intensive production systems interact with stockpeople on a regular basis as part of routine husbandry and management. These interactions occur through various sensory channels and may involve tactile, visual, olfactory and auditory contact with stockpeople (Tallet et al., 2018), and can be perceived by pigs on a scale ranging from positive to negative (Hemsworth & Coleman, 2011). Interactions such as gentle stroking and scratching upon approach are perceived positively by pigs (Tallet et al., 2014), while interactions such as shouting and slapping pigs are understandably negative (Hemsworth & Coleman, 2011). Through repeated interactions, relationships can develop between humans and pigs that allow both parties to make inferences about the nature of future interactions (Hemsworth & Coleman, 2011). The valence and frequency of pigs' previous interactions with humans determines the quality of this human-animal relationship from the perspective of the pig (Hemsworth & Coleman, 2011).

Since the quality of the human-animal relationship defines how each partner perceives future interactions with the other, from the perspective of the animal it can be assessed by measuring the response of the animal to the presence of humans (Hemsworth & Coleman, 2011). For example, the behavioural response of the animal to handling or to a stationary or approaching human. The primary response of most domestic animals to humans is fear (Jones, 1997). High levels of animal fear towards humans are generally indicated by more avoidance and less exploration of a human. To some, it may seem counter-intuitive to assess fear by measuring exploration. However, as high levels of fear inhibit other motivational systems including exploration, a lack of exploratory behaviour can be taken as evidence of relatively high levels of fear and vice versa (Feenders et al., 2011).

The aforementioned approach to examining fear is supported by several experiments in which pigs that had previous positive or negative experiences with humans responded differently in terms of their avoidance and exploration of a stationary human. Compared to pigs that were patted and stroked when they approached a person, pigs that were slapped, hit or received an electric shock by a person took longer to approach and physically interact with a stationary human in an arena, spent less time in close proximity to the stationary human and initiated fewer physical interactions with them (Gonyou et al., 1986; Hemsworth & Barnett, 1991; Hemsworth et al., 1981a; Hemsworth et al., 1986a; Hemsworth et al., 1987; Paterson & Pearce, 1989; Pearce et al., 1989). Moreover, there is evidence of a relationship between pigs' exploration of a stationary human in an arena and their physiological stress response to a human in the home pen. For example, the latency of pigs to both approach and physically interact with a stationary human in an arena have been positively related to the magnitude of pigs' change in cortisol concentrations after exposure to a human in the home pen (Hemsworth & Barnett, 1987).

In addition to direct interactions that occur between stockpeople and pigs, there are several factors that can affect the human-animal relationship and its development in this setting. Conditioned responses to humans can develop as a result of pigs associating stockpeople with rewarding or punishing events that occur as part of routine management. Tallet and colleagues (2019) found that piglets that were tail docked by a stockperson before 3 days of age were slower to interact with an unfamiliar human at 14 days of age compared to piglets that hadn't been tail docked but had received the same management, suggesting that pigs can associate humans with stressful and painful husbandry procedures imposed by humans, and this affects their subsequent fear responses. Additionally, Hemsworth and colleagues (1996b) found that gilts that were fed in the close presence of a human were faster to approach a familiar and an unfamiliar person compared to gilts fed with minimal human contact, showing that pigs' fear of humans is reduced when they associate people with positive events such as feeding. Reduced fear of humans may also occur as a result of pigs habituating to human presence through repeated exposure to humans in a neutral context, such as through frequent visual contact with people (Hemsworth & Coleman, 2011). Studies on poultry have shown that visual contact with a human reduces birds' avoidance of a person (Jones, 1993; Taylor et al., 2022) and their fear and stress responses to handling (Zulkifli et al., 2002).

Pigs can discriminate between handlers that are familiar vs. unfamiliar as well as familiar handlers that they have had previous positive vs. aversive interactions with (Brajon et al., 2015a; Sommavilla et al., 2016; Tanida et al., 1995). Pigs can also generalize their previous interactions with one person to other people (Brajon et al., 2015a; Hemsworth et al., 1994; Tanida et al., 1995) and to different locations (Hemsworth et al., 1994), however this generalisation process can be context-dependent (Brajon et al., 2015a). Therefore, while pigs can distinguish between different people, positive or negative interactions with one human can affect pigs' perceptions of other humans.

Characteristics of the human, such as their body language and posture, have been shown to affect how pigs perceive their interactions with humans. Hemsworth and colleagues (1986c) found that pigs showed increased approach and interaction with a person when they squatted, remained stationary, did not wear gloves or did not initiate interactions, compared to when the person stood erect, approached, wore gloves or touched the pig on the snout respectively, suggesting that the characteristics of the person in the latter treatments were more threatening. Other features, such as how predictable interactions with humans are as well as the level of control the animal has in interacting with humans are also likely to be important, although they have not been investigated in great detail for pigs (Hemsworth et al., 1987; Rault et al., 2020).

Through social or emotional contagion, one pigs' perception of humans may also affect the perception of other pigs in the same group. Reimert and colleagues (2017) showed evidence of emotional contagion in pigs, whereby the pen mates of pigs that were exposed to positive and negative treatments behaved similarly to the pigs exposed to the treatments. After exposure to a negative treatment which was imposed outside of the home pen and involved social isolation, restraint and loud noises, both pigs that received the treatment and their pen mates that had remained in the home pen, showed more inactivity and less exploration compared to pigs exposed to a positive treatment involving access to peat, straw and food rewards. Positive or negative interactions with one pig may therefore have consequences for other pigs in the same group. Luna and colleagues (2021) suggested that through social learning, pigs became less fearful of a human when they had observed other pigs positively interacting with a person. Compared to pigs that had received minimal human contact, pigs that observed other pigs being fed a sucrose solution and being gently stroked by a person for 5 weeks were faster to approach and interact with a stationary and approaching stockperson, spent more time near and more time investigating the stockperson, and accepted physical contact by the stockperson more readily. While the authors suggested these effects were a result of observational social learning, it is also possible that the observer pigs showed lower fear of humans from simply habituating to human presence.

The behaviour and physiology of the dam may also affect the responses of her offspring to humans. Rooney and colleagues (2021) classified gilts as either 'friendly' when they quietly accepted physical contact and approached or stayed close to a human, or 'fearful' when they refused physical contact and avoided a human. The authors found that offspring from friendly gilts demonstrated less freezing in an open field test and accepted human contact more readily than piglets from fearful gilts. During late gestation, fearful gilts had higher basal cortisol concentrations than friendly gilts, suggesting that prenatal stress may have contributed to increased fear levels in the offspring, although, genetic factors as well as social learning from the dam may have also played a role.

1.3.2 Effects of Human-Animal Interactions on Stress Resilience in Pigs

There is a considerable body of literature which outlines how the quality of the human-animal relationship can affect stress in farm animals. As discussed in the previous section, negative handling by humans increases pigs' fear of humans in an arena and increases their cortisol response to human presence in the home pen, indicating that a poor human-animal relationship can induce acute stress in pigs. Previous negative interactions with humans have also been associated with a chronic stress response in animals, since for example in many of the experiments where negative handling increased pigs' fear of humans, negative handling also resulted in higher basal cortisol concentrations in the absence of humans (Hemsworth et al., 1981a; Hemsworth et al., 1986a; Hemsworth et al., 1987). This chronic stress response is thought to be responsible for detrimental effects on productivity that have been associated with high levels of fear towards humans. For example as discussed in Section 1.2.2, high levels of fear of humans in pigs have been linked to indicators of poor reproductive performance, such as reduced pregnancy (Hemsworth et al., 1986a) and farrowing rate (Hemsworth et al., 1989), smaller litter sizes (Hemsworth et al., 1981b), longer durations of farrowing and longer inter-piglet birth intervals, (Janczak et al., 2003) and an increased incidence of piglets being stillborn (Hemsworth et al., 1999) crushed (Lensink et al., 2009) savaged (Marchant Forde, 2002) and dying without milk in their stomach (Janczak et al., 2003). High levels of fear of humans have also been associated with reduced growth (Gonyou et al., 1986; Hemsworth & Barnett, 1991; Hemsworth et al., 1996a; Hemsworth et al., 1981a, 1987). Furthermore, rough handling prior to slaughter has been shown to reduce meat quality (Channon et al., 2000; Dokmanovic et al., 2014; Van de Perre et al., 2010; Vermeulen et al., 2015). There are clearly several negative consequences of increased stress induced by high levels of fear towards humans in pigs.

While the effects of negative experiences with humans have been well documented, there has been growing interest in understanding how positive interactions with humans and a positive human-animal relationship affect the welfare of domestic animals (Rault et al., 2020). Since stockpeople are involved in many of the stressors that pigs face as part of routine production (e.g., imposing husbandry procedures), a positive perception of humans may be beneficial in terms of alleviating the cost of routine stressors. Rewarding interactions that can create a positive perception of humans include tactile contacts such as patting, stroking and scratching pigs when they approach humans, as well as moving slowly and talking softly to pigs. Evidence that pigs find these types of interactions rewarding is based on the fact that pigs show increased motivation to be in the presence of handlers that impose them, for example increased voluntary approach and proximity seeking behaviour to handlers is observed after positive human-interactions (Miura et al., 1996; Tanida et al., 1995). Although studied to a lesser extent, other types of behavioural responses during contact with humans also indicate that pigs are motivated to interact with people. Rault and colleagues (2019) found that piglets which were stroked on the abdomen when they approached a familiar person demonstrated a behavioural response that involved lying on the side, stretching the limbs, fast grunts and eye closure, which may have suggested trust in humans. Brajon and colleagues (2015b) found that in the presence of a familiar human, weaned pigs pulled on the human's clothes and displayed vigorous head shaking which resembled behaviour that piglets show when playing with objects, suggesting that humans may even be perceived as play mates or play objects. Positive interactions with humans may even provide a source of environmental enrichment for pigs, as has been suggested for animals in companion (Wells, 2004; Willen et al., 2019), laboratory (Cloutier et al., 2018) and zoo settings (Claxton, 2011). As there is continued interest in providing domestic animals with positive states of welfare rather than only alleviating negative ones, the following sections will primarily focus on the effects of positive human-animal interactions and a positive human-animal relationship for pigs.

1.3.2.1 Fear

Relative to minimal or routine interactions that occur as part of regular husbandry and management, positive interactions with humans, such as patting and stroking upon approach, can improve the human-animal relationship and reduce pigs' fear of humans. Tanida and colleagues (1995) found that 4 week old pigs that individually received 10 minutes of positive handling 5 days per week for 3 weeks, were faster to approach a familiar and an unfamiliar person and spent more time interacting with them compared to pigs that received routine contact from humans. Although there were no differences in responses to being caught by an unfamiliar person in Tanida and colleague's experiment, positively handled pigs showed less escape behaviour when being caught by a familiar person and showed a lower flight distance to an unfamiliar moving person. Hemsworth and colleagues (1986a) imposed positive handling for 5 minutes 3 times per week in the home pens of boars and gilts that were 11 weeks of age at the start of the experiment. At 14 weeks of age, pigs receiving positive handling were faster than pigs receiving routine handling to interact with a human in a novel arena, and at 18 weeks of age were faster to approach and interact with the human and initiated more physical interactions with them. Brajon and colleagues (2015b) studied the effects on fear of several different positive and negative treatments imposed on weaned pigs, including gentle handling and stroking that was delivered in the home pens over 5 days in 18 sessions of 5 minutes each. The responses of pigs to the handler, when the handler was stationary as well as when they approached, were assessed in the home pens weekly after the treatments had been imposed. Compared to pigs that received routine handling, positively handled pigs spent more time in contact with the stationary handler until 3 weeks after the treatment had been imposed, after which the routine handled pigs showed similar responses to the positively handled pigs presumably as they had habituated to the handler from repeated testing. However, in response to the approaching handler, positively handled pigs showed less reactivity (based on less escape behaviour) compared to routine handled pigs throughout the 5 weeks of testing. These experiments provide evidence that patting and stroking upon approach, when imposed at an individual level or a group level, can reduce pigs' fear of the handler. Additionally, these experiments show that there is some generalisation of the effects of positive human interactions. For example, positively handled pigs generalized their response to different humans (i.e., familiar and unfamiliar), locations (i.e., home pen and arena), and contexts (i.e., moving and stationary human).

Although there appears to be some generalisation of the effects of positive and negative interactions with humans, these effects are still likely to be stimulus specific, whereby positive and negative interactions with humans affect animals' fear of humans but have little impact on levels of fear towards other stimuli (Hemsworth & Coleman, 2011). In support of this, Hemsworth and colleagues (1986b) found that 2 minutes of patting and stroking pigs when they approached a handler from 0-8 weeks of age reduced pigs' fear of humans, but had no effect on the locomotion of pigs in a novel arena. In contrast, work by de Oliveira (2015), Zupan (2016) and colleagues found that 2 minutes of handling and stroking imposed mostly daily from 5-35 days of age, increased the willingness of pigs to accept contact from familiar and unfamiliar humans and also reduced their fear in a novel environment. Pigs from the handling treatment vocalised less and showed more play behaviour in a novel arena compared to pigs that received routine handling, suggesting that handling reduced pigs' fear of humans as well as novel situations. It is possible that reduced fear of humans attenuated reduced fear of novel situations in de Oliveira and Zupan's studies since people were involved in the testing procedure, although the same was true in Hemsworth's experiment that found no effects of handling on pigs' responses to a novel arena. In de Oliveira and Zupan's studies, the handling treatment was 'enforced' and involved holding and stroking pigs irrespective of their behaviour, while in the earlier experiment by Hemsworth, pigs were patted and stroked only if they voluntarily approached the handler. Handling that is imposed early in life in a forced fashion rather than opportunistically may provide a minor challenge for pigs to overcome that improves their ability to cope with a wide range of challenges, as is the case with rodents (see Section 1.2.1).

1.3.2.2 Response to Routine Challenges

Most of the challenges that farm animals encounter involve close or intense interactions with humans, and thus interactions with humans can affect animals' stress responses to these challenges. Rushen and colleagues (2001) found that when cows were isolated from other cows and being milked in a novel environment, brushing by a familiar person reduced cows' heart rate, vocalisations, activity and eliminative behaviour. Similarly, previous positive handling has been reported to reduce kicking and restless behaviour in dairy cows during rectal palpation (Waiblinger et al., 2004), and reduce the heart rates of calves during transport and prior to slaughter (Lensink et al., 2001). For pigs, close contact with stockpeople may occur during daily inspections, at weaning and when pigs are loaded for transport or being moved across facilities. Furthermore, pigs may be physically handled during husbandry procedures such as tail docking, vaccination, teeth resection, ear notching and castration that occur at a young age. Handling by stockpeople has been suggested to be one of the most stressful elements of many of these routine husbandry procedures (Marchant-Forde et al., 2009).

Muns and colleagues (2015) studied the effects of providing positive human contact (caressing and talking to piglets) for 6 min during at least 6 suckling bouts on the first day of life on piglets' responses to husbandry procedures. During tail docking at 2 days of age, piglets from the positive human contact treatment showed escape behaviour that was of a shorter duration, but a similar intensity, compared to piglets that received only routine contact from humans. Furthermore, during capture at 15 days of age, positive human contact piglets tended ($p < 0.1$) to show escape behaviour that was of both a shorter duration and lower intensity. Unexpectedly, cortisol concentrations after husbandry procedures at 2 days of age were higher in positively handled pigs, although the authors suggest the HPA axis may not be fully developed by this age. Sommavilla and colleagues (2011) found that piglets showed more resting and fewer escape attempts and agonistic interactions after weaning if they had experience during the lactation period with a handler who moved carefully and used a soft tone of voice, compared to a handler that moved unpredictably and shouted during routine inspections and feeding. These studies show that previous positive interactions with humans can ameliorate the aversiveness of many routine husbandry and management practices.

Positive interactions with humans may also buffer stress when pigs are exposed to challenging situations. Villain and colleagues (2020) found that after a short period of social isolation, weaned pigs spent less time in an attentive state and vocalised less if a familiar human was present, suggesting that the presence of a known person reduced piglets' stress after isolation. Pederson and colleagues (1998) found that positive handling of sows reduced the magnitude of the physiological stress response to individual housing in tethered stalls. Positively handled sows that were patted and talked to for 3 minutes a day had lower basal cortisol concentrations compared to sows that received routine handling, indicating that the positive handling treatment may have alleviated chronic stress associated with the housing system. Furthermore, while sows in the positive handling treatment showed similar behavioural responses to humans compared to sows that received routine handling, the positively handled sows had lower cortisol concentrations after being in the close presence of a human.

1.3.2.3 Cognition

Research by Brajon and colleagues (2015c; 2016) has investigated how the human-animal relationship affects aspects of cognition in pigs. Three handling treatments were imposed on weaned pigs: 1. Gentle contact – involving stroking and talking softly to pigs and walking slowly around the pen; 2. Rough contact – involving approaching pigs, hitting the walls and floor, talking loudly and imposing rough tactile interactions or startling auditory stimuli; and 3. Minimal contact – involving no additional contact with humans. The pigs were trained to discriminate between a 'positive' auditory cue that predicted food rewards and a 'negative' auditory cue that predicted punishments. When exposed to a novel cue intermediate in tone and frequency to the others, pigs from the gentle contact group were more likely than rough and minimal contact piglets to judge the new cue as positive rather than negative, indicating that the gentle contact treatment induced an optimistic judgement bias (Brajon et al., 2015c). There was no effect of the handling treatment on the ability of pigs to successfully learn to discriminate between the positive and negative cues. However, for rough contact pigs, there was a relationship between fear of humans and learning performance. Rough contact pigs which showed highly fearful responses to an unfamiliar human were slower to learn the task than piglets from the same handling treatment that showed less fearful responses to the human (Brajon et al., 2016). Learning mechanisms are important for the development of stress resilience, as resilience encompasses being able to learn to adapt in response to challenge.

1.3.2.4 Social and Maternal Behaviour

There is limited evidence of the human-animal relationship affecting social behaviour in pigs. Rault (2016) reported that talking softly and gently patting weaned pigs on the head and neck when they approached, stimulated the release of oxytocin, which is most often associated with positive social behaviour. Furthermore, increases in oxytocin concentrations were positively correlated with the frequency of interactions pigs initiated with a familiar human. In another study, positive handling for 15 minutes imposed 3 days a week appeared to reduce harmful social behaviour in weaned pigs, as evidenced by a lower prevalence of tail biting in positively handled pigs (Büttner et al., 2018). However, the positive handling treatment in this experiment involved attracting pigs with chopped straw which confounded the comparison of the two handling treatments. Sommavilla and colleagues (2011) found that agonistic behaviour after weaning was reduced in piglets that had experience pre-weaning with a handler that moved carefully and spoke softly compared to a handler that moved unpredictably and shouted. In contrast, Brown and colleagues (2009) reported that pigs which were faster to approach an unfamiliar human tended ($p < 0.1$) to be more aggressive with other pigs at mixing.

Baxter and colleagues (2016) studied the effects of different handling treatments imposed on dairy goats during pregnancy, and found evidence of positive handling improving maternal behaviour. During gestation, goats received either gentle handling (gentle tactile contact upon approach, imposed when pigs voluntarily entered a straw enriched pen containing food rewards), aversive handling (loud vocalisations and unpredictable movements imposed after forced movement to an empty pen, random presence of a dog outside the pen) or minimal handling (routine handling only). In the first 2 hours after parturition, gently handled goats demonstrated greater attentiveness towards their offspring, as evidenced by more time grooming and a higher frequency of nosing behaviours towards their kids compared to goats from the minimal and aversive groups. Furthermore, kids from aversively handled goats took longer to perform play behaviours and longer to stand, reach the udder and suck compared to kids from goats that received minimal or gentle handling. There may have also been confounding factors in this experiment as the treatments involved positive and negative experiences (e.g., access to straw and food rewards) in addition to interactions with the human.

1.3.2.5 Productivity

Several studies have shown that frequent negative interactions with humans, through inducing fear and stress, can compromise animal health and productivity (Rushen et al., 1999; Waiblinger et al., 2006). Comparatively, less is known about the impacts of positive human-animal interactions.

There is limited evidence that positive handling relative to minimal or routine handling improves reproductive performance in sows. In research by English and colleagues (1999), talking to, stroking and rubbing sows for 1 minute daily appeared to improve ease of handling and reduce sows' fear of humans, but also reduce farrowing duration and the occurrence of piglets being savaged. Similarly, Andersen and colleagues (2006) found that the same handling treatment appeared to reduce farrowing duration, but only in sows that had high levels of fear towards humans at the start of the experiment. The statistical evidence in both experiments was not strong, although the authors report that the direction of effects for most variables measured were consistent in indicating there were benefits from the positive handling treatment. Andersen and colleagues (2006) found no effects of positive handling on piglet survival. In contrast, De Meyer and colleagues (2020) reported reduced piglet mortality by providing sows with daily back scratching and music, although the music may have been a confounding factor. Additionally, Pol and colleagues (2021) reported that confident sows which showed low avoidance of stockpeople, produced and weaned more piglets than fearful sows.

Hemsworth and colleagues (1987) showed that gilts which were stroked when they approached a human had higher growth rates and feed conversion efficiencies compared to gilts that received unpleasant or inconsistent handling treatments. However, the growth and feed conversion rates of positively handled gilts did not differ significantly from gilts that received minimal handling. Furthermore, unpleasant and inconsistently handled gilts had higher cortisol concentrations and were more fearful of humans, suggesting that these treatments impaired productivity rather than the positive handling treatment 'boosting' productivity. In contrast, Day and colleagues (2002b) found walking slowly around the pen and stroking growing pigs upon approach, led to increased daily feed intake in positively handled compared to minimally handled pigs. Although, this did not translate into significant increases to daily weight gain or feed conversion ratios. The duration of positive handling was longer in Day and colleague's experiment (5 minutes daily) compared to that in Hemsworth and colleague's experiment (3 minutes 3 times per week). Muns and colleagues (2015) found no differences in weights at 2 days of age or at weaning of piglets that were patted and stroked on the first day of life compared to piglets that weren't. Research by de Oliveira and colleagues (2015, 2019) examined the effects on growth of stroking piglets when they were being held or restrained from 1-5 weeks of age. Pigs which were habituated to human presence in the home pen showed improved growth at 12 weeks of age compared to their pen mates which were caught and stroked while being held or restrained, suggesting that the stressor of being disturbed but not physically handled stimulated weight gain. Furthermore, the authors also found that for pigs which were handled, growth from 0-12 weeks of age was higher in those that habituated to the handling procedure rather than sensitized to it, as evident by less reactivity during handling over time.

1.4 Early Experiences and Stress Resilience

1.4.1 Important Early Experiences for Pigs

It's been widely acknowledged in several species that during early periods of postnatal development, brain plasticity is most intense which allows experiences to have a profound effect in shaping how animals behave and respond to challenges during adulthood (Knudsen, 2004). While much of the literature on early stress and its effects on subsequent stress resilience has focused on laboratory species, there is evidence in farm animals that early life experiences can have prolonged effects on the behavioural and physiological responses of animals to stressors. Some examples include: maternal deprivation from 20-40 days of age increasing plasma cortisol concentrations and heart rate in calves challenged with restraint at 6 months of age (Lay et al., 1998); stroking during feeding from 1-3 days of age reducing fear of humans in lambs at 25 days of age (Markowitz et al., 1998); rearing in structurally complex aviaries compared to cages from 0-16 weeks of age improving chickens' memory in a cognitive test at 23 weeks of age (Tahamtani et al., 2015); and stress on the first day of life involving hatching in noisy incubators, handling for sex sorting, vaccination and transport, increasing chickens' corticosterone response to restraint at 6 weeks of age, pessimistic judgement bias at 10 weeks of age, and tail feather damage at 20 weeks of age (Hedlund et al., 2019; 2021). For pigs, there are several aspects of the early environment that may cause similar long term changes to behaviour, physiology and cognition (Telkänranta & Edwards, 2018). A summary by Telkänranta and Edwards (2018) of positive and negative effects of different early life experiences for pigs is shown in Figure 1.1.

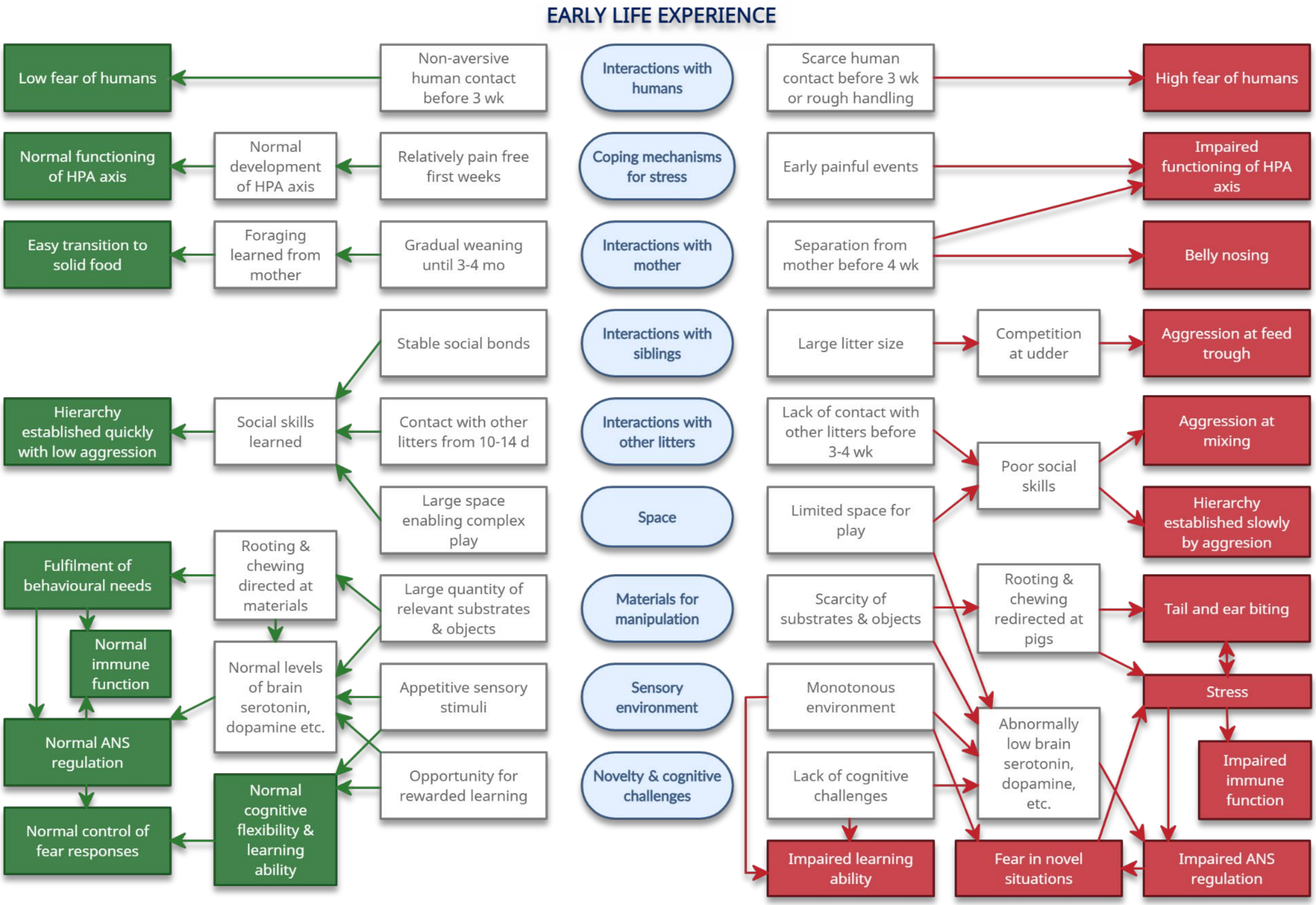


Figure 1.1. Summary adapted from Telkänranta and Edwards (2018) of the positive and negative consequences of different early life experiences for piglets. Permission was granted from the copyright owners to include this information in this thesis.

Firstly, contact with humans early in life is likely to be important, since as discussed in Section 1.3, humans are a key determining factor in the welfare of pigs. Many of the major challenges that pigs face in production are experienced early in life, and most of if not all of these challenges involve humans to some extent. For example, husbandry procedures often conducted in the first few days of life such as tail docking, vaccination, teeth resection, ear notching and castration are carried out by stockpeople (Marchant-Forde et al., 2009). Moreover, weaning imposes several stressors simultaneously on pigs and involves intense contact and handling by stockpeople (Weary et al., 2008). Since positive interactions with humans can reduce pigs' fear of humans and may also have other benefits associated with stress resilience (see Section 1.3.2.), it may be particularly important for pigs to experience positive interactions with humans early in life when they are exposed to several stressors. Furthermore, as most routine interactions with humans in production systems invoke stress, fear or pain (Hemsworth & Coleman, 2011; Waiblinger et al., 2006), developing a positive human-animal relationship may be easier at a young age when pigs have experienced fewer negative interactions with humans.

Secondly, the early housing environment during lactation, in terms of its physical characteristics and how it influences maternal care, may affect the development of stress coping mechanisms in piglets. Farrowing crates are the most common form of housing for sows and piglets during the farrowing and lactation period as they reduce the risk of piglets being crushed by the sow, are space efficient and allow stockpeople to safely access pigs (Barnett et al., 2001; Baxter et al., 2018). However, they may pose a risk to sow welfare (Barnett et al., 2001; Baxter et al., 2018; Johnson & Marchant-Forde, 2009). In Australia, sows generally spend most of gestation in group accommodation and are housed in farrowing crates from one week prior to expected parturition until piglets are weaned at around 4 weeks of age (Australian Pork Limited, 2022). Therefore, sows spend around 5 weeks in farrowing crates with a restricted range of movement and limited opportunities to engage in highly motivated behaviours such as nest building (Barnett et al., 2001; Johnson & Marchant-Forde, 2009). Furthermore, sows in crates have a reduced ability to express maternal behaviours and have limited agency over interactions with their piglets (Barnett et al., 2001; Johnson & Marchant-Forde, 2009). Due to these welfare concerns, several alternative housing systems have been developed and studied that provide the sow with less confinement during the farrowing and lactation period (Baxter et al., 2018; Johnson & Marchant-Forde, 2009). These alternative 'loose' systems vary considerably in terms of space, design features and the level of confinement of the sow (Baxter et al., 2011; Goumon et al., 2022; Johnson & Marchant-Forde, 2009). Some systems allow the sow freedom of movement throughout the farrowing and lactation period, while others are used to temporarily confine the sow for a short period of time (often 3-4 days) around parturition and early lactation when the mortality risk to liveborn piglets is greatest (Goumon et al., 2022).

The farrowing and lactation housing system can affect the way in which piglets and sows interact with one another since sow behavioural expression is limited in farrowing crates compared to in loose systems (Barnett et al., 2001; Johnson & Marchant-Forde, 2009). Interactions between sows and piglets occur sooner (Martin et al., 2015) and are more frequent in loose lactation pens than in farrowing crates (Chidgey et al., 2016; Ison et al., 2015; Singh et al., 2017), and some of these sow-piglet interactions, such as nose contact, are vital for the development of the mother-offspring bond which may influence other aspects of maternal behaviour (Blackshaw & Hagelsø, 1990; Petersen et al., 1990). For example, compared to gilts in farrowing crates, gilts in loose farrowing and lactation pens showed more nose contact with their piglets in addition to a lower incidence of piglet directed aggression (Ison et al., 2015). There is also evidence of sows in loose lactation pens showing increased maternal responsiveness, based on more vocalising, alertness, piglet directed behaviour and faster posture changes in response to piglet screams (Cronin et al., 1996; Singh et al., 2017; Thodberg et al., 2002). Therefore, through restricting or facilitating post-natal maternal care, the farrowing and lactation environment may greatly impact piglet neural, behavioural and physiological development and thus stress resilience.

Alongside maternal behaviour, there are several features of the farrowing and lactation housing environment that may impact piglet development though providing piglets with opportunities for learning and to perform natural behaviours. These include space, sensory stimulation, the provision of bedding or enrichment materials, opportunities for interactions with conspecifics, and the overall complexity of the environment. When reviewing the literature, it is difficult to interpret the effects of different features of the early environment separately, since in many cases for example, in loose housing systems where piglets have increased opportunity for maternal care, they also have more space and/or are provided with straw bedding. The focus of much of this thesis is the early human and housing environment. The subsequent sections will primarily concentrate on the effects of the early human and housing environment on stress resilience and will consider effects of various features of the early housing environment, including confinement of the sow, space, complexity etc., together.

1.4.2 Effects of Early Experiences on Stress Resilience in Pigs

1.4.2.1 Behaviour and Physiology during the Lactation Period

Stress experienced by the dam can impact stress in her offspring (Jarvis et al., 2006; Rooney et al., 2021). Confinement may cause stress to sows that could be transferred to her piglets, although based on cortisol concentrations during the lactation period the evidence for confinement in crates inducing short and long term stress in sows is highly contradicting (reviewed by: (Goumon et al., 2022)). A positive relationship has been reported between the length of confinement of sows in crates and hair cortisol concentrations in both sows and their piglets, indicating increased stress at the end of the lactation period in piglets reared in farrowing crates (Morgan et al., 2021). In contrast, no differences in faecal cortisol were found between 12-26 days of age in piglets reared in crates and then loose lactation pens from 4 days after farrowing compared to piglets reared continuously in farrowing crates (Kinane et al., 2021). Faecal cortisol tended ($p < 0.1$) to be higher in piglets from the loose system at 5 days of age, but this was likely affected by the sow being released from the crate the day prior.

Rearing piglets in farrowing crates rather than loose housing systems during the lactation period appears to harm their social development. Martin and colleagues (2015) found that social interactions occurred earlier in life in piglets from loose farrowing and lactation pens compared to farrowing crates, with straw available to piglets in both systems. Play behaviour also occurred earlier in life and more frequently during the lactation period in the loose system. Clarkson and colleagues (2021) found that piglets in loose farrowing and lactation pens showed more play behaviour compared to piglets in farrowing crates with less straw, and in loose pens, foster piglets were more likely to be included in play with non-foster piglets, indicating better social connectedness in the loose system. Singh and colleagues (2017) found that piglets housed in loose lactation pens from 3 days after farrowing showed more play behaviour and less manipulative behaviour directed towards pen mates compared to piglets from farrowing crates, with no straw or bedding in either system. Chaloupková and colleagues (2007a) found tendencies ($p < 0.10$) for the same effects on social and locomotor play behaviour: piglets reared in loose pens with straw that were 60 % larger than standard farrowing crates tended to play more than piglets from standard farrowing crates without straw, while play behaviour was intermediate in piglets from farrowing crates with straw that were 20 % larger than standard crates.

In contrast to the findings above, both Oostindjer and colleagues (2011) and Kinane and colleagues (2021) found no differences in play behaviour during lactation in piglets reared in farrowing crates and piglets reared in loose pens from 4 days after farrowing. No bedding was provided in the former experiment, while in the latter, hessian sacks and plant fibers were provided in both crates and loose pens. In these two experiments, floor space in the loose housing system was either the same (Oostindjer et al.) or only 16 % larger (Kinane et al.) to that in the crated system. In the experiments mentioned earlier which reported more play behaviour in loose systems compared to crates (Clarkson et al., 2021; Martin et al., 2015; Singh et al., 2017), floor space differed between the housing systems by more than 33 %, suggesting that space may be key factor facilitating play behaviour. Kinane and colleagues found no effect of housing system on fighting, although there tended ($p < 0.1$) to be less ear and tail biting in the loose system than in crates. Oostindjer and colleagues found no effect of housing system on aggression, belly nosing, manipulation or nosing of pen mates during the lactation period. They also found that irrespective of the level of sow confinement, more space and the addition of wood shavings, peat, branches and straw reduced belly nosing and manipulative behaviour and increased play behaviour, indicating that enrichment materials as well as space affect piglet behavioural development.

Most research examining the effects on stress resilience of interactions with humans has been conducted on weaned pigs, although there are some studies showing benefits of positive handling during the lactation period. Stroking during suckling bouts on the first day of life reduced the reactivity of piglets to tail docking at 2 days of age and tended ($p < 0.1$) to reduce reactivity to capture at 2 wk of age (Muns et al., 2015). Tail docking on its own (performed on the first day of life) was shown to increase pigs fear of humans at 2 wk of age (Tallet et al., 2019). Furthermore, stroking while being held from 5-35 days of age reduced fear of humans and fear of a novel environment at 4 wk of age (de Oliveira et al., 2015).

1.4.2.2 Response to Weaning

Weaning imposes several stressors simultaneously on pigs including social stress induced by mixing with unfamiliar pigs (Weary et al., 2008). Therefore, how pigs cope with weaning may be affected by their social development pre-weaning, although the evidence for this is confounding. In the previously mentioned experiment by Oostindger and colleagues (2011) that reported no effects of rearing piglets in crates or loose lactation pens on their behaviour during the lactation period, piglets from farrowing crates showed less play behaviour and more belly nosing, manipulative behaviour and food exploration after weaning. While in the previously mentioned experiment by Martin and colleagues (2015), piglets from farrowing crates showed less play behaviour during the lactation period but after weaning showed similar levels to piglets reared in loose farrowing and lactation pens. Martin and colleagues found that piglets reared in loose farrowing and lactation pens had larger increases in lesion scores from 0-3 days after weaning compared to piglets reared in farrowing crates. However, between 3-7 days after weaning, changes in the lesion scores of loose pen piglets had dramatically reduced while changes were sustained in the farrowing crate piglets. The authors suggested this indicated that the loose pen piglets showed more aggression in the initial period after weaning (0-3 days post weaning), but less chronic aggression following this (3-7 days post weaning). In contrast, other research has shown pigs from farrowing crates have more injuries than those from loose systems at 4 days after weaning (Kutzer et al., 2009) or similar injuries at 6 hours after weaning (Chaloupková et al., 2007a). It is possible that through increased opportunity with the sow, loose housing systems assist piglets in adapting to the post weaning environment, but further evidence is necessary to support this.

Lange and colleagues (2020) found that while there were no differences in skin lesions, cortisol concentrations and aggression post-weaning between piglets reared in farrowing crates and loose lactation pens, rearing piglets in groups reduced all of these parameters. Others have also shown that rearing piglets in group lactation pens rather than farrowing crates reduces aggression after weaning (Kutzer et al., 2009; Li & Wang, 2011; Verdon et al., 2016). The same effect is seen by providing piglets in farrowing crates and loose pens with opportunities to socialise with non-littermates during the lactation period (Hessel et al., 2006; Kutzer et al., 2009; Salazar et al., 2018). Increased space in group lactation systems may contribute to the development of normal social skills by facilitating behaviours such as play fighting, in addition to group lactation systems providing piglets with more social experience which may improve their ability to form a social dominance hierarchy quickly and efficiently after weaning (D'Eath, 2005).

Experiences with humans during the lactation period may also affect how pigs cope with weaning. More resting and less escape attempts and agonistic behaviour were found in piglets that had experience with a handler that moved carefully and spoke softy during routine inspections and feeding, compared to piglets that had experience with a handler that moved unpredictably and shouted (Sommavilla et al., 2011). Although, no differences in plasma cortisol concentrations were observed 60 minutes after weaning in piglets that were patted and stroked at 1 day of age compared to pigs that weren't (Muns et al., 2015).

1.4.2.3 Behaviour and Physiology during the Weaner, Grower and Finisher Periods

Overall, there is a lack of research examining persistent effects of early experiences on the behaviour and physiology of pigs later in life, particularly in terms of effects of the early housing environment.

While there was no difference in the cortisol response to weaning of pigs from farrowing crates and pigs from larger straw enriched loose pens, Chaloupková and colleagues (2007a) found that rearing in farrowing crates increased the cortisol response to transport at 6 months of age and reduced meat pH after slaughter. Ear tagging after weaning increased plasma and salivary cortisol concentrations in pigs from both indoor and larger outdoor pens, but the increase was greater in pigs from the indoor system (Yonezawa et al., 2012). Rearing in poor conditions (confined sow, weaning at 4 weeks of age, no straw) compared to enriched conditions (loose sow, weaning at 5-6 weeks of age, straw and more space) reduced manipulative behaviour in terms of nibbling of pen mates, but not tail biting, from 12 weeks of age until slaughter (Simonsen, 1995). Moreover, rearing in farrowing crates compared to outdoor group pens with access to mud, straw and pasture, increased aggression at 32-33 weeks of age (De Jonge et al., 1996). These findings suggest that sow confinement, reduced space, and a lack of enrichment early in life can negatively affect the welfare of pigs in the long-term.

There is evidence to show that housing in an enriched environment during the lactation period and subsequently housing in a barren environment increases stress in pigs. Pigs that had access to straw during the lactation and weaner periods but not subsequent to this, showed more biting of other pigs when no straw was available compared to pigs that never had substrates to begin with (Day et al., 2002a). Similarly, providing wood shavings and straw during the lactation period but not subsequently, increased tail biting and redirected exploration towards pen fittings during the finishing stage (Munsterhjelm et al., 2009). Although in the same experiment, aggressive behaviour at 14 weeks of age was also reduced in pigs that had access to substrates early in life, indicating there was some benefit. Luo and colleagues (2020) found that pigs which were upgraded from barren pens to enriched pens (substrates and toys, more space) at 7 weeks of age showed more play behaviour and exploration, while pigs that moved from enriched to barren housing showed more manipulative behaviour than pigs that remained in barren housing. Bolhuis and colleagues (2006) found that pigs which were moved from enriched pens with straw to barren pens of the same size but without straw at 10 weeks of age, showed less inactivity compared to pigs that remained in barren housing. However, the current presence of straw had more of an effect on behaviour than rearing history, with barren housed pigs showing less activity, play behaviour and exploration, and more manipulation of pen mates compared to pigs with access to straw. Therefore, both the early and current environment can impact the behaviour of pigs.

Early human contact can impact the fear responses of pigs to humans (Hemsworth & Barnett, 1992; Hemsworth et al., 1986b). Pigs can remember previous positive or negative interactions with humans for at least 5 weeks (Brajon et al., 2015b), and when these interactions take place early in life the effects on fear of humans may be sustained for even longer. Hemsworth and colleagues (1986b) studied the effects of three handling treatments imposed from 0-8 weeks of age on the persistency of pigs' responses to humans. Compared to pigs that received only routine contact, pigs that were artificially reared and handled (brief but regular stroking by a human in an arena), and, pigs that were handled but reared with their dam, spent more time in close proximity to a human in tests from 10-24 weeks of age. These findings suggest that handling in the first two months of life reduced pigs' fear of humans, and that this effect persisted months after the handling treatments had been applied.

In order to more precisely define which periods early in life were important for handling to occur, Hemsworth and Barnett (1992) imposed positive handling, as described above, for 2 minutes daily on pigs from either 0-3, 3-6, 6-9 and 9-12 weeks of age, or, not at all. At 18 weeks of age, the strongest reductions in fear of humans were observed in pigs that had been handled from 0-3 and 9-12 weeks of age. This suggests that the first three weeks of life may represent a sensitive period of development during which positive interactions with humans have a profound effect on the human-animal relationship. It was likely that the strong reductions in fear of humans in pigs that were handled from 9-12 weeks of age were a consequence of this being the most recent handling treatment. There were no effects of handling at any age on the responses of pigs to a human at 20, 22 or 24 weeks of age, implying that subsequent negative handling by stockpeople (e.g., during husbandry and management) overrode the effects of the positive handling treatments. Therefore, this experiment showed that positive interactions in the first few weeks of life can have a significant and prolonged effect on the fear responses of pigs to humans, but that subsequent interactions with humans can also modify the effects of this early learning.

While there is support for early human handling having a considerable impact on the fear responses of pigs to humans later in life, there are still details relating to early human-pig interactions that remain unclear. For example, the specific timing and duration of positive human interaction that would produce the most benefit in terms of sustaining reduced fear responses throughout the pig's life in production. There is some evidence that only a small amount of positive human contact early in life can reduce pigs' subsequent fear of humans. Around 36 minutes of patting and stroking imposed on the whole litter while piglets were suckling on the first day of life tended ($p < 0.1$) to reduce piglets' reactivity during capture at 2 weeks of age (Muns et al., 2015). Whether early positive interactions with humans can result in prolonged improvements to general stress resilience, rather than only prolonged reductions in fear of humans, requires further investigation.

Few studies have examined the impact of early housing, sow confinement or enrichment on pigs' subsequent fear responses. In response to a human at 26 days of age during the lactation period, piglets from standard farrowing crates, were more likely to vocalise, faster to move and tended ($p < 0.1$) to be more likely to interact with an unfamiliar person compared to piglets from enriched loose pens (60 % larger and contained straw) but not piglets from enriched farrowing crates (20 % larger and contained straw) (Chaloupková et al., 2007b). But when tested at 3 and 6 months of age, there was no effects of the early housing system on pigs' responses to the human. In another study, rearing piglets in loose lactation pens from 4 days after farrowing compared to crates had no effect on their responses to familiar human presence or human contact at 8, 15, or 22 days after weaning (Kinane et al., 2021). Although, piglets from the loose system were slower to approach a novel object at 8 days after weaning which may suggest greater fear of novelty. There were no differences in the responses of piglets to a novel object at 15 or 22 days after weaning, but the same novel object was used in all tests and there was an indication that pigs had habituated to it. Olsson and colleagues (1999) examined the effects of two rearing conditions on pigs' responses to novelty: 1. Poor conditions: the sow was tethered during gestation and crated during lactation, no bedding or straw was offered, piglets were weaned at 6 weeks of age; and, 2. Enriched conditions: sows were in groups during gestation and lactation, free range conditions on sand with access to a mud pool, straw and pasture, piglets were weaned at 10 weeks of age. All pigs were housed under the same conditions after 10 weeks of age. At around 9 months of age, pigs from the poor conditions showed less fear of novelty as indicated by less avoidance and a faster latency to contact a novel metal bucket. It is impossible to know which specific features of the early life rearing conditions were responsible for these effects on fear.

Providing opportunities for cognitive challenges early in life may reduce fear responses later in life. Siegford and colleagues (2008) found that exposing 2 week old piglets to a maze task that required spatial learning, tended ($p < 0.1$) to reduce pigs' fear of humans at 50 days of age, based on a lower latency to interact with an unfamiliar person and a higher frequency of physical interactions with them. Pigs that were exposed to the maze task showed less fear of humans than both pigs that were separated from the sow and/or handled for the same duration, indicating that it was exposure to the cognitive challenge of the maze itself, rather than handling or maternal separation, that resulted in reduced fear levels.

1.5 Aims

Limited evidence suggests that positive interactions with humans, including patting, stroking and scratching pigs, may improve pigs' stress resilience, on the basis of measurements such as fear responses to novelty and humans, behavioural and physiological responses to challenges such as routine husbandry and management procedures, social and maternal behaviour and biological fitness (see Section 1.3.2.). Furthermore, a wide body of research on laboratory animals has shown that resilience to stress is fostered by learning to overcome experiences that are 'challenging but not overwhelming' early in life during sensitive periods of development (Lyons et al., 2009; Lyons et al., 2010; Lyons & Schatzberg, 2019; Parker & Maestripieri, 2011). While the existence of a sensitive period in pigs is not well known, there is some indication that pigs are more receptive to experiences that occur before weaning (see Section 1.4.). Differing experiences in terms of the level of maternal care and interactions with the dam, the complexity of the physical environment, and the amount and quality of interactions with humans, may offer pigs with differing opportunities for learning to overcome challenges early in life, and may therefore shape both the immediate and long-term responses of pigs to stress. The aim of this thesis was to examine the effects of positive interactions with humans and early life human and housing experiences on stress resilience in pigs. Three experiments, each presented in a separate chapter in this thesis, were conducted under this overarching aim. More specifically, the aim of each experiment was as follows:

1. *Chapter 2 research aim* – to examine the effects of daily positive human contact **during gestation** and age/experience on stress resilience in **sows during gestation and lactation.**

2. *Chapter 3 research aim* – to examine the **immediate effects** of regular positive human contact during lactation, and, the farrowing and lactation housing system, on stress resilience in **piglets during the lactation period and at weaning.**

3. *Chapter 4 research aim* – to examine both the **immediate and long-term effects** of regular positive human contact during lactation, and, the farrowing and lactation housing system, on stress resilience in **pigs from birth until slaughter**.

1.6 References

Andersen, I. L., Berg, S., Bøe, K. E., & Edwards, S. (2006). Positive handling in late pregnancy and the consequences for maternal behaviour and production in sows. *Applied Animal Behaviour Science, 99*(1), 64-76.

Arroyo, L., Valent, D., Carreras, R., Peña, R., Sabrià, J., Velarde, A., & Bassols, A. (2019). Housing and road transport modify the brain neurotransmitter systems of pigs: Do pigs raised in different conditions cope differently with unknown environments? *PLOS ONE, 14*(1), 1-20.

Australian Pork Limited. (2022). How we farm: About pig farming. Retrieved from https://australianpork.com.au/about-pig-farming/housing

Barnett, J. L., Hemsworth, P. H., Cronin, G. M., Jongman, E. C., & Hutson, G. D. (2001). A review of the welfare issues for sows and piglets in relation to housing. *Australian Journal of Agricultural Research, 52*(1), 1-28.

Baxter, E. M., Andersen, I. L., & Edwards, S. A. (2018). Sow welfare in the farrowing crate and alternatives. In M. Špinka (Ed.), *Advances in pig welfare* (pp. 27-72). Duxford, United Kingdom: Woodhead Publishing.

Baxter, E. M., Lawrence, A. B., & Edwards, S. A. (2011). Alternative farrowing systems: Design criteria for farrowing systems based on the biological needs of sows and piglets. *Animal, 5*(4), 580-600.

Baxter, E. M., Mulligan, J., Hall, S. A., Donbavand, J. E., Palme, R., Aldujaili, E., Zanella, A. J., & Dwyer, C. M. (2016). Positive and negative gestational handling influences placental traits and mother-offspring behavior in dairy goats. *Physiology & Behavior, 157*, 129-138.

Blackshaw, J. K., & Hagelsø, A. M. (1990). Getting-up and lying-down behaviours of loose-housed sows and social contacts between sows and piglets during day 1 and day 8 after parturition. *Applied Animal Behaviour Science, 25*(1), 61-70.

Bolhuis, J. E., Schouten, W. G. P., Schrama, J. W., & Wiegant, V. M. (2006). Effects of rearing and housing environment on behaviour and performance of pigs with different coping characteristics. *Applied Animal Behaviour Science, 101*(1), 68-85.

Brajon, S., Laforest, J.-P., Bergeron, R., Tallet, C., & Devillers, N. (2015a). The perception of humans by piglets: Recognition of familiar handlers and generalisation to unfamiliar humans. *Animal Cognition 18*(6), 1299-1316.

Brajon, S., Laforest, J.-P., Bergeron, R., Tallet, C., Hötzel, M.-J., & Devillers, N. (2015b). Persistency of the piglet's reactivity to the handler following a previous positive or negative experience. *Applied Animal Behaviour Science, 162*, 9-19.

Brajon, S., Laforest, J.-P., Schmitt, O., & Devillers, N. (2015c). The way humans behave modulates the emotional state of piglets. *PLOS ONE, 10*(8), 1-17.

Brajon, S., Laforest, J.-P., Schmitt, O., & Devillers, N. (2016). A preliminary study of the effects of individual response to challenge tests and stress induced by humans on learning performance of weaned piglets (sus scrofa). *Behavioural Processes, 129*, 27-36.

Brown, J. A., Dewey, C., Delange, C. F. M., Mandell, I. B., Purslow, P. P., Robinson, J. A., Squires, E. J., & Widowski, T. M. (2009). Reliability of temperament tests on finishing pigs in group-housing and comparison to social tests. *Applied Animal Behaviour Science, 118*(1), 28-35.

Bus, J. D., Boumans, I. J. M. M., Webb, L. E., & Bokkers, E. A. M. (2021). The potential of feeding patterns to assess generic welfare in growing-finishing pigs. *Applied Animal Behaviour Science, 241*, 105383.

Büttner, K., Czycholl, I., Basler, H., & Krieter, J. (2018). Effects of an intensified human–animal interaction on tail biting in pigs during the rearing period. *Journal of Agricultural Science, 156*(8), 1039-1046.

Buwalda, B., Scholte, J., de Boer, S. F., Coppens, C. M., & Koolhaas, J. M. (2012). The acute glucocorticoid stress response does not differentiate between rewarding and aversive social stimuli in rats. *Hormones and Behavior, 61*(2), 218-226.

Carlstead, K., & Shepherdson, D. J. (2000). Alleviating stress in zoo animals with environmental enrichment. In G. P. Moberg & J. A. Mench (Eds.), *The biology of animal stress: Basic principles and implications for animal welfare* (pp. 337-354). Wallingford, United Kingdom: CABI Publishing.

Chaloupková, H., Illmann, G., Bartoš, L., & Špinka, M. (2007a). The effect of pre-weaning housing on the play and agonistic behaviour of domestic pigs. *Applied Animal Behaviour Science, 103*(1), 25-34.

Chaloupková, H., Illmann, G., Neuhauserova, K., Tomanek, M., & Valis, L. (2007b). Preweaning housing effects on behavior and physiological measures in pigs during the suckling and fattening periods. *Journal of Animal Science*, 1741-1749.

Channon, H. A., Payne, A. M., & Warner, R. D. (2000). Halothane genotype, pre-slaughter handling and stunning method all influence pork quality. *Meat Science, 56*(3), 291-299.

Chen, A. (2019). Preface. In A. Chen (Ed.), *Stress resilience: Molecular and behavioral aspects* (pp. xvii-xx). London, England: Academic Press.

Chidgey, K. L., Morel, P. C. H., Stafford, K. J., & Barugh, I. W. (2016). Observations of sows and piglets housed in farrowing pens with temporary crating or farrowing crates on a commercial farm. *Applied Animal Behaviour Science, 176*, 12-18.

Clarkson, J. M., Baxter, E. M., & Martin, J. E. (2021). Who plays with whom: Farrowing environment influences isolation of foster piglets in play. *Frontiers in Animal Science, 2*, 724080.

Claxton, A. M. (2011). The potential of the human–animal relationship as an environmental enrichment for the welfare of zoo-housed animals. *Applied Animal Behaviour Science, 133*(1), 1-10.

Cloutier, S., LaFollette, M. R., Gaskill, B. N., Panksepp, J., & Newberry, R. C. (2018). Tickling, a technique for inducing positive affect when handling rats. *Journal of Visualized Experiments*(135), 57190.

Cook, C. J., Mellow, D. J., Harris, P. J., Ingram, J. R., & Matthews, L. R. (2000). Hands-on and hands-off measurements of stress. In G. P. Moberg & J. A. Mench (Eds.), *The biology of animal stress: Basic principles and implications for animal welfare* (pp. 123-146). Wallingford, United Kingdom: CABI Publishing.

Crofton, E. J., Zhang, Y., & Green, T. A. (2015). Inoculation stress hypothesis of environmental enrichment. *Neuroscience & Biobehavioral Reviews, 49*, 19-31.

Cronin, G. M., & Glatz, P. C. (2021). Causes of feather pecking and subsequent welfare issues for the laying hen: A review. *Animal Production Science, 61*(10), 1005-1990.

Cronin, G. M., Simpson, G. J., & Hemsworth, P. H. (1996). The effects of the gestation and farrowing environments on sow and piglet behaviour and piglet survival and growth in early lactation. *Applied Animal Behaviour Science, 46*(3), 175-192.

D'Eath, R. B. (2005). Socialising piglets before weaning improves social hierarchy formation when pigs are mixed post-weaning. *Applied Animal Behaviour Science, 93*, 199-211.

Dang, D. X., & Kim, I. H. (2021). The effects of road transportation with or without homeopathic remedy supplementation on growth performance, apparent nutrient digestibility, fecal microbiota, and serum cortisol and superoxide dismutase levels in growing pigs. *Journal of Animal Science, 99*(4), 1-7.

Day, J. E. L., Burfoot, A., Docking, C. M., Whittaker, X., Spoolder, H. A. M., & Edwards, S. A. (2002a). The effects of prior experience of straw and the level of straw provision on the behaviour of growing pigs. *Applied Animal Behaviour Science, 76*(3), 189-202.

Day, J. E. L., Spoolder, H. A. M., Burfoot, A., Chamberlain, H. L., & Edwards, S. A. (2002b). The separate and interactive effects of handling and environmental enrichment on the behaviour and welfare of growing pigs. *Applied Animal Behaviour Science, 75*(3), 177-192.

De Jonge, F. H., Bokkers, E. A. M., Schouten, W. G. P., & Helmond, F. A. (1996). Rearing piglets in a poor environment: Developmental aspects of social stress in pigs. *Physiology & Behavior, 60*(2), 389-396.

De Meyer, D., Amalraj, A., Van Limbergen, T., Fockedey, M., Edwards, S., Moustsen, V. A., Chantziaras, I., & Maes, D. (2020). Short communication: Effect of positive handling of sows on litter performance and pre-weaning piglet mortality. *Animal*, 1-7.

de Oliveira, D., Keeling, L. J., & Paranhos da Costa, M. J. R. (2019). Individual variation over time in piglet´s reactions to early handling and its association to weight gain. *Applied Animal Behaviour Science, 215*, 7-12.

de Oliveira, D., Paranhos da Costa, M. J. R., Zupan, M., Rehn, T., & Keeling, L. J. (2015). Early human handling in non-weaned piglets: Effects on behaviour and body weight. *Applied Animal Behaviour Science, 164*, 56-63.

de Ruyter, E. M., van Wetter, W. H. E. J., Lines, D. S., & Plush, K. J. (2017). Gradually reducing sow contact in lactation is beneficial for piglet welfare around weaning. *Applied Animal Behaviour Science, 193*, 43-50.

De, U. K., Nandi, S., Mukherjee, R., Gaur, G. K., & Verma, M. R. (2017). Identification of some plasma biomarkers associated with early weaning stress in crossbred piglets. *Comparative Clinical Pathology, 26*(2), 343-349.

DeBoer, S. P., Garner, J. P., McCain, R. R., Lay Jr, 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*(1), 15-27.

Dokmanovic, M., Velarde, A., Tomovic, V., Glamoclija, N., Markovic, R., Janjic, J., & Baltic, M. Z. (2014). The effects of lairage time and handling procedure prior to slaughter on stress and meat quality parameters in pigs. *Meat Science, 98*(2), 220-226.

Donaldson, T. M., Newberry, R. C., Špinka, M., & Cloutier, S. (2002). Effects of early play experience on play behaviour of piglets after weaning. *Applied Animal Behaviour Science, 79*(3), 221-231.

English, P. R., Grant, S. A., McPherson, O., & Edwards, S. A. (1999). Evaluation of the effects of the positive 'befriending' of sows and gilts ('pleasant' treatment) prior to parturition and in early lactation on sow behaviour, the process of parturition and piglet survival. *BSAP Occasional Publication, 23*, 132-136.

Escribano, D., Gutiérrez, A. M., Tecles, F., & Cerón, J. J. (2015). Changes in saliva biomarkers of stress and immunity in domestic pigs exposed to a psychosocial stressor. *Research in Veterinary Science, 102*, 38-44.

Escribano, D., Ko, H.-L., Chong, Q., Llonch, L., Manteca, X., & Llonch, P. (2019). Salivary biomarkers to monitor stress due to aggression after weaning in piglets. *Research in Veterinary Science, 123*, 178-183.

Feenders, G., Klaus, K., & Bateson, M. (2011). Fear and exploration in european starlings (sturnus vulgaris): A comparison of hand-reared and wild-caught birds. *PLOS ONE, 6*(4), 1-8.

Forkman, B., Boissy, A., Meunier-Salaün, M. C., Canali, E., & Jones, R. B. (2007). A critical review of fear tests used on cattle, pigs, sheep, poultry and horses. *Physiology & Behavior, 92*(3), 340-374.

Gonyou, H. W., Hemsworth, P. H., & Barnett, J. L. (1986). Effects of frequent interactions with humans on growing pigs. *Applied Animal Behaviour Science, 16*(3), 269-278.

Goumon, S., Illmann, G., Moustsen, V. A., Baxter, E. M., & Edwards, S. A. (2022). Review of temporary crating of farrowing and lactating sows. *Frontiers in Veterinary Science, 9*.

Hedlund, L., Palazon, T., & Jensen, P. (2021). Stress during commercial hatchery processing induces long-time negative cognitive judgement bias in chickens. *Animals, 11*(4).

Hedlund, L., Whittle, R., & Jensen, P. (2019). Effects of commercial hatchery processing on short- and long-term stress responses in laying hens. *Scientific Reports 9*(1), 2367.

Hemsworth, P. H., & Barnett, J. L. (1987). Human-animal interactions. *Veterinary Clinics of North America: Food Animal Practice, 3*(2), 339-356.

Hemsworth, P. H., & Barnett, J. L. (1991). The effects of aversively handling pigs, either individually or in groups, on their behaviour, growth and corticosteroids. *Applied Animal Behaviour Science, 30*(1), 61-72.

Hemsworth, P. H., & Barnett, J. L. (1992). The effects of early contact with humans on the subsequent level of fear of humans in pigs. *Applied Animal Behaviour Science, 35*, 83-90.

Hemsworth, P. H., Barnett, J. L., & Campbell, R. G. (1996a). A study of the relative aversiveness of a new daily injection procedure for pigs. *Applied Animal Behaviour Science, 49*(4), 389-401.

Hemsworth, P. H., Barnett, J. L., Coleman, G. J., & Hansen, C. (1989). A study of the relationships between the attitudinal and behavioural profiles of stockpersons and the level of fear of humans and reproductive performance of commercial pigs. *Applied Animal Behaviour Science, 23*(4), 301-314.

Hemsworth, P. H., Barnett, J. L., & Hansen, C. (1981a). The influence of handling by humans on the behavior, growth, and corticosteroids in the juvenile female pig. *Hormones and Behavior, 15*, 396-403.

Hemsworth, P. H., Barnett, J. L., & Hansen, C. (1986a). The influence of handling by humans on the behaviour, reproduction and corticosteroids of male and female pigs. *Applied Animal Behaviour Science, 15*(4), 303.

Hemsworth, P. H., Barnett, J. L., & Hansen, C. (1987). The influence of inconsistent handling by humans on the behaviour, growth and corticosteroids of young pigs. *Applied Animal Behaviour Science, 17*(3/4), 245-252.

Hemsworth, P. H., Barnett, J. L., Hansen, C., & Gonyou, H. W. (1986b). The influence of early contact with humans on subsequent behavioural response of pigs to humans. *Applied Animal Behaviour Science, 15*, 55-63.

Hemsworth, P. H., Brand, A., & Willems, P. (1981b). The behavioural response of sows to the presence of human beings and its relation to productivity. *Livestock Production Science, 8*(1), 67-74.

Hemsworth, P. H., & Coleman, G. J. (2011). *Human-livestock interactions: The stockperson and the productivity and welfare of intensively farmed animals* (2nd ed.). Wallingford, United Kingdom: CABI Publishing.

Hemsworth, P. H., Coleman, G. J., Cox, M., & Barnett, J. L. (1994). Stimulus generalization: The inability of pigs to discriminate between humans on the basis of their previous handling experience. *Applied Animal Behaviour Science, 40*(2), 129-142.

Hemsworth, P. H., Gonyou, H. W., & Dziuk, P. J. (1986c). Human communication with pigs: The behavioural response of pigs to specific human signals. *Applied Animal Behaviour Science, 15*(1), 54-45.

Hemsworth, P. H., Morrison, R. S., Tilbrook, A. J., Butler, K. L., Rice, M., & Moeller, S. J. (2016). Effects of varying floor space on aggressive behavior and cortisol concentrations in group-housed sows. *Journal of Animal Science, 94*(11), 4809-4818.

Hemsworth, P. H., Pederson, V., Cox, M., Cronin, G. M., & Coleman, G. J. (1999). A note on the relationship between the behavioural response of lactating sows to humans and the survival of their piglets. *Applied Animal Behaviour Science, 65*, 43-52.

Hemsworth, P. H., Rice, M., Nash, J., Giri, K., Butler, K. L., Tilbrook, A. J., & Morrison, R. S. (2013). Effects of group size and floor space allowance on grouped sows: Aggression, stress, skin injuries, and reproductive performance. *Journal of Animal Science, 91*(10), 4953-4964.

Hemsworth, P. H., Verge, J., & Coleman, G. J. (1996b). Conditioned approach-avoidance responses to humans: The ability of pigs to associate feeding and aversive social experiences in the presence of humans with humans. *Applied Animal Behaviour Science, 50*(1), 71-82.

Hessel, E. F., Reiners, K., & Van den Weghe, H. F. A. (2006). Socializing piglets before weaning: Effects on behavior of lactating sows, pre- and postweaning behavior, and performance of piglets. *Journal of Animal Science, 84*(10), 2847-2855.

Hyun, Y., Ellis, M., & Johnson, R. W. (1998). Effects of feeder type, space allowance, and mixing on the growth performance and feed intake pattern of growing pigs. *Journal of Animal Science, 76*(11), 2771-2778.

Iacoviella, B. M., & Charney, D. S. (2019). Cognitive and behavioral components of resilience to stress. In A. Chen (Ed.), *Stress resilience: Molecular and behavioral aspects* (pp. 23). London, England: Academic Press.

Ison, S. H., Wood, C. M., & Baxter, E. M. (2015). Behaviour of pre-pubertal gilts and its relationship to farrowing behaviour in conventional farrowing crates and loose-housed pens. *Applied Animal Behaviour Science, 170*, 26-33.

Janczak, A. M., Pedersen, L. J., Rydhmer, L., & Bakken, M. (2003). Relation between early fear- and anxiety-related behaviour and maternal ability in sows. *Applied Animal Behaviour Science, 82*(2), 121-135.

Jarvis, S., Moinard, C., Robson, S. K., Baxter, E., Ormandy, E., Douglas, A. J., Seckl, J. R., Russell, J. A., & Lawrence, A. B. (2006). Programming the offspring of the pig by prenatal social stress: Neuroendocrine activity and behaviour. *Hormones and Behavior, 49*(1), 68-80.

Johnson, A. K., & Marchant-Forde, J. N. (2009). Welfare of pigs in the farrowing environment. In J. N. Marchant-Forde (Ed.), *The welfare of pigs* (pp. 141-188). Dordrecht, Netherlands: Springer.

Jones, R. B. (1993). Reduction of the domestic chick's fear of human beings by regular handling and related treatments. *Animal Behaviour, 46*(5), 991-998.

Jones, R. B. (1997). Fear and distress. In M. C. Appleby & B. O. Hughes (Eds.), *Animal welfare* (pp. 75-87). Wallingford, United Kingdom: CABI Publishing.

Kinane, O., Butler, F., & O’Driscoll, K. (2021). Freedom to grow: Improving sow welfare also benefits piglets. *Animals, 11*(4).

Knudsen, E. I. (2004). Sensitive periods in the development of the brain and behavior. *Journal of Cognitive Neuroscience, 16*(8), 1412-1425.

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. *Applied Animal Behaviour Science, 121*(1), 16-24.

Lange, A., Gentz, M., Hahne, M., Lambertz, C., Gauly, M., Burfeind, O., & Traulsen, I. (2020). Effects of different farrowing and rearing systems on post-weaning stress in piglets. *Agriculture, 10*(6), 230.

Larsen, M. L. V., Gustafsson, A., Marchant-Forde, J. N., & Valros, A. (2019). Tear staining in finisher pigs and its relation to age, growth, sex and potential pen level stressors. *Animal, 13*(8), 1704-1711.

Lawrence, A. B., & Terlouw, E. M. C. (1993). A review of behavioral factors involved in the development oand continued performance of stereotypic behaviors in pigs. *Journal of Animal Science, 71*(10), 2815-2825.

Lay, D. C., Friend, T. H., Randel, R. D., Bowers, C. L., Grissom, K. K., Neuendorff, D. A., & Jenkins, O. C. (1998). Effects of restricted nursing on physiological and behavioral reactions of brahman calves to subsequent restraint and weaning. *Applied Animal Behaviour Science, 56*(2), 109-119.

Lensink, B. J., Fernandez, X., Cozzi, G., Florand, L., & Veissier, I. (2001). The influence of farmers' behavior on calves' reactions to transport and quality of veal meat. *Journal of Animal Science, 79*(3), 642-652.

Lensink, B. J., Leruste, H., De Bretagne, T., & Bizeray-Filoche, D. (2009). Sow behaviour towards humans during standard management procedures and their relationship to piglet survival. *Applied Animal Behaviour Science, 119*(3), 151-157.

Levine, S., Chevalier, J. A., Korchin, S. J., Levine, S., Chevalier, J. A., & Korchin, S. J. (1956). The effects of early shock and handling on later avoidance learning. *Journal of Personality, 24*(4), 475-493.

Levine, S., Haltmeyer, G. C., Karas, G. G., & Denenberg, V. H. (1967). Physiological and behavioral effects of infantile stimulation. *Physiology & Behavior, 2*(1), 55-59.

Li, Y., & Wang, L. (2011). Effects of previous housing system on agonistic behaviors of growing pigs at mixing. *Applied Animal Behaviour Science, 132*(1), 20-26.

Liu, D., Diorio, J., Tannenbaum, B., Caldji, C., Francis, D., Freedman, A., Sharma, S., Pearson, D., Plotsky, P. M., & Meaney, M. J. (1997). Maternal care, hippocampal glucocorticoid receptors, and hypothalamic- pituitary-adrenal responses to stress. *Science, 277*(5332), 1659-1662.

Llamas Moya, S., Boyle, L. A., Lynch, P. B., & Arkins, S. (2008). Effect of surgical castration on the behavioural and acute phase responses of 5-day-old piglets. *Applied Animal Behaviour Science, 111*(1), 133-145.

Luna, D., González, C., Byrd, C. J., Palomo, R., Huenul, E., & Figueroa, J. (2021). Do domestic pigs acquire a positive perception of humans through observational social learning? *Animals, 11*(1), 127.

Luo, L., Reimert, I., Middelkoop, A., Kemp, B., & Bolhuis, J. E. (2020). Effects of early and current environmental enrichment on behavior and growth in pigs. *Frontiers in Veterinary Science, 7*(268).

Lyons, D. M., Parker, K. J., Katz, M., & Schatzberg, A. F. (2009). Developmental cascades linking stress inoculation, arousal regulation, and resilience. *Frontiers in Behavioral Neuroscience, 3*(32).

Lyons, D. M., Parker, K. J., & Schatzberg, A. F. (2010). Animal models of early life stress: Implications for understanding resilience. *Developmental Psychobiology, 52*(5), 402-410.

Lyons, D. M., & Schatzberg, A. F. (2019). Resilience as a process instead of a trait. In A. Chen (Ed.), *Stress resilience: Molecular and behavioral aspects* (pp. 33-44). London, England: Academic Press.

Macrì, S., & Würbel, H. (2006). Developmental plasticity of hpa and fear responses in rats: A critical review of the maternal mediation hypothesis. *Hormones and Behavior, 50*(5), 667-680.

Marchant-Forde, J. N., Lay, D. C., McMunn, K. A., Cheng, H. W., Pajor, E. A., & Marchant-Forde, R. M. (2009). Postnatal piglet husbandry practices and well-being: The effects of alternative techniques delivered in combination. *Journal of Animal Science*(3), 1150-1161.

Marchant Forde, J. N. (2002). Piglet- and stockperson-directed sow aggression after farrowing and the relationship with a pre-farrowing, human approach test. *Applied Animal Behaviour Science, 75*(2), 115-132.

Marco-Ramell, A., Arroyo, L., Peña, R., Pato, R., Saco, Y., Fraile, L., Bendixen, E., & Bassols, A. (2016). Biochemical and proteomic analyses of the physiological response induced by individual housing in gilts provide new potential stress markers. *BMC Veterinary Research 12*(1), 265-265.

Markowitz, T. M., Dally, M. R., Gursky, K., & Price, E. O. (1998). Early handling increases lamb affinity for humans. *Animal Behaviour, 55*(3), 573-587.

Martin, J. E., Ison, S. H., & Baxter, E. M. (2015). The influence of neonatal environment on piglet play behaviour and post-weaning social and cognitive development. *Applied Animal Behaviour Science, 163*, 69-79.

Mason, G., & Rushen, J. (2006). *Stereotypic animal behaviour: Fundamentals and applications to welfare* (2nd ed.). Wallingford, United Kingdom: CABI Publishing.

Mason, G., Wilson, D., Hampton, C., & Würbel, H. (2004). Non-invasively assessing disturbance and stress in laboratory rats by scoring chromodacryorrhoea. *Alternatives to Laboratory Animals, 32*, 153-159.

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. *The Veterinary Journal, 217*, 43-51.

Meaney, M. J. (2001). Maternal care, gene expression, and the transmission of individual differences in stress reactivity across generations. *Annual Review of Neuroscience, 24*, 1161-1192.

Meaney, M. J., Aitken, D. H., Bhatnagar, S., & Sapolsky, R. M. (1991). Postnatal handling attenuates certain neuroendocrine, anatomical, and cognitive dysfunctions associated with aging in female rats. *Neurobiology of Aging, 12*(1), 31-38.

Meaney, M. J., Aitken, D. H., van Berkel, C., Bhatnagar, S., & Sapolsky, R. M. (1988). Effect of neonatal handling on age-related impairments associated with the hippocampus. *Science, 239*(4841), 766-768.

Meaney, M. J., Bhatnagar, S., Larocque, S., McCormick, C., Shanks, N., Sharma, S., Smythe, J., Viau, V., & Plotsky, P. M. (1993). Individual differences in the hypothalamic-pituitary-adrenal stress response and the hypothalamic crf system. *Annals of the New York Academy of Sciences, 697*, 70-85.

Mellor, D. J., Beausoleil, N. J., Littlewood, K. E., McLean, A. N., McGreevy, P. D., Jones, B., & Wilkins, C. (2020). The 2020 five domains model: Including human–animal interactions in assessments of animal welfare. *Animals, 10*(10), 1870.

Miura, A., Tanida, H., Tanaka, T., & Yoshimoto, T. (1996). Behavioral response to humans of weanling pigs exposed to a short period of individual handling. *Animal Science and Technology, 67*(8), 693-701.

Moberg, G. P. (2000). Biological response to stress: Implications for animal welfare. In G. P. Moberg & J. A. Mench (Eds.), *The biology of animal stress: Basic principles and implications for animal welfare* (pp. 1-21). Wallingford, United Kingdom: CABI Publishing.

Morgan, L., Meyer, J., Novak, S., Younis, A., Ahmad, W. A., & Raz, T. (2021). Shortening sow restraint period during lactation improves production and decreases hair cortisol concentrations in sows and their piglets. *Animal, 15*(2).

Muns, R., Rault, J. L., & Hemsworth, P. (2015). Positive human contact on the first day of life alters the piglet's behavioural response to humans and husbandry practices. *Physiology & Behavior, 151*, 162-167.

Munsterhjelm, C., Peltoniemi, O. A. T., Heinonen, M., Hälli, O., Karhapää, M., & Valros, A. (2009). Experience of moderate bedding affects behaviour of growing pigs. *Applied Animal Behaviour Science, 118*(1), 42-53.

Murata, H. (2007). Stress and acute phase protein response: An inconspicuous but essential linkage. *Veterinary Journal, 173*(3), 473-474.

Numberger, J., Ritzmann, M., Übel, N., Eddicks, M., Reese, S., & Zöls, S. (2016). Ear tagging in piglets: The cortisol response with and without analgesia in comparison with castration and tail docking. *Animal, 10*(11), 1864-1870.

Olsson, I. A. S., de Jonge, F. H., Schuurman, T., & Helmond, F. A. (1999). Poor rearing conditions and social stress in pigs: Repeated social challenge and the effect on behavioural and physiological responses to stressors. *Behavioural Processes, 46*(3), 201-215.

Oostindjer, M., van den Brand, H., Kemp, B., & Bolhuis, J. E. (2011). Effects of environmental enrichment and loose housing of lactating sows on piglet behaviour before and after weaning. *Applied Animal Behaviour Science, 134*(1), 31-41.

Ott, S., Soler, L., Moons, C. P., Kashiha, M. A., Bahr, C., Vandermeulen, J., Janssens, S., Gutiérrez, A. M., Escribano, D., Cerón, J. J., Berckmans, D., Tuyttens, F. A., & Niewold, T. A. (2014). Different stressors elicit different responses in the salivary biomarkers cortisol, haptoglobin, and chromogranin a in pigs. *Research in Veterinary Science, 97*(1), 124-128.

Parker, K. J., Buckmaster, C. L., Sundlass, K., Schatzberg, A. F., & Lyons, D. M. (2006). Maternal mediation, stress inoculation, and the development of neuroendocrine stress resistance in primates. *Proceedings of the National Academy of Sciences of the United States of America, 103*(8), 3000-3005.

Parker, K. J., & Maestripieri, D. (2011). Identifying key features of early stressful experiences that produce stress vulnerability and resilience in primates. *Neuroscience & Biobehavioral Reviews, 35*(7), 1466-1483.

Paterson, A. M., & Pearce, G. P. (1989). Boar-induced puberty in gilts handled pleasantly or unpleasantly during rearing. *Applied Animal Behaviour Science, 22*(3/4), 233-225.

Payne, A. P. (1994). The harderian gland: A tercentennial review. *Journal of Anatomy, 185*, 1-49.

Pearce, G. P., Paterson, A. M., & Pearce, A. N. (1989). The influence of pleasant and unpleasant handling and the provision of toys on the growth and behaviour of male pigs. *Applied Animal Behaviour Science, 23*(1/2), 37-27.

Pederson, V., Barnett, J. L., Hemsworth, P. H., Newman, E. A., & Schirmer, B. (1998). The effects of handling on behavioural and physiological responses to housing in tether-stalls among pregnant pigs. *Animal Welfare, 7*(2), 137-150.

Petersen, V., Recén, B., & Vestergaard, K. (1990). Behaviour of sows and piglets during farrowing under free-range conditions. *Applied Animal Behaviour Science, 26*(1), 169-179.

Piñeiro, M., Piñeiro, C., Carpintero, R., Morales, J., Campbell, F. M., Eckersall, P. D., Toussaint, M. J. M., & Lampreave, F. (2007). Characterisation of the pig acute phase protein response to road transport. *Veterinary Journal, 173*(3), 669-674.

Pol, F., Kling-Eveillard, F., Champigneulle, F., Fresnay, E., Ducrocq, M., & Courboulay, V. (2021). Human–animal relationship influences husbandry practices, animal welfare and productivity in pig farming. *Animal, 15*(2).

Price, E. O. (1985). Evolutionary and ontogenetic determinants of animal suffering and well-being. In G. P. Moberg (Ed.), *Animal stress* (pp. 15-26). New York, New York: Springer.

Pryce, C. R., & Feldon, J. (2003). Long-term neurobehavioural impact of the postnatal environment in rats: Manipulations, effects and mediating mechanisms. *Neuroscience and Biobehavioral Reviews, 27*(1), 57-71.

Puppe, B., Tuchscherer, M., & Tuchscherer, A. (1997). The effect of housing conditions and social environment immediately after weaning on the agonistic behaviour, neutrophil/lymphocyte ratio, and plasma glucose level in pigs. *Livestock Production Science, 48*(2), 157-164.

Ralph, C. R., & Tilbrook, A. J. (2016). The usefulness of measuring glucocorticoids for assessing animal welfare. *Journal of Animal Science, 94*(2), 457-470.

Rault, J.-L., Truong, S., Hemsworth, L., Le Chevoir, M., Bauquier, S., & Lai, A. (2019). Gentle abdominal stroking ('belly rubbing') of pigs by a human reduces eeg total power and increases eeg frequencies. *Behavioural Brain Research, 374*, 111892.

Rault, J.-L., Waiblinger, S., Boivin, X., & Hemsworth, P. (2020). The power of a positive human-animal relationship for animal welfare. *Frontiers in Veterinary Science, 7*(857).

Rault, J. L. (2016). Effects of positive and negative human contacts and intranasal oxytocin on cerebrospinal fluid oxytocin. *Psychoneuroendocrinology, 69*, 60-66.

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. *Domestic Animal Endocrinology, 65*, 67-70.

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. *Applied Animal Behaviour Science, 193*, 37-42.

Rooney, H. B., Schmitt, O., Courty, A., Lawlor, P. G., & O'Driscoll, K. (2021). Like mother like child: Do fearful sows have fearful piglets? *Animals, 11*(5), 1232.

Royo, F., Lyberg, K., Abelson, K. S. P., Carlsson, H. E., & Hau, J. (2005). Effect of repeated confined single housing of young pigs on faecal excretion of cortisol and iga. *Scandinavian Journal of Animal Science, 32*(1), 33.

Rushen, J., Munksgaard, L., Marnet, P. G., & DePassillé, A. M. (2001). Human contact and the effects of acute stress on cows at milking. *Applied Animal Behaviour Science, 73*(1), 1-14.

Rushen, J., Taylor, A. A., & de Passillé, A. M. (1999). Domestic animals' fear of humans and its effect on their welfare. *Applied Animal Behaviour Science, 65*(3), 285-303.

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. *Applied Animal Behaviour Science, 206*, 25-31.

Sapolsky, R. M. (2000). Stress hormones: Good and bad. *Neurobiology of Disease, 7*(5), 540-542.

Sapolsky, R. M., Romero, L. M., & Munck, A. U. (2000). How do glucocorticoids influence stress responses? Integrating permissive, suppressive, stimulatory, and preparative actions. *Endocrine Reviews, 21*(1), 55-89.

Schrøder-Petersen, D. L., & Simonsen, H. B. (2001). Tail biting in pigs. *The Veterinary Journal, 162*(3), 196-210.

Siegford, J. M., Rucker, G., & Zanella, A. J. (2008). Effects of pre-weaning exposure to a maze on stress responses in pigs at weaning and on subsequent performance in spatial and fear-related tests. *Applied Animal Behaviour Science, 110*(1), 189-202.

Simonsen, H. B. (1995). Effect of early rearing environment and tail docking on later behaviour and production in fattening pigs. *Acta Agriculturae Scandinavica, 45*(2), 139-144.

Singh, C., Verdon, M., Cronin, G. M., & Hemsworth, P. H. (2017). The behaviour and welfare of sows and piglets in farrowing crates or lactation pens. *Animal, 11*(7), 1210-1221.

Smit, M. N., Zhou, X., Landero, J. L., Young, M. G., & Beltranena, E. (2021). Dietary energy level, feeder space, and group size on growth performance and carcass characteristics of growing-finishing barrows and gilts. *Translational Animal Science, 5*(3), 1-15.

Smotherman, W. P., Brown, C. P., & Levine, S. (1977). Maternal responsiveness following differential pup treatment and mother-pup interactions. *Hormones and Behavior, 8*(2), 242-253.

Sommavilla, R., Cristiane Goncalves, T., Evaldo Antonio Lencioni, T., & Maria José, H. t. (2016). Ninety one-days-old piglets recognize and remember a previous aversive handler. *Livestock Science, 194*, 7-9.

Sommavilla, R., Hötzel, M. J., & Dalla Costa, O. A. (2011). Piglets' weaning behavioural response is influenced by quality of human–animal interactions during suckling. *Animal, 5*(9), 1426-1431.

Spinka, M., Newberry, R. C., & Bekoff, M. (2001). Mammalian play: Training for the unexpected. *Quarterly Review of Biology, 76*(2), 141-168.

Sutherland, M. A., Backus, B. L., Brooks, T. A., & McGlone, J. J. (2017). The effect of needle-free administration of local anesthetic on the behavior and physiology of castrated pigs. *Journal of Veterinary Behavior: Clinical Applications and Research, 21*, 71-76.

Sutherland, M. A., Davis, B. L., & McGlone, J. J. (2011). The effect of local or general anesthesia on the physiology and behavior of tail docked pigs. *Animal, 5*(8), 1237-1246.

Tahamtani, F. M., Nordgreen, J., Nordquist, R. E., & Janczak, A. M. (2015). Early life in a barren environment adversely affects spatial cognition in laying hens (gallus gallus domesticus). *Frontiers in Veterinary Science, 2*.

Taliaz, D., Loya, A., Gersner, R., Haramati, S., Chen, A., & Zangen, A. (2011). Resilience to chronic stress is mediated by hippocampal brain-derived neurotrophic factor. *The Journal of Neuroscience, 31*(12), 4475.

Tallet, C., Brajon, S., Devillers, N., & Lensink, J. (2018). Pig-human interactions: Creating a positive perception of humans to ensure pig welfare. In M. Špinka (Ed.), *Advances in pig welfare* (pp. 382). Duxford, United Kingdom: Woodhead Publishing.

Tallet, C., Rakotomahandry, M., Herlemont, S., & Prunier, A. (2019). Evidence of pain, stress, and fear of humans during tail docking and the next four weeks in piglets (sus scrofa domesticus). *Frontiers in Veterinary Science, 6*(462).

Tallet, C., Sy, K., Prunier, A., Nowak, R., Boissy, A., & Boivin, X. (2014). Behavioural and physiological reactions of piglets to gentle tactile interactions vary according to their previous experience with humans. *Livestock Science, 167*, 331-341.

Tanida, H., Miura, A., Tanaka, T., & Yoshimoto, T. (1995). Behavioural response to humans in individually handled weanling pigs. *Applied Animal Behaviour Science, 42*, 249-259.

Taylor, P. S., Hemsworth, P. H., & Rault, J.-L. (2022). Environmental complexity: Additional human visual contact reduced meat chickens' fear of humans and physical items altered pecking behavior. *Animals, 12*(3), 310.

Telkänranta, H., & Edwards, S. A. (2018). Lifetime consequences of the early physical and social environment of piglets. In M. Špinka (Ed.), *Advances in pig welfare* (pp. 101-136). Cambridge, England: Woodhead Publishing.

Telkänranta, H., Marchant-Forde, J. N., & Valros, A. (2016). Tear staining in pigs: A potential tool for welfare assessment on commercial farms. *Animal, 10*(2), 318-325.

Thodberg, K., Jensen, K. H., & Herskin, M. S. (2002). Nursing behaviour, postpartum activity and reactivity in sows: Effects of farrowing environment, previous experience and temperament. *Applied Animal Behaviour Science, 77*(1), 53-76.

Tilbrook, A. J., & Ralph, C. R. (2018). Hormones, stress and the welfare of animals. *Animal Production Science, 58*(3), 408-415.

Turpin, D. L., Langendijk, P., Sharp, C., & Pluske, J. R. (2017). Improving welfare and production in the peri-weaning period: Effects of co-mingling and intermittent suckling on the stress response, performance, behaviour, and gastrointestinal tract carbohydrate absorption in young pigs. *Livestock Science, 203*, 82-91.

Turpin, D. L., Langendijk, P., Tai-Yuan, C., Lines, D., & Pluske, J. R. (2016). Intermittent suckling causes a transient increase in cortisol that does not appear to compromise selected measures of piglet welfare and stress. *Animals, 6*(3), 24.

Van de Perre, V., Permentier, L., De Bie, S., Verbeke, G., & Geers, R. (2010). Effect of unloading, lairage, pig handling, stunning and season on ph of pork. *Meat Science, 86*(4), 931-937.

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. *Applied Animal Behaviour Science, 183*, 35-41.

Vermeulen, L., Van de Perre, V., Permentier, L., De Bie, S., Verbeke, G., & Geers, R. (2015). Pre-slaughter handling and pork quality. *Meat Science, 100*, 118-123.

Villain, A. S., Lanthony, M., Guérin, C., Noûs, C., & Tallet, C. (2020). Manipulable object and human contact: Preferences and modulation of emotional states in weaned piglets. *Frontiers in Veterinary Science, 7*.

Waiblinger, S., Boivin, X., Pedersen, V., Tosi, M.-V., Janczak, A. M., Visser, E. K., & Jones, R. B. (2006). Assessing the human–animal relationship in farmed species: A critical review. *Applied Animal Behaviour Science, 101*(3-4), 185-242.

Waiblinger, S., Menke, C., Korff, J., & Bucher, A. (2004). Previous handling and gentle interactions affect behaviour and heart rate of dairy cows during a veterinary procedure. *Applied Animal Behaviour Science, 85*(1-2), 31-42.

Weary, D. M., Jasper, J., & Hötzel, M. J. (2008). Understanding weaning distress. *Applied Animal Behaviour Science, 110*(1-2), 24-41.

Wells, D. L. (2004). A review of environmental enrichment for kennelled dogs, canis familiaris. *Applied Animal Behaviour Science, 85*(3-4), 307-317.

Willen, R. M., Schiml, P. A., & Hennessy, M. B. (2019). Enrichment centered on human interaction moderates fear-induced aggression and increases positive expectancy in fearful shelter dogs. *Applied Animal Behaviour Science, 217*, 57-62.

Yonezawa, T., Takahashi, A., Imai, S., Okitsu, A., Komiyama, S., Irimajiri, M., Matsuura, A., Yamazaki, A., & Hodate, K. (2012). Effects of outdoor housing of piglets on behavior, stress reaction and meat characteristics. *Asian-Australasian Journal of Animal Sciences, 25*(6), 886-894.

Zulkifli, I., Gilbert, J., Liew, P. K., & Ginsos, J. (2002). The effects of regular visual contact with human beings on fear, stress, antibody and growth responses in broiler chickens. *Applied Animal Behaviour Science, 79*(2), 103-112.

Zupan, M., Rehn, T., de Oliveira, D., & Keeling, L. J. (2016). Promoting positive states: The effect of early human handling on play and exploratory behaviour in pigs. *Animal, 10*(1), 135-141.

Chapter 2

Effects of Human Contact during Gestation on Stress Resilience in Sows

This chapter is presented as the author-accepted manuscript of a peer-reviewed article:

Hayes, M. E., Hemsworth, L. M., Morrison, R. S., Butler, K. L., Rice, M., Rault, J.-L., & Hemsworth, P. H. (2021). Effects of positive human contact during gestation on the behaviour, physiology and reproductive performance of sows. *Animals, 11*(1), 214.

2.1 Abstract

Previous positive interactions with humans may ameliorate the stress response of farm animals to aversive routine practices such as painful or stressful procedures, particularly those associated with stockpeople. We studied the effects of positive handling by providing younger (parity 1–2) and older (parity 3–8) sows housed in pens of fifteen (*n* = 24 pens in total) with either positive human contact (+HC) or routine human contact (control) during gestation. The +HC treatment involved a familiar stockperson patting and scratching sows and was imposed at a pen-level for 2 min daily. Measurements studied included behavioural, physiological and productivity variables. The +HC sows showed reduced avoidance of the stockperson conducting pregnancy testing and vaccination in the home pens, however the behavioural and cortisol responses of sows in a standard unfamiliar human approach test did not differ. There were no effects of +HC on aggression between sows, serum cortisol or serum brain-derived neurotrophic factor concentrations during gestation, or on the behavioural and cortisol response to being moved to farrowing crates. There were also no effects of +HC on the maternal responsiveness of sows, farrowing rate or the number of piglets born alive, stillborn or weaned. Sows in the +HC pens reduced their physical interaction with the stockpeople imposing the treatment after 2 weeks, which suggests the sows may have habituated to the novel or possible rewarding elements of the handling treatment. This experiment shows that regular positive interaction with stockpeople does reduce sows' fear of stockpeople, but does not always confer stress resilience.

2.2 Introduction

Stress, which refers to the biological response of an individual to an environmental change, can threaten animal welfare and compromise the productivity of farmed animals (Moberg, 2000). The degree to which animal welfare and productivity are affected by stress is dependent on the strength of the stressor, and how resilient the animal is to stress. In animal production systems where husbandry and housing practices involve close human contact, human interaction of a positive nature may facilitate stress resilience (Rault et al., 2020). For example, previous positive handling reduced heart rate in calves during transport and slaughter (Lensink et al., 2001), and reduced kicking and restless behaviour in dairy cows during rectal palpation (Waiblinger et al., 2004). Stroking in the first 2 weeks of life increased daily weight gain in calves from birth until weaning (Lürzel et al., 2015), and slow predictable movements and stroking during handling improved fertility rates in hens (Bertin et al., 2019) and enhanced the expression of maternal care in dairy goats postpartum (Baxter et al., 2016).

The majority of studies assessing the impact of handling on pigs have demonstrated that frequent negative interactions, such as shouting, slapping and hitting, increase pigs' fear of humans and can induce both acute and chronic stress. Reduced growth, feed conversion efficiency and reproduction have all been reported in pigs as a consequence of aversive handling (Hemsworth & Coleman, 2011). Although stockpeople can be associated with positive experiences (e.g., feeding: (Hemsworth et al., 1996)), most interactions between animals and stockpeople in commercial production systems are inadvertently negative for pigs. Almost all husbandry practices, that can elicit stress and pain in animals, are imposed by humans. However, positive interactions with humans may ameliorate the aversiveness of many husbandry practices, including those involving surgical interventions, given that handling *per se* may be just as stressful as the pain associated with the procedure (Marchant-Forde et al., 2009). For example, piglets that were patted and stroked during suckling bouts on the first day of life showed shorter durations of escape behaviour during tail docking at 2 days of age and capture at 15 days of age, in comparison to non-handled piglets (2015).

Few other studies have researched the effects of additional positive human interaction for pigs relative to minimal or routine handling. Positive handling (talking to and patting sows when they approached) for 3 min per day reduced the magnitude of the physiological stress response to tether housing in sows (Pederson et al., 1998). Talking to, stroking and rubbing sows for 1 min per day improved ease of handling and reduced fear of humans, farrowing duration and the occurrence of piglets being savaged (English et al., 1999). Though not all of the differences in the latter study were statistically significant, the authors report that the trends from all variables measured were consistent in terms of indicating there were benefits from the positive contact treatment. De Meyer et al. (2020) reported reduced piglet mortality by providing sows with daily back scratching and music. Anderson et al. (2006) found no effects of positive handling on piglet survival or early piglet weight gain; however, positive handling resulted in shorter durations of farrowing for sows which had high levels of fear towards humans at the start of the study.

Furthermore, there is evidence that stroking and talking softly induces an optimistic cognitive bias in piglets (Brajon et al., 2015b), and stimulates the release of oxytocin, a hormone most often associated with positive social behaviour, in weaner pigs (Rault, 2016). These findings, in addition to the ones above, indicate that positive human contact has the potential to mitigate deleterious stress effects that pigs may encounter in commercial environments, but also confer broader stress resilience by offering stimulation in an otherwise barren environment. Stress resilience can be measured by the behavioural and physiological responses of pigs to stressors (Lyons et al., 2009). Brain-derived neurotrophic factor (BDNF), a neurotrophin which plays an important role in neuroplasticity, is also thought to mediate stress resilience by regulating HPA axis activity early in life (Taliaz et al., 2011), and is being increasingly studied in farm animals (Arroyo et al., 2019; Rault et al., 2018). An individual's response to a stressor is influenced by the animal's previous experience with stress (Moberg, 2000), and thus age and/or experience may impact stress resilience. The aim of this experiment was therefore to examine the effects of regular positive human contact during gestation on stress resilience to routine management practices of sows from two different parity groups.

2.3 Materials and Methods

2.3.1 Animals and Housing

This experiment was conducted at a large commercial piggery in southern New South Wales, Australia. All animal procedures were conducted with prior institutional ethical approval under the requirements of the New South Wales Prevention of Cruelty to Animals Act 1985 in accordance with the National Health and Medical Research Council/Commonwealth Scientific and Industrial Research Organization/Australian Animal Commission Code of Practice for the Care and Use of Animals for Scientific Purposes (Rivalea Animal Ethics Committee #17B019C).

Three hundred and sixty mixed-parity Landrace × Large White sows that were in good health at the start of the experiment were studied throughout gestation (treatment period) and lactation. Sows were studied over two-time replicates, conducted in late spring and late winter, and were housed in groups of fifteen ($n = 24$ pens) with sows of the same parity group (parity 1–2 or parity 3–8) during gestation. Within each time replicate, half of the sows were introduced to the experiment in week 1 of the experiment while the remaining half were introduced to the experiment in week 2. In each of these weeks, 90 sows (45 parity 1–2 and 45 parity 3–8) which had been inseminated within the previous 4 d were selected and assigned to the gestation pens that had been allocated to the appropriate parity (see subsection section "Design" for further detail). There were two adjacent rows of partially slatted floored pens (6 pens/row) separated by a central corridor, each with a space allowance of 2 m^2/sow. Overhead water sprinklers covering 50% of each pen were activated when the internal temperature exceeded 26 °C. Feed (2.5 kg/sow/day of a commercial diet; 13.1 MJ/kg DM, and 13.5% CP) was delivered onto the solid section of the floor via automatic overhead drop feeders (5/pen) twice per day (approximately 07:00 and 07:30 h). The shed was renovated for the experiment, so that all pens had solid sides to limit visual contact between adjacent pens; however, the gates on the front of the pens were not solid, allowing visual contact with people and pigs in and across the corridor. Two CCTV cameras were placed over each pen. Sows were introduced to farrowing crates (2.3 × 1.7 m) at least 5 d prior to expected parturition, with sows from the same gestation pen generally being in nearby farrowing crates. Fostering piglets within treatment was performed when necessary. No bedding or nesting material was provided during the gestation or farrowing/lactation periods.

2.3.2 Treatments

A factorial treatment design with the following main effects was used to study the effects of positive human contact and age/experience on sow behaviour, physiology and reproductive performance:

1. Human contact
    I. *'Positive human contact (+HC)'*—At 13:00 h daily, one of five male stockpeople trained to impose this handling treatment entered each +HC treatment pen for 2 min, walked slowly through the group and stopped to pat, stroke, scratch and talk softly to sows in their pathway and sows approaching. The +HC treatment was imposed from post-insemination mixing until sows were moved to farrowing accommodation in wk 16 of treatment. The stockpeople imposing this handling treatment were responsible for routine management of all sows.
    II. *'Control contact'*—this treatment involved human contact only associated with routine management. Routine management of all sows involved twice daily health and welfare checks by the same stockpeople responsible for imposing the +HC treatment. These welfare checks involved one stockperson visually inspecting the animals and the facilities. Welfare checks generally took less than 30 s per pen and were usually conducted in the aisle, although sometimes it was necessary for stockpeople to enter the pen.
2. Parity
    I. *'Younger'*—Parity 1–2 sows
    II. *'Older'*—Parity 3–8 sows

2.3.3 Design

Within each row the pens were assigned to three pairs of adjacent, or nearly adjacent, pens. In each of these pairs one pen had younger sows and the other had older sows. Additionally, in each of these pairs, one pen received the +HC treatment and the other pen received the control contact treatment. Thus, each pair of pens had one pen of younger sows with +HC and one pen of older sows with control contact (Pair combination A) or alternatively had one pen of younger sows with control contact and one pen of older sows with +HC (Pair combination B). Within each of the two time-replicates, there was one row with two pairs of pair combination A and one pair of pair combination B, and the other row had one pair of pair combination A and two pairs of pair combination B. Within these restrictions, the combinations of human contact treatment and sow parity group were assigned randomly to the 24 pens. The ANOVA structure of this design is presented in Table 2.1.

Table 2.1. Structure of analysis of variance, including information summary, for statistical analysis of the study.

Source of Variation	Degrees of Freedom
Time replicate stratum	1
Row within time replicate stratum	
Human contact treatment by parity interaction	1
Residual	1
Pair within row stratum	
Human contact treatment by parity interaction	1
Residual	7
Pen within pair stratum	
Human contact treatment	1
Parity	1
Residual	10

Information summary for analysis of variance

Model Term	Efficiency factor	Non-orthogonal Terms
Row within time replicate stratum		
Human contact treatment by parity interaction	0.111	
Pair within row stratum		
Human contact treatment by parity interaction	0.889	Row within time replicate

2.3.4 Measurements

2.3.4.1 Sow and Stockperson Behaviour during +HC Treatment Imposition

Using CCTV camera footage, sow and stockperson behaviour was recorded during the imposition of +HC on d 2, 5, 7, 9, 11, 15, 17, 28, 43, 58 and 73 of treatment. Measures of stockperson behaviour included the proportion of sows in each +HC pen the stockperson physically interacted with in terms of patting, stroking or scratching a sow, and the total number of tactile interactions initiated by stockpeople. Measures of sow behaviour included the total number of tactile interactions with the stockperson (sniffing, nosing or chewing the stockperson or their attire) and the mean proportion of sows in each +HC pen within 1 m of the stockperson (recorded using instantaneous scan sampling every 10 s during the 2 min treatment imposition).

2.3.4.2 Responses towards Routine Management and Husbandry Practices

All sows underwent routine husbandry and management practices imposed in commercial production. This included pregnancy testing with an external ultrasound probe (wk 6 of treatment), intramuscular vaccination (wk 12 of treatment) and being moved to farrowing accommodation (end of treatment, wk 16). During the pregnancy testing and vaccination that were conducted in the home pens, CCTV footage was used to record the approach–withdrawal responses of sows to the stockperson. When the approaching stockperson was within 1 m of a sow before conducting pregnancy check and vaccination, the sow was recorded as either withdrawing (moved away from the stockperson within 2 s) or not withdrawing (approached the stockperson or remained stationary). The response of each sow was recorded again immediately after (within 2 s) pregnancy check and vaccination had been conducted. The number of sow-initiated tactile interactions was also recorded, but only during pregnancy testing as the stockperson was holding a stockboard during vaccination which prevented sows from physically interacting. Interactions during pregnancy testing were defined as any tactile contact including sniffing, nosing or chewing the stockperson or their attire. The stockpeople conducting pregnancy testing and vaccination were different for each husbandry practice and different between time replicates. However, the stockperson was always one of the people responsible for routine management of all pigs, and thus was familiar to both +HC and control sows. Additionally, the stockperson was also one of the five people responsible for imposing the +HC treatment. When sows were being moved into farrowing accommodation, direct observations were used to record the behaviour of sows while entering the farrowing crates. When each sow reached within 1 m of the crate, they were recorded as either voluntarily entering (walked into the crate without stopping or turning in the aisle) or resisting entry (attempted to turn around or stopped for more than 2 s in the aisle, requiring the stockperson to use pushing or slapping to move the sow into the crate). At 1.5, 3.5 and 4.5 h after introduction to the farrowing crates, the behaviour of 6 randomly selected sows from each gestation pen was observed. For periods of 30 s at each of the three time points, one-zero sampling was used to record whether or not sows were lying, bar biting (chewing/mouthing crate fittings), sham chewing (repetitive chewing in the absence of feed) or vocalising. Saliva samples for subsequent analysis of cortisol were collected from 6 randomly selected sows from each gestation pen 2.5 h after introduction to farrowing crates (see subsequent section “Physiological Sample Collection Details and Assay Characteristics” for further detail). Four weeks after housing in the farrowing crates, saliva samples for subsequent analysis of cortisol were collected again from another 6 sows randomly selected from each gestation pen, as previous research has demonstrated that cortisol concentrations in gilts housed in farrowing crates increase at 4 weeks postpartum relative to gilts in lactation pens (Cronin et al., 1991).

2.3.4.3 Responses towards Humans in a Standard Human Approach Test

The behavioural response to an unfamiliar stationary human in a novel arena was assessed in 6 sows randomly selected from each pen during wk 9 of treatment, following the method described by Hemsworth and colleagues (1989). The human approach test (HAT) arena (3 × 3.8 m) was constructed with solid black boarding within the shed where sows were housed. Painted lines were used to mark quadrants on the floor of the arena as well as a 0.5 m radius half-circle on the floor adjacent to the mid-point of the wall opposite the arena entry, in which the human stood. Testing was undertaken from 08:30 to 15:00 h, starting at least 1 h after feeding. Prior to testing, the 6 test sows from the first pen were moved into a holding area located 9 m away from the test arena. These 6 sows were given 4 min to settle in the holding area, before the first test sow was removed from the group and individually introduced to the empty test arena for a familiarisation period of 2 min. During the familiarisation period, the number of entries into quadrants was recorded as a measure of exploration in the novel arena. At the end of the familiarisation period, the human slowly entered and walked to the marked mid-point of the wall. The human stood stationary in this position for 3 min, and verbally relayed the sow's behaviour to an assistant standing outside the arena who was not visible to the sow, to record the following behavioural variables: latency to approach within 0.5 m of the human, time spent within 0.5 m of the human, latency to interact and number of tactile interactions with the human. Interactions were defined as any tactile contact including sniffing, nosing or chewing the human or their attire, and a bout criterion of 5 s was chosen to separate one bout of interaction from another bout. Upon completion of the test, the human collected a saliva sample from the sow for later analysis of cortisol (see subsequent section "Physiological Sample Collection Details and Assay Characteristics" for further detail). The test sow was then moved back to the holding pen and the next sow from the group was walked into the arena. Once all sows from the group had been tested, they were moved back to the home pen and the 6 test sows in the next pen along the aisle were moved to the holding area. All sows were moved by experienced handlers, unfamiliar to all pigs, with the assistance of a solid pig board.

2.3.4.4 Aggressive Behaviour between Sows during Feeding

Using CCTV camera footage, aggression during feeding was sampled for 1 day in wk 1, 4 and 8 of treatment. Aggressive behaviour was defined as slashes, butts, pushes, and bites, and these were distinguished from other tactile interactions with sows on the basis that the former were associated with avoidance or retaliation by one sow as a consequence of the interaction. The number of aggressive interactions between sows in each pen was recorded continuously by one observer for 15 min after each of two morning feed drops, spaced 30 min apart. This time period was chosen as aggression between sows during feeding is most intense in the first 15 min after feed is dropped (Verdon, 2014). Only aggressive interactions in which the head of the sow (defined as extending from the snout to the ears) displaying the aggressive behaviour was clearly visible were recorded, and so the number of aggressive interactions in each pen was expressed based on the average number of sows visible in the camera's field of view. For each 15 min sampling period, instantaneous scan sampling at 30 s intervals was used to count the number of sows in the field of view. Aggression in each pen was then calculated by multiplying the number of aggressive interactions by the inverse of the average number of sows in the field of view.

2.3.4.5 Basal Physiological Measurements during Gestation

In wk 5 and 10 of treatment, blood samples were collected from 3 sows randomly selected from each pen for later analysis of serum cortisol and serum brain-derived neurotrophic factor (BDNF) (see subsequent section "Physiological Sample Collection Details and Assay Characteristics" for further detail).

2.3.4.6 Maternal Responsiveness Test

In wk 2 of lactation (i.e., 3 wk after treatment had finished), the maternal behaviour of all sows was assessed by recording their behavioural responses towards an audio recording of an unfamiliar piglet squealing. Following the protocol for the maternal responsiveness test as described by Singh and colleagues (2017), an experimenter wearing a portable stereo unit around their neck entered the farrowing shed 5 min after sows had received their first feed delivery for the day (approximately 8:00 h). The experimenter then turned on the stereo unit and broadcasted a pre-recorded sound of a squealing piglet (80 decibels at a distance of 1 m) while walking slowly through the farrowing shed, pausing for 3 s in front of each sow's crate. The experimenter repeated the test six times, with each test taking approximately 10 min to complete. Two Go-Pro cameras were mounted on either side of the experimenter's head, which allowed the sows' behavioural responses during each 3 s bout of the audio broadcasting to later be assessed by one observer. Maternal responsiveness in the test was measured through changes in posture (changed from lying or sitting to standing), disruptions to feeding (lifted head from feeder) and the occurrence of sows vocalising, displaying behaviour directed towards their piglets (turned head to look at, sniff or nose piglets) and bar biting (chewing or mouthing crate fittings).

2.3.4.7 Reproductive Performance

Records of farrowing rate (proportion of sows inseminated that farrowed), the total number of piglets born alive, stillborn and mummified and the total number of piglets weaned were collected for all sows.

2.3.4.8 Physiological Sample Collection Details and Assay Characteristics

A random number generator was used to select sows from each pen for the collection of physiological samples. Saliva and blood samples were all collected within 2 min of approaching the sow, and in the event that a sow could not be sampled within this period, another sow from the pen was chosen. All samples were collected between 10:00 and 12:00 h, with the exception of post-HAT saliva samples which were obtained after each sow had been tested. For collection of saliva, 2 experimenters collected samples from sows in the pen simultaneously. A synthetic swab (Salimetrics; Carlsbad, California, USA) was secured to the end of a cable tie and offered to the sow to chew on for 1–2 min or until it was suitably moistened. The swab was then placed into a saliva collection tube (Sarstedt; South Australia, Australia) and kept on ice before being centrifuged at 2500× *g* for 2 min. After centrifugation, saliva was pipetted into polypropylene tubes and stored at −20 °C until later analysis of cortisol. For collection of blood, 2 experimenters were used for each sow; the first restrained the sow with a snout snare and the second collected the blood sample. Samples were obtained in 6 mL serum collection tubes (BD Vacutainer, New South Wales, Australia) via jugular venepuncture. After collection, tubes were gently inverted five times and allowed to clot for 1 h at room temperature. Samples were then centrifuged at 1000× *g* for 15 min, and the designated serum was pipetted off into two polypropylene tubes and stored at −20 °C until later analysis of cortisol and BDNF. Salivary and serum cortisol were quantified using commercially available RIA kits from MP Biomedicals (Item #07221105). The intra- and inter-assay co-efficient of variations for cortisol assays were all between 2.9 and 4.2%. Serum BDNF was analysed at 1:25 dilution and quantified using a commercially available ELISA kit from Biosensis (Item #BEK-2211). The mean intra- and inter-assay coefficient of variations were 2.9 and 7.8%, respectively.

2.3.5 Statistical Analysis

The unit of analysis for all statistical analyses was the group of sows from a single pen. For measurements taken on individual sows, pen values were calculated as averages of each individual sow measurement, or as a proportion of sows with a particular response. Prior to analysis, the number of sow-initiated tactile interactions during pregnancy testing, aggressive interactions in wk 1, 4 and 8 of treatment and serum cortisol and BDNF concentrations in wk 5 and 10 of treatment were logarithmically transformed on a pen basis, so that the amount of residual variation did not increase as the mean increased and/or so that the residuals did not have a markedly skewed distribution. There were no BDNF concentration measurements available for any sows in two pens in wk 5 and one pen in wk 10, due to blood samples being haemolysed or returning results outside the normal detectable range of the assay. Thus, there were 2 missing values for BDNF at wk 5 and 1 missing value for BDNF at wk 10.

Apart from measurements of sow behaviour during +HC treatment imposition, all sow measurements were analysed using an analysis of variance of the form presented in Table 2.1. Human contact treatment by parity interactions were estimated in both the row within time replicate and the pair within row stratums. However, there was only a residual degree of freedom in the row within time replicate stratum and almost 90% of the structural information was estimated in the pair within row stratum (efficiency factor = 0.889; Table 2.1). Thus, all results presented that involve the human contact by parity interaction are only from the pair within row stratum (i.e., we used an intra-block analysis). Sow behaviour during +HC treatment measurements used a modified version of the intra-block analysis of variance that only included a treatment effect for parity and did not include a pair within row stratum. In these analyses, the effect of parity was estimated on 7 degrees of freedom. Non-parametric permutation tests were used to calculate p-values for the proportion of sows that were bar biting, sham chewing and vocalising after entry to farrowing crates, and the proportion of sows that were bar biting during the maternal responsiveness test. In all these cases there were many pens with no sows partaking in the behaviour. These permutation tests were calculated using the usual F statistic obtained from the analysis of variance in Table 2.1, but with the permutation distribution implied from the structure of this analysis of variance. Results for stockperson behaviour during +HC treatment imposition are presented as box and whisker plots profiled over the period of treatment imposition. All analyses were carried out using the ANOVA directive, the APERMTEST procedure, the AREPMEASURES procedure and the BOXPLOT procedure of GenStat for Windows 18th Edition (VSN International, 2018).

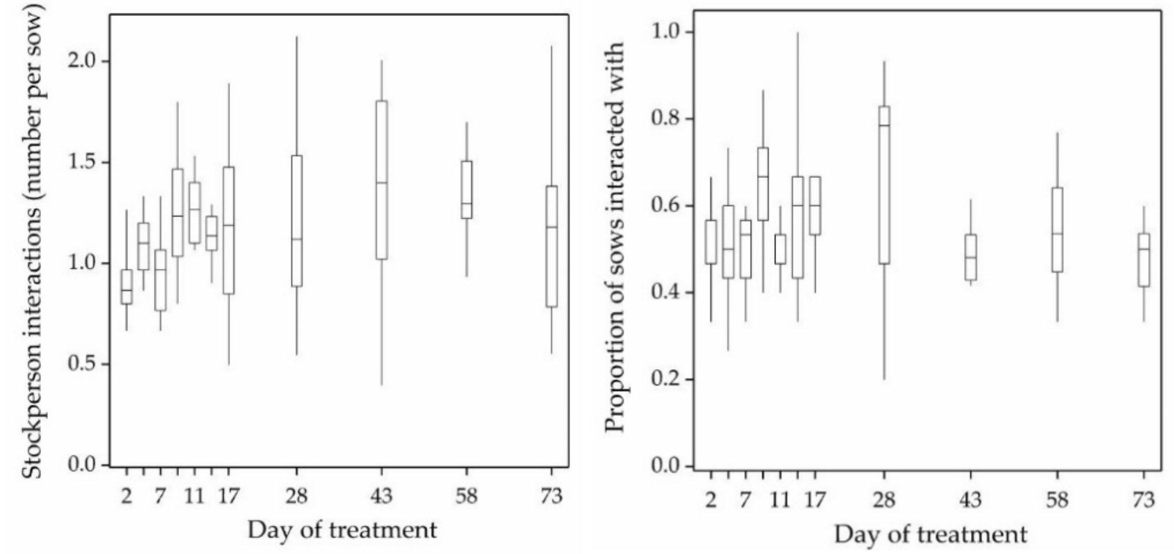
2.4 Results

There were no significant human contact × parity interactions ($p > 0.05$) on any sow variables measured, and thus the means and standard error of differences are reported for only the main effects of human contact and parity.

2.4.1 Sow and Stockperson Behaviour during +HC Treatment Imposition

2.4.1.1 Stockperson Behaviour during +HC Treatment Imposition

During the 2 min treatment imposition, stockpeople interacted with an average of 54% of sows from each +HC pen and initiated 1.2 tactile interactions per sow (Figure 2.1a, b).

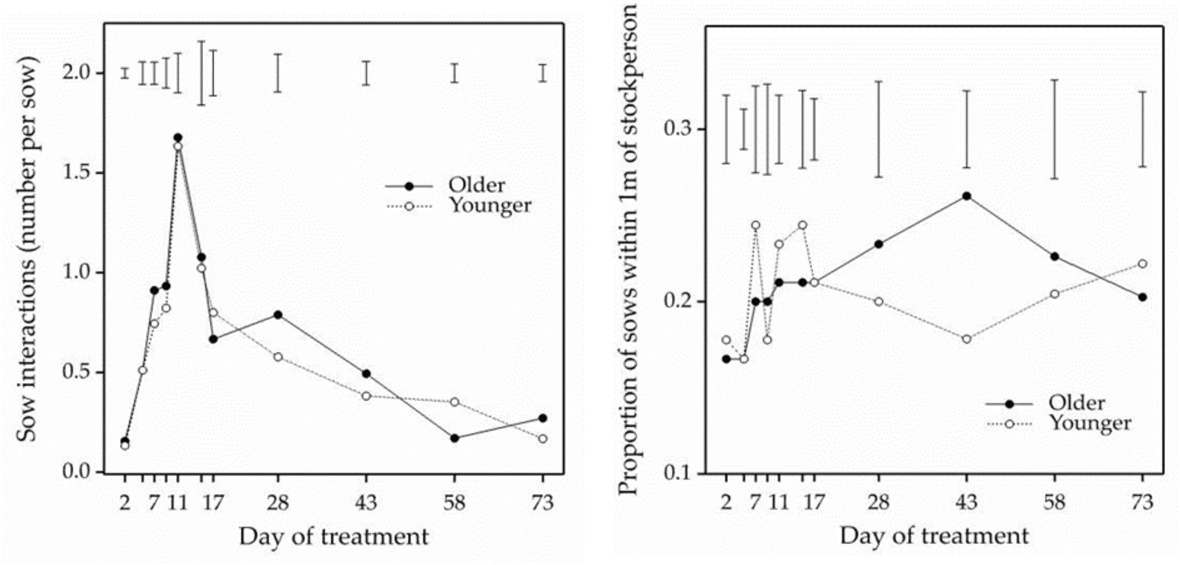


(a) **(b)**

Figure 2.1. Box and whisker plots of stockperson behaviour during positive human contact imposition on d 2, 5, 7, 9, 11, 15, 17, 28, 43, 58 and 73 of treatment. 2.1a shows the number of tactile interactions (patting, stroking, scratching) by stockpeople in each +HC treatment pen. 2.1b shows the proportion of sows in each +HC pen that stockpeople interacted with.

2.4.1.2 Sow Behaviour during +HC Treatment Imposition

There were no parity effects ($p > 0.05$) on the number of tactile interactions with stockpeople initiated by +HC sows during imposition of the handling treatment. There were also no parity effects ($p > 0.05$) on the mean proportion of +HC sows within 1 m of the stockperson during treatment imposition. The number of tactile interactions by sows increased from 0.1 to 1.7 interactions per sow from day 2 until day 11 of treatment, after which the number of tactile interactions by sows reduced (Figure 2.2a; $F_{3.62,36.2} = 38.02$, $p = 4.5 \times 10^{-12}$ for test of day of treatment effect using a repeated measures analysis of variance with Greenhouse Geisser correction ($\varepsilon = 0.3622$) on square root transformed data). The mean proportion of sows from each +HC pen within 1 m of the stockperson imposing the treatment was 21% (Figure 2.2b; $F_{4.07,40.7} = 1.13$, $p = 0.36$ for test of day of treatment effect using a repeated measures analysis of variance with Greenhouse Geisser correction ($\varepsilon = 0.4067$)).



(a) **(b)**

Figure 2.2. Sow behaviour during positive human contact imposition on d 2, 5, 7, 9, 11, 15, 17, 28, 43, 58 and 73 of treatment. 2.2a shows the mean number of tactile interactions (sniffing, nosing, chewing) with stockpeople initiated by older (solid line) and younger (dashed line) +HC sows. 2.2b shows the mean proportion of older (solid line) and younger (dashed line) +HC sows within 1 m of the stockperson during treatment imposition. Error bars represent standard error of the difference using single day analyses of variance, which did not require data transformation for number of sow interactions.

2.4.2 Treatment and Parity Effects

2.4.2.1 Responses towards Routine Management and Husbandry Practices

Sows from +HC pens showed fewer withdrawal responses than sows from control pens when the stockperson approached within 1 m to conduct pregnancy testing in wk 6 (12 vs. 37 % of sows; sed = 4.2; $p < 0.001$), and vaccination in wk 12 of treatment (43 vs. 67 % of sows; sed = 7.2; $p = 0.008$; Table 2.2). There were also fewer withdrawal responses by +HC sows immediately after pregnancy testing (16 vs. 52 % of sows; sed = 3.9; $p < 0.001$) and vaccination (84 vs. 92 % of sows; sed = 3.9; $p = 0.05$). During the imposition of pregnancy testing on the entire pen, +HC sows initiated significantly more tactile interactions with the stockperson (95% confidence interval for % increase = (120, 430); $p < 0.001$). There was a higher proportion of younger sows avoiding the stockperson before (32 vs. 18 % of sows; sed = 4.2; $p = 0.009$) and after (39 vs. 29 % of sows; sed = 3.9; $p = 0.03$) the pregnancy test. However, a similar effect was not present at vaccination ($p > 0.05$). Despite good statistical precision, there were no significant effects ($p > 0.05$) of treatment or parity on the behavioural or cortisol responses of sows towards being moved from group gestation pens to individual farrowing crates.

Table 2.2. Effects of human contact and parity on sows' responses towards pregnancy testing, vaccination and being moved into farrowing crates. Means and standard error of the difference (s.e.d.) for main effects are presented. The number of tactile interactions during pregnancy testing was logarithmically (base e) transformed; back-transformed means are presented in parentheses.

Measurement	**Human Contact**		**Parity**		**s.e.d.**	***p*-Value**		
	Positive	**Control**	**Younger**	**Older**		**Human Contact**	**Parity**	**Contact × Parity**
Pregnancy testing								
Proportion of sows withdrawing from the approaching stockperson	0.12	0.37	0.32	0.18	0.042	**0.00016**	**0.0089**	0.060
Proportion of sows withdrawing post pregnancy test	0.16	0.52	0.39	0.29	0.039	**3.6×10^{-6}**	**0.027**	0.063
Tactile interactions initiated with the stockperson (number per pen)	2.8 (16)	1.6 (4.7)	2.3 (10)	2.0 (7.3)	0.20	**0.00011**	0.13	0.52
Vaccination								
Proportion of sows withdrawing from the approaching stockperson	0.43	0.67	0.54	0.56	0.072	**0.0079**	0.75	0.44
Proportion of sows withdrawing post vaccination	0.84	0.92	0.90	0.86	0.039	0.054	0.25	0.79
Moving to farrowing crates								
Proportion of sows resisting entry to crate	0.51	0.46	0.54	0.44	0.048	0.31	0.072	0.82
Proportion of sows lying down after entry [1]	0.69	0.70	0.65	0.73	0.068	0.86	0.21	0.46
Proportion of sows bar biting after entry [1]	0.060	0.060	0.040	0.070	0.022	0.84 [2]	0.14 [2]	1.0 [2]
Proportion of sows sham chewing after entry [1]	0.090	0.070	0.090	0.070	0.017	0.25 [2]	0.33 [2]	0.81 [2]
Proportion of sows vocalising after entry [1]	0.32	0.26	0.34	0.25	0.072	0.39 [2]	0.21 [2]	0.11 [2]
Salivary cortisol concentrations 2.5 h after entry (ng/mL)	1.3	1.3	1.2	1.3	0.066	0.40	0.16	0.97
Salivary cortisol concentrations 4 wk after entry (ng/mL)	1.6	1.4	1.5	1.5	0.14	0.25	0.71	0.68

[1] Data represent the mean proportion of sows engaging in each behaviour when observed 1.5, 3.5 and 4.5 h after entry to farrowing crates.

[2] *p*-values calculated using permutation tests.

2.4.2.2 Responses towards Humans in a Standard Human Approach Test

Despite good precision, there were no effects (*p* > 0.05) of treatment or parity on the latency to approach, latency to initiate tactile interaction, the total time spent in close proximity or the total number of tactile interactions with a stationary human in the HAT conducted in wk 9 of treatment (Table 2.3). There were also no effects (*p* > 0.05) of treatment or parity on the number of quadrants entered in the 2 min familiarisation period, or on cortisol concentrations in saliva collected after the HAT.

2.4.2.1 Aggressive Behaviour between Sows during Feeding

There were no significant effects (*p* > 0.05) of treatment or parity on the number of aggressive interactions between sows during morning feed drops in wk 1, 4 and 8 of treatment (Table 2.4).

2.4.2.2 Basal Physiological Measurements during Gestation and Lactation

There were no significant effects (*p* > 0.05) of treatment or parity on serum cortisol or brain-derived neurotrophic factor concentrations in wk 5 or 10 of treatment (Table 2.5).

2.4.2.3 Maternal Responsiveness Test

In response to an audio recording of unfamiliar piglet squeals, there were no significant treatment or parity effects (*p* > 0.05) on the proportion of sows that moved from resting to standing, stopped feeding, vocalised, directed their behaviour towards their piglets or began biting crate fixtures (Table 2.6).

2.4.2.1 Reproductive Performance

There were no effects of treatment or parity on the proportion of sows that farrowed (Table 2.7). There were more piglets stillborn from older parity sows compared to younger parity sows (1.4 vs. 1.0; sed = 0.14; *p* = 0.013). There were no effects (*p* > 0.05) of human contact or parity on the number of piglets born alive, mummified or weaned from each sow.

Table 2.3. Effects of human contact and parity on sows' responses in the human approach test. A maximum response time of 180 s was given to sows which did not approach or interact with the human. Means and standard error of the difference (S.e.d.) for main effects are presented.

Measurement	Human Contact		Parity		S.e.d.	*p*-Value		
	Positive	Control	Younger	Older		Human Contact	Parity	Contact × Parity
No. entries into different quadrants during familiarisation period	11	11	11	11	0.70	0.55	0.78	0.68
Latency to approach within 0.5 m of human (s)	76	78	77	77	0.19	0.87	0.96	0.53
Time spent within 0.5 m of human (s)	28	31	33	26	0.10	0.64	0.30	0.58
Latency to physically interact with human (s)	109	108	106	111	0.19	0.98	0.66	0.65
Tactile interactions initiated with human (number per sow)	2.8	4.0	3.9	3.0	0.72	0.11	0.24	0.76
Salivary cortisol concentrations on completion of testing (ng/mL)	3.6	3.4	3.9	3.1	0.44	0.59	0.084	0.058

Table 2.4. Effects of human contact and parity on aggression between sows during feeding in the morning. Data represent the mean number of aggressive interactions per sow, based off the average number of sows in the camera's field of view. Means and standard error of the difference (S.e.d.) for main effects are presented. Data were logarithmically (base *e*) transformed; back-transformed means are presented in parentheses.

Measurement	Human Contact		Parity		S.e.d.	*p*-Value		
	Positive	Control	Younger	Older		Human Contact	Parity	Contact × Parity
Aggressive interactions wk 1 of treatment	1.9 (7.0)	2.1 (7.9)	2.0 (7.6)	2.0 (7.2)	0.19	0.54	0.81	0.65
Aggressive interactions wk 4 of treatment	2.3 (9.5)	2.3 (9.5)	2.2 (9.3)	2.3 (9.7)	0.11	0.99	0.66	0.40
Aggressive interactions wk 8 of treatment	2.0 (7.6)	2.0 (7.4)	2.1 (7.9)	2.0 (7.1)	0.12	0.84	0.30	0.44

Table 2.5. Effects of human contact and parity on basal serum cortisol and BDNF concentrations during the treatment period. Means and standard error of the difference (S.e.d.) for main effects are presented. Data were logarithmically (base *e*) transformed; back-transformed means are presented in parentheses.

Measurement	Human Contact		Parity		S.e.d.	*p*-Value		
	Positive	Control	Younger	Older		Human Contact	Parity	Contact × Parity
Cortisol								
Wk 5 of treatment (ng/mL)	1.8 (5.8)	1.7 (5.4)	1.7 (5.6)	1.7 (5.6)	0.13	0.55	0.94	0.17
Wk 10 of treatment (ng/mL)	2.2 (8.7)	2.3 (9.7)	2.2 (9.2)	2.2 (9.1)	0.12	0.41	0.94	0.27
BDNF								
Wk 5 of treatment (pg/mL)	7.1 (1190)	7.3 (1420)	6.7 (830)	7.6 (2020)	0.70	0.80	0.24	0.92
Wk 10 of treatment (pg/mL)	7.2 (1310)	6.8 (930)	6.6 (710)	7.5 (1720)	0.50	0.50	0.11	0.72

Table 2.6. Effects of human contact and parity on the behaviour of sows in response to an audio recording of unfamiliar piglet vocalisations. Data represent the mean proportion of sows engaging in each behaviour during the maternal responsiveness test. Means and standard error of the difference (S.e.d.) for main effects are presented.

Measurement	**Human Contact**		**Parity**			***p*-Value**		
	Positive	**Control**	**Younger**	**Older**	**S.e.d.**	**Human Contact**	**Parity**	**Contact × Parity**
Changed posture from sitting or lying to upright	0.22	0.31	0.28	0.24	0.063	0.19	0.58	0.15
Disrupted from feeding	0.25	0.33	0.28	0.31	0.043	0.093	0.43	0.58
Vocalising	0.61	0.76	0.66	0.71	0.069	0.06	0.42	0.40
Behaviour towards piglets	0.39	0.40	0.36	0.43	0.068	0.98	0.29	0.98
Bar biting	0.16	0.080	0.13	0.11	0.052	0.12 [1]	0.68 [1]	0.06 [1]

[1] *p*-values calculated using permutation tests.

Table 2.7. Effects of human contact and parity on the reproductive performance of sows. Means and standard error of the difference (S.e.d.) for main effects are presented.

Measurement	**Human Contact**		**Parity**			***p*-Value**		
	Positive	**Control**	**Younger**	**Older**	**S.e.d.**	**Human Contact**	**Parity**	**Contact × Parity**
Farrowing rate	0.83	0.79	0.82	0.81	0.038	0.40	0.78	0.66
Number of piglets born alive	12	12	12	12	0.41	0.71	0.57	0.53
Number of stillborn piglets	1.3	1.2	1.0	1.4	0.14	0.46	**0.013**	0.99
Number of mummified piglets	0.17	0.15	0.14	0.18	0.042	0.65	0.31	0.23
Number of piglets weaned	9.3	9.8	9.7	9.5	0.25	0.070	0.52	0.37

2.5 Discussion

This experiment examined the effects of regular positive human contact during gestation on stress resilience in sows from young and old parity groups. Two min of daily positive handling reduced the avoidance responses of sows to stockpeople imposing two common husbandry practices in their home pens, pregnancy check and vaccination. However, there were no effects of human contact on the behavioural and cortisol responses to the presence of an unfamiliar human or to being moved into farrowing crates, suggesting that the sows did not generalise to other humans or other contexts. Furthermore, there were no treatment effects on aggression during feeding, basal cortisol or BDNF concentrations, or the reproductive performance of the sows.

There were fewer sows from the +HC treatment that showed avoidance of the stockperson before and after the imposition of pregnancy testing and vaccination, indicating reduced fear of these practices. It is likely these behavioural responses reflect reduced fear of the stockpeople imposing the husbandry practices, rather than reduced fear of the procedures *per se*; however, close human presence may be the most stressful element of many husbandry practices (Marchant-Forde et al., 2009). Although measuring the physiological responses towards these stressors would be valuable in further understanding the effects of positive handling on sows, these findings on the behavioural responses of sows show that previous positive interactions with stockpeople can ameliorate the stress responses of pigs towards routine husbandry practices imposed by those same persons. Reduced stress is a beneficial outcome for animal welfare, and may have additional benefits for the stockperson, as pigs which are less fearful of humans are easier to handle (English et al., 1999) and thus impose husbandry practices upon. Pigs with lower fear of humans may also be less likely to sustain injuries arising from avoiding stockpeople during routine inspections and handling, as demonstrated with calves (Lensink et al., 2001). There was an effect of parity with a higher proportion of younger sows withdrawing from the stockperson in comparison to older sows during pregnancy testing. These results indicate that younger sows show greater fear of humans during routine practices in commercial production systems.

Although sows in the human contact treatment showed reduced avoidance of the stockperson imposing husbandry practices in the group pens, and despite good precision of the statistical analysis, there were no effects of +HC on the behaviour of sows when moved to and after entering farrowing crates, or on salivary cortisol concentrations after entry. There were also no effects of the handling treatment on the frequency of aggressive interactions during gestation, although aggression was only studied during feeding bouts when it is more likely to be intense due to competition between sows.

To our knowledge, there have been no other studies assessing the impact of positive handling on aggression or abnormal behaviours in sows, such as sham chewing and bar biting, as observed after introduction to farrowing crates in this experiment. In weaned pigs, positive human interaction has been reported to reduce the incidence of tail biting (Büttner et al., 2018), although the handling treatment in this experiment involved offering chopped straw to piglets which may have increased foraging and thus reduced tail biting behaviour.

There were no effects of +HC on the behavioural or cortisol responses of sows towards an unfamiliar human in the HAT. Although the positive handling treatment was primarily of a tactile nature in the present experiment, interactions between pigs and stockpeople occur through various sensory channels (Tallet et al., 2018). One of the difficulties in conducting this experiment was eliminating human interaction in the control treatment that was not associated with routine management. Both control and +HC sows were allocated to one area of the gestation shed to control for positional effects in the facility. While group pens had solid sides to limit visual contact between adjacent pens, the gates on the front of the pens were not solid, and allowed visual contact with people and pigs in the aisle and in the opposite pens. The imposition of the positive handling treatment therefore unavoidably involved control sows receiving up to 30 min daily of additional visual, auditory and olfactory contact with stockpeople, which greatly exceeded the general stockperson contact in the home pen associated with routine management. Studies on poultry have shown that visual contact with an experimenter reduces birds' fear of handling (Zulkifli et al., 2002), and can be more effective at reducing fear of humans than gentle tactile contact (Jones, 1993). Recent research has also demonstrated that pigs' fear of humans is reduced when they observe a human stroking other pigs (Luna et al., 2021). It is therefore possible that any treatment effects on responses in the HAT were diluted as a result of control sows receiving additional visual exposure to humans.

There are several explanations for the conflicting behavioural responses of sows to familiar stockpeople imposing the two husbandry practices and to the unfamiliar human in the HAT. It may be that a human closely approaching and imposing a husbandry procedure is more likely to differentiate pigs of varying levels of fear of humans. It is also possible that the stockperson contact control sows received during the imposition of the +HC treatment in nearby pens may have reduced the fear responses of control sows to the extent that they were similarly motivated to +HC sows to explore and interact with the human in the HAT. Furthermore, the +HC sows may not have generalised their response to unfamiliar humans in a different location. However, it should be recognised that there is evidence pigs can generalise handling experiences to other humans (Brajon et al., 2015a) in different locations (Hemsworth et al., 1994), and it has been well documented that positive human contact relative to minimal handling reduces pigs' fear of humans in a standard test such as the HAT (Hemsworth & Coleman, 2011; Tallet et al., 2014; Tanida et al., 1995).

A feature of the positive handling treatment that differentiates this experiment from others is that patting and stroking occurred irrespective of sows voluntarily seeking interaction from the stockperson (although if the stockperson initiated interaction and the sow retreated, the stockperson did not pursue the sow). In the majority of studies reporting benefits of positive handling, contact was "unforced", and only occurred if pigs approached the human (e.g., reduced fear of humans (Hemsworth & Barnett, 1992; Hemsworth et al., 1986); reduced cortisol response (Pederson et al., 1998); reduced tail biting (Büttner et al., 2018); increased oxytocin (Rault, 2016); and increased feed intake (Day et al., 2002). There is increasing attention being paid to the importance of providing animals with a sense of control over aspects of their environment, including in their interactions with humans (Rault et al., 2020). The +HC treatment in the present experiment may have been more effective if it was contingent on pigs voluntarily soliciting interaction from the stockperson, therefore providing sows with greater choice and control in the interaction. Although the proportion of +HC sows within 1 m of the stockperson remained consistent from the start until the end of the treatment period, voluntary interaction by +HC sows with the stockpeople imposing the handling increased sharply from day 2 until day 11 of treatment, and then reduced until the end of the treatment period. While reduced interaction at the end of the treatment period may correlate with reduced activity later into gestation, it is surprising that there was a reduction in the sows' tactile contact with the stockperson after only 2 weeks. The reduced physical interaction initiated by sows suggests habituation to any novel or reinforcing elements of the handling treatment.

Positive human contact had no effect on the maternal behaviour or reproductive performance of sows. High levels of fear towards humans can induce chronic stress, and have been associated with an increased incidence of stillborn piglets (Hemsworth et al., 1999), reduced pregnancy rate (Hemsworth et al., 1986), smaller litter sizes (Hemsworth et al., 1981), and a higher prevalence of crushing piglets (Lensink et al., 2009). There were no treatment effects on sows' fear responses in the HAT or on basal cortisol or BDNF concentrations during gestation, and so any stress-related effects of treatment were unlikely to affect the reproductive performance of sows in this experiment. Other studies comparing the effects of positive handling of sows relative to minimal handling have reported reduced farrowing duration (Andersen et al., 2006) and lower pre-weaning mortality (De Meyer et al., 2020). However, farrowing duration was only reduced in highly fearful sows in the former study, and sows and piglets were also exposed to music in the latter study. In regards to the effects of parity in the present experiment, there were higher numbers of stillborn piglets from older sows than younger sows, but as reproductive performance is known to decline with age this was expected (Klimas et al., 2020).

2.6 Conclusion

Providing group housed sows with 2 min of daily patting and scratching reduced the fear responses of pigs towards stockpeople imposing pregnancy testing and vaccination in the home pens. However, the positive handling treatment had no effect on sows' behavioural and cortisol responses towards an unfamiliar human standing stationary, or towards being moved into farrowing crates. Aggression during feeding, basal cortisol and BDNF concentrations, and reproductive performance were not affected by the handling treatment. There was evidence that sows in the positive human contact groups habituated to the handling treatment after 2 weeks. While this experiment showed that previous positive interactions with stockpeople can ameliorate the stress responses of pigs towards routine husbandry practices, further research is necessary to understand the effects of positive handling on stress resilience when pigs have control over the interactions with humans, as well as the possible effects of habituation to positive handling.

2.7 References

Andersen, I. L., Berg, S., Bøe, K. E., & Edwards, S. (2006). Positive handling in late pregnancy and the consequences for maternal behaviour and production in sows. *Applied Animal Behaviour Science, 99*(1), 64-76.

Arroyo, L., Valent, D., Carreras, R., Peña, R., Sabrià, J., Velarde, A., & Bassols, A. (2019). Housing and road transport modify the brain neurotransmitter systems of pigs: Do pigs raised in different conditions cope differently with unknown environments? *PLOS ONE, 14*(1), 1-20.

Baxter, E. M., Mulligan, J., Hall, S. A., Donbavand, J. E., Palme, R., Aldujaili, E., Zanella, A. J., & Dwyer, C. M. (2016). Positive and negative gestational handling influences placental traits and mother-offspring behavior in dairy goats. *Physiology & Behavior, 157*, 129-138.

Bertin, A., Anne-Sophie, D., Cécile, A., Cécilia, H., Frédérique, M., Ludovic, C., Ludovic, D., Rupert, P., & Sophie, L. (2019). Human behaviour at the origin of maternal effects on offspring behaviour in laying hens (gallus gallus domesticus). *Physiology & Behavior, 201*, 175-183.

Brajon, S., Laforest, J.-P., Bergeron, R., Tallet, C., & Devillers, N. (2015a). The perception of humans by piglets: Recognition of familiar handlers and generalisation to unfamiliar humans. *Animal Cognition 18*(6), 1299-1316.

Brajon, S., Laforest, J.-P., Schmitt, O., & Devillers, N. (2015b). The way humans behave modulates the emotional state of piglets. *PLOS ONE, 10*(8), 1-17.

Büttner, K., Czycholl, I., Basler, H., & Krieter, J. (2018). Effects of an intensified human–animal interaction on tail biting in pigs during the rearing period. *Journal of Agricultural Science, 156*(8), 1039-1046.

Cronin, G. M., Barnett, J. L., Hodge, F. M., Smith, J. A., & McCallum, T. H. (1991). The welfare of pigs in two farrowing/lactation environments: Cortisol responses of sows. *Applied Animal Behaviour Science, 32*(2), 117-127.

Day, J. E. L., Spoolder, H. A. M., Burfoot, A., Chamberlain, H. L., & Edwards, S. A. (2002). The separate and interactive effects of handling and environmental enrichment on the behaviour and welfare of growing pigs. *Applied Animal Behaviour Science, 75*(3), 177-192.

De Meyer, D., Amalraj, A., Van Limbergen, T., Fockedey, M., Edwards, S., Moustsen, V. A., Chantziaras, I., & Maes, D. (2020). Short communication: Effect of positive handling of sows on litter performance and pre-weaning piglet mortality. *Animal*, 1-7.

English, P. R., Grant, S. A., McPherson, O., & Edwards, S. A. (1999). Evaluation of the effects of the positive 'befriending' of sows and gilts ('pleasant' treatment) prior to parturition and in early lactation on sow behaviour, the process of parturition and piglet survival. *BSAP Occasional Publication, 23*, 132-136.

Hemsworth, P. H., & Barnett, J. L. (1992). The effects of early contact with humans on the subsequent level of fear of humans in pigs. *Applied Animal Behaviour Science, 35*, 83-90.

Hemsworth, P. H., Barnett, J. L., Coleman, G. J., & Hansen, C. (1989). A study of the relationships between the attitudinal and behavioural profiles of stockpersons and the level of fear of humans and reproductive performance of commercial pigs. *Applied Animal Behaviour Science, 23*(4), 301-314.

Hemsworth, P. H., Barnett, J. L., & Hansen, C. (1986). The influence of handling by humans on the behaviour, reproduction and corticosteroids of male and female pigs. *Applied Animal Behaviour Science, 15*(4), 303.

Hemsworth, P. H., Brand, A., & Willems, P. (1981). The behavioural response of sows to the presence of human beings and its relation to productivity. *Livestock Production Science, 8*(1), 67-74.

Hemsworth, P. H., & Coleman, G. J. (2011). *Human-livestock interactions: The stockperson and the productivity and welfare of intensively farmed animals* (2nd ed.). Wallingford, United Kingdom: CABI Publishing.

Hemsworth, P. H., Coleman, G. J., Cox, M., & Barnett, J. L. (1994). Stimulus generalization: The inability of pigs to discriminate between humans on the basis of their previous handling experience. *Applied Animal Behaviour Science, 40*(2), 129-142.

Hemsworth, P. H., Pederson, V., Cox, M., Cronin, G. M., & Coleman, G. J. (1999). A note on the relationship between the behavioural response of lactating sows to humans and the survival of their piglets. *Applied Animal Behaviour Science, 65*, 43-52.

Hemsworth, P. H., Verge, J., & Coleman, G. J. (1996). Conditioned approach-avoidance responses to humans: The ability of pigs to associate feeding and aversive social experiences in the presence of humans with humans. *Applied Animal Behaviour Science, 50*(1), 71-82.

Jones, R. B. (1993). Reduction of the domestic chick's fear of human beings by regular handling and related treatments. *Animal Behaviour, 46*(5), 991-998.

Klimas, R., Klimienė, A., Sobotka, W., Kozera, W., & Matusevičius, P. (2020). Effect of parity on reproductive performance sows of different breeds. *South African Journal of Animal Science, 50*(3), 434-441.

Lensink, B. J., Fernandez, X., Cozzi, G., Florand, L., & Veissier, I. (2001). The influence of farmers' behavior on calves' reactions to transport and quality of veal meat. *Journal of Animal Science, 79*(3), 642-652.

Lensink, B. J., Leruste, H., De Bretagne, T., & Bizeray-Filoche, D. (2009). Sow behaviour towards humans during standard management procedures and their relationship to piglet survival. *Applied Animal Behaviour Science, 119*(3), 151-157.

Luna, D., González, C., Byrd, C. J., Palomo, R., Huenul, E., & Figueroa, J. (2021). Do domestic pigs acquire a positive perception of humans through observational social learning? *Animals, 11*(1), 127.

Lürzel, S., Münsch, C., Windschnurer, I., Futschik, A., Palme, R., & Waiblinger, S. (2015). The influence of gentle interactions on avoidance distance towards humans, weight gain and physiological parameters in group-housed dairy calves. *Applied Animal Behaviour Science, 172*, 9-16.

Lyons, D. M., Parker, K. J., Katz, M., & Schatzberg, A. F. (2009). Developmental cascades linking stress inoculation, arousal regulation, and resilience. *Frontiers in Behavioral Neuroscience, 3*(32).

Marchant-Forde, J. N., Lay, D. C., McMunn, K. A., Cheng, H. W., Pajor, E. A., & Marchant-Forde, R. M. (2009). Postnatal piglet husbandry practices and well-being: The effects of alternative techniques delivered in combination. *Journal of Animal Science*(3), 1150-1161.

Moberg, G. P. (2000). Biological response to stress: Implications for animal welfare. In G. P. Moberg & J. A. Mench (Eds.), *The biology of animal stress: Basic principles and implications for animal welfare* (pp. 1-21). Wallingford, United Kingdom: CABI Publishing.

Muns, R., Rault, J. L., & Hemsworth, P. (2015). Positive human contact on the first day of life alters the piglet's behavioural response to humans and husbandry practices. *Physiology & Behavior, 151*, 162-167.

Pederson, V., Barnett, J. L., Hemsworth, P. H., Newman, E. A., & Schirmer, B. (1998). The effects of handling on behavioural and physiological responses to housing in tether-stalls among pregnant pigs. *Animal Welfare, 7*(2), 137-150.

Rault, J.-L., Waiblinger, S., Boivin, X., & Hemsworth, P. (2020). The power of a positive human-animal relationship for animal welfare. *Frontiers in Veterinary Science, 7*(857).

Rault, J. L. (2016). Effects of positive and negative human contacts and intranasal oxytocin on cerebrospinal fluid oxytocin. *Psychoneuroendocrinology, 69*, 60-66.

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. *Domestic Animal Endocrinology, 65*, 67-70.

Singh, C., Verdon, M., Cronin, G. M., & Hemsworth, P. H. (2017). The behaviour and welfare of sows and piglets in farrowing crates or lactation pens. *Animal, 11*(7), 1210-1221.

Taliaz, D., Loya, A., Gersner, R., Haramati, S., Chen, A., & Zangen, A. (2011). Resilience to chronic stress is mediated by hippocampal brain-derived neurotrophic factor. *The Journal of Neuroscience, 31*(12), 4475.

Tallet, C., Brajon, S., Devillers, N., & Lensink, J. (2018). Pig-human interactions: Creating a positive perception of humans to ensure pig welfare. In M. Špinka (Ed.), *Advances in pig welfare* (pp. 382). Duxford, United Kingdom: Woodhead Publishing.

Tallet, C., Sy, K., Prunier, A., Nowak, R., Boissy, A., & Boivin, X. (2014). Behavioural and physiological reactions of piglets to gentle tactile interactions vary according to their previous experience with humans. *Livestock Science, 167*, 331-341.

Tanida, H., Miura, A., Tanaka, T., & Yoshimoto, T. (1995). Behavioural response to humans in individually handled weanling pigs. *Applied Animal Behaviour Science, 42*, 249-259.

Verdon, M. (2014). *Sow aggression in groups: Predicting and implications for sow welfare.* (Doctor of Philosophy). The University of Melbourne, Retrieved from http://hdl.handle.net/11343/42128

VSN International. (2018). Genstat for windows 19th edition. Hemel Hempstead, United Kingdom: VSN International.

Waiblinger, S., Menke, C., Korff, J., & Bucher, A. (2004). Previous handling and gentle interactions affect behaviour and heart rate of dairy cows during a veterinary procedure. *Applied Animal Behaviour Science, 85*(1-2), 31-42.

Zulkifli, I., Gilbert, J., Liew, P. K., & Ginsos, J. (2002). The effects of regular visual contact with human beings on fear, stress, antibody and growth responses in broiler chickens. *Applied Animal Behaviour Science, 79*(2), 103-112.

Chapter 3

Effects of Early Human Contact and Housing on Stress Resilience in Piglets during the Lactation Period and at Weaning

This chapter is presented as the author-accepted manuscript of a peer-reviewed article:

Hayes, M. E., Hemsworth, L. M., Morrison, R. S., Tilbrook, A. J., & Hemsworth, P. H. (2021). Positive human contact and housing systems impact the responses of piglets to various stressors. *Animals, 11*(6), 1619.

3.1 Abstract

This experiment studied the effects of lactation housing systems and human interaction on piglets' responses to routine stressors. Forty litters of piglets were reared in either a standard farrowing crate (FC) or a loose farrowing and lactation pen (LP; PigSAFE pen) and received either routine contact with humans (C) or regular opportunities for positive human contact (+HC; 3 min of patting, stroking and scratching 5 times/week). Behavioural and physiological responses to routine husbandry procedures, weaning, novelty and humans were studied in addition to effects on piglet growth, injuries and survival. Compared to C piglets, +HC piglets vocalised for shorter durations ($p = 0.018$) during husbandry procedures and showed a lower intensity of escape behaviour during iron injection ($p = 0.042$) and oral vaccination ($p = 0.026$) at 3 d of age, capture at 2 wk of age ($p < 0.001$), and intramuscular vaccination ($p = 0.005$) at 3 wk of age. +HC piglets at 2 wk of age were faster than C piglets to approach ($p = 0.048$) and interact ($p = 0.042$) with a stationary unfamiliar human. Compared to LP piglets, FC piglets showed a lower intensity of escape behaviour during capture and iron administration by a stockperson at 3 d of age ($p = 0.043$). FC piglets at 2 wk of age were faster than LP piglets to approach ($p = 0.005$) and interact ($p = 0.027$) with a novel object and approach ($p = 0.009$) and interact ($p = 0.008$) with an unfamiliar human. FC piglets had fewer injuries than LP piglets at 2 wk of age ($p = 0.004$). +HC pigs had fewer injuries than C pigs after weaning ($p = 0.003$). After weaning there were more pigs from LP than FC observed to be upright (both stationary, $p = 0.002$ and walking, $p = 0.024$), vocalizing ($p = 0.004$), nosing another pig ($p = 0.035$) and nosing the pen floor ($p = 0.038$). There were no significant effects on neutrophil:lymphocyte ratios or plasma cortisol concentrations 1.5 h after weaning. However, 25 h after weaning +HC pigs had higher haptoglobin concentrations than C pigs ($p = 0.002$), and C/LP pigs had higher cortisol concentrations than +HC/LP and C/FC pigs ($p = 0.012$). There were no significant effects on piglet growth, the number of piglets born alive or the number stillborn, however there were more piglets weaned from FC than LP ($p = 0.035$). The results from this experiment raise questions that require further research on the ability of pigs reared in loose pens to cope with stressors such as exposure to humans, novelty, husbandry procedures and weaning. This experiment also provides evidence that regular positive human interaction reduces pigs' fear of humans and husbandry procedures imposed by stockpeople. More research is required to determine if any of these effects are sustained long-term.

3.2 Introduction

Commercial pigs are exposed to several stressful situations as part of routine production, including painful husbandry procedures, close contact with stockpeople, exposure to novel environments, weaning and mixing with unfamiliar pigs. Stress resilience, which refers to the ability of an individual to cope with and recover from stress (Iacoviella & Charney, 2019), has obvious implications for the pig industry as an impaired ability of pigs to cope with these routine stressors is likely to negatively affect pig welfare and productivity based on studies in non-human primates and rodents (Lyons et al., 2009; Lyons et al., 2010; Parker & Maestripieri, 2011). Stress resilience can be shaped by several early life environmental inputs, and for pigs, the physical environment, maternal care from the sow and interactions with humans are likely candidates.

Farrowing crates remain the most widely used system for rearing pigs during the lactation phase, although increasing concern for animal welfare has led to the design of several alternative farrowing and lactation housing systems, which allow free movement of the sow. One example is the PigSAFE pen (Piglet and Sow Alternative Farrowing Environment), a loose farrowing and lactation system that was designed to optimise sow welfare and piglet survival while maintaining ease of management and commercial viability (Baxter et al., 2011). PigSAFE pens offer greater space and contain features such as sloped walls, varied flooring and separate sections for feeding, nursing and elimination, and as such are considered to be a more complex environment than the traditional farrowing crate (Martin et al., 2015). In addition, PigSAFE pens offer greater opportunity for sow–piglet interaction, which may have implications for the level of maternal care piglets receive. Sows in other loose lactation systems show improved maternal behaviour, as evident by increased responsiveness to piglet vocalisations and more frequent interactions with their piglets in comparison to sows from farrowing crates (Cronin et al., 1996; Singh et al., 2017; Thodberg et al., 2002b). In loose housing systems such as PigSAFE, the increased environmental complexity and the greater opportunity for maternal care may contribute to improved stress resilience in piglets.

While research on the PigSAFE system is sparse, there is evidence that pigs reared under different maternal and/or physical conditions vary in their responses to stressors routinely encountered in production. Pigs from farrowing crates showed greater cortisol responses after ear tagging (Yonezawa et al., 2012) and restraint (De Jonge et al., 1996) compared to pigs from outdoor pens, and greater cortisol responses after transport compared to pigs from larger crates and pens enriched with straw (Chaloupkova et al., 2007). The ratio of neutrophil to lymphocyte cells after husbandry procedures was higher in pigs from barren environments than pigs from environments enriched with newspaper, soil, balls and rope (Backus & McGlone, 2018). Play behaviour was performed more frequently (Martin et al., 2015; Singh et al., 2017) and occurred earlier in life (Martin et al., 2015) in piglets in loose pens, which suggests the farrowing crate environment negatively impacts the development of normal social skills. This can affect how pigs respond to social stressors, since after mixing with unfamiliar pigs at weaning, belly nosing and manipulative behaviours were performed more frequently by pigs from farrowing crates than loose pens (Oostindjer et al., 2011). Furthermore, pigs reared in loose pens spent more time exploring food after weaning (Oostindjer et al., 2011), and tended to show improved growth compared to piglets from farrowing crates (Oostindjer et al., 2010). Acute phase proteins such as haptoglobin have also been used to study stress in pigs (Piñeiro et al., 2007; Salamano et al., 2008) and may provide information on the magnitude of the stress response to weaning (Murata, 2007).

Many challenges faced by commercial pigs involve close contact or handling by stockpeople, and thus responses to these challenges are affected by the level of fear pigs have towards humans. Piglets that had experience with a handler who moved unpredictably and shouted during routine feeding and inspections demonstrated less resting, and more escape attempts and agonistic interactions after weaning, in comparison to piglets that had experience with a handler who moved carefully and used a soft tone of voice (Sommavilla et al., 2011). Talking softly and imposing gentle tactile contact such as stroking and scratching upon approach is perceived positively by pigs (Tallet et al., 2014) and reduces fear of humans (Hemsworth & Coleman, 2011; Tallet et al., 2014; Tanida et al., 1995). Patting and stroking during suckling bouts reduced the duration of escape behaviour in piglets during husbandry procedures conducted at 2 d of age and tended ($p < 0.1$) to reduce escape behaviour during capture at 15 d of age (Muns et al., 2015), and daily patting and scratching reduced the avoidance responses of sows to stockpeople imposing pregnancy testing and vaccination (Hayes et al., 2021). Pigs can remember positive interactions with humans for at least 5 wk (Brajon et al., 2015), and when these interactions take place early in life the effects may be sustained for up to 18 wk (Hemsworth & Barnett, 1992).

The effects of providing pigs with opportunities for positive human interaction extend beyond reducing fear of humans. Piglets that were stroked while being held showed reduced fear of tactile contact by familiar and unfamiliar people, but also performed more play behaviour and vocalised less in a novel arena, suggesting that the handling treatment reduced both fear of humans and novel situations (de Oliveira et al., 2015; Zupan et al., 2016). Positive handling has also been reported to reduce tail biting behaviour in weaner pigs, although the handling treatment in this experiment involved attracting piglets with chopped straw which is a potentially confounding factor (Büttner et al., 2018). Weight gain was higher in piglets habituated to human handling (de Oliveira et al., 2019) and human presence (de Oliveira et al., 2015). The presence of a familiar human after social isolation reduced the duration and frequency of piglets' vocalisations, which suggests that familiar people may even buffer stress when pigs are exposed to challenging situations (Villain et al., 2020).

Since maternal care, the physical environment and interactions with humans can impact stress resilience, the aim of this experiment was to study the effects of farrowing and lactation housing systems and positive human contact on piglets' responses to routine stressors. The hypothesis for this experiment was that piglets reared in loose farrowing and lactation pens (PigSAFE pens) with opportunities for positive human interaction show improved stress resilience compared to piglets reared in traditional farrowing crates with routine contact from stockpeople.

3.3 Materials and Methods

This experiment was conducted at a large commercial piggery in NSW, Australia. All animal procedures were conducted with prior institutional ethical approval under the requirements of the New South Wales Prevention of Cruelty to Animals Act 1985 in accordance with the National Health and Medical Research Council/Commonwealth Scientific and Industrial Research Organization/Australian Animal Commission Code of Practice for the Care and Use of Animals for Scientific Purposes (Rivalea Animal Ethics Committee #19B012C).

3.3.1 Animals and Treatments

Forty litters of piglets from primiparous sows (Landrace x Large White) were studied from birth until 2 d after weaning in a 2 x 2 factorial design, with the main effects as listed below and detailed in subsequent sections:

1. Housing system
    - I. FC—*Farrowing crate*
    - II. LP—*Loose pen*
2. Human contact treatment
    - I. +HC—*Positive human contact*
    - II. C—*Routine human contact*

3.3.1.1 Housing Systems during the Farrowing and Lactation Period

The layouts of both housing systems studied are depicted in Figure 3.1. The two housing systems were in one shed but in separate rooms adjacent to one another. The footprint of each farrowing crate was 2.3 × 1.7 m, with an internal space for the sow of 2.3 × 0.60 m, which allowed the sow to stand or lay down, but not turn around. Farrowing crates had a creep area that was heated by an overhead lamp and contained a 1.1 × 0.41 m solid floored mat. The rest of the floor was slatted steel (10 mm width between slats). The walls of each farrowing crate were solid and 0.51 m high, allowing sows and piglets to have visual contact with stockpeople in the aisles.

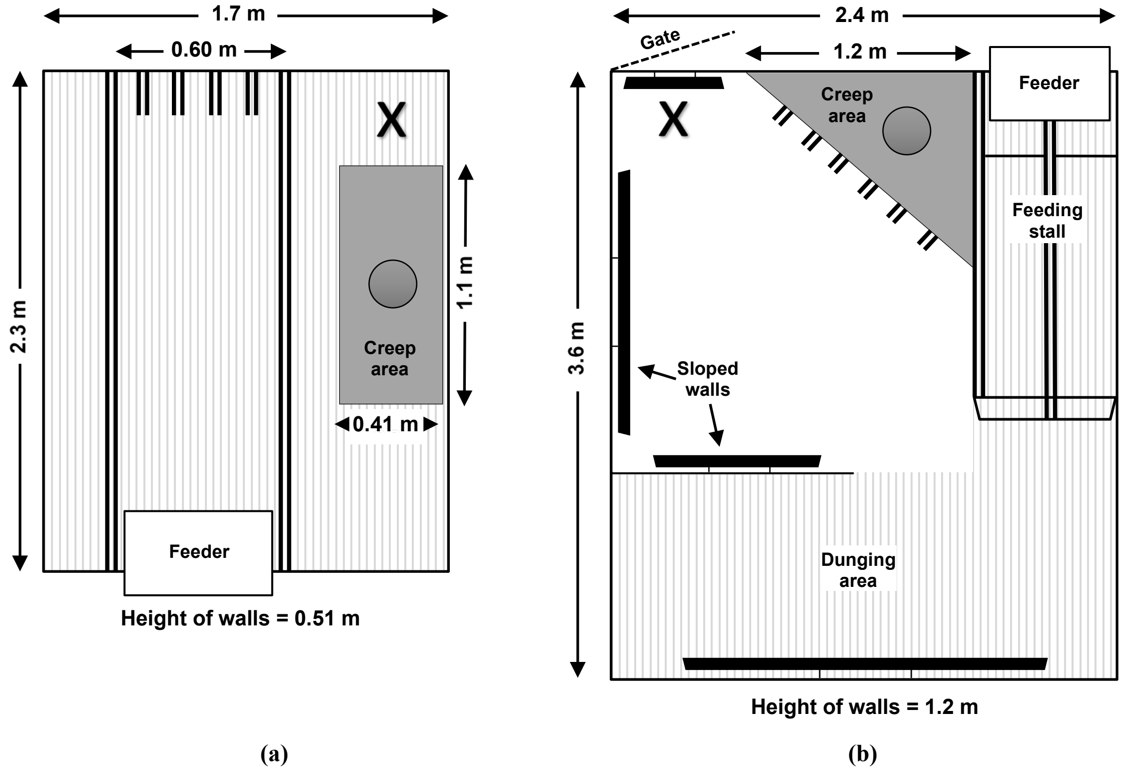


Figure 3.1. Layout and dimensions of the two housing systems: (a) farrowing crate (FC); (b) loose pen (LP). X denotes the position of the experimenter during the imposition of the positive human contact treatment.

The design of the loose pen system studied was PigSAFE (Piglet and Sow Alternative Farrowing Environment). Loose pens were 3.6 × 2.4 m and allowed free movement of the sow during farrowing and the entire lactation period. Loose pens contained a triangular creep area only accessible to piglets, which had an overhead lamp and a removable solid roof. The sows' feeder was located within a stalled area where the sow could be confined temporarily if stockpeople required access to the pen or piglets, although stockpeople very rarely used the feeding stall for this purpose. The flooring in the central area, including the creep, was solid plastic while the flooring in the back-dunging area and the feeder stall was slatted plastic (MIK Rubin flooring, 10 mm width between slats). The walls of the loose pens were solid and 1.2 m high, which restricted piglets' visual contact with humans in the room unless they were standing directly in front of the pen. The loose pens contained barred windows which allowed interaction between adjacent sows. Interaction between adjacent piglets was restricted early in life but was possible at 2–3 wk of age once piglets were large enough reach the windows. The walls of each pen were also sloped to reduce the incidence of piglets being crushed by the sow.

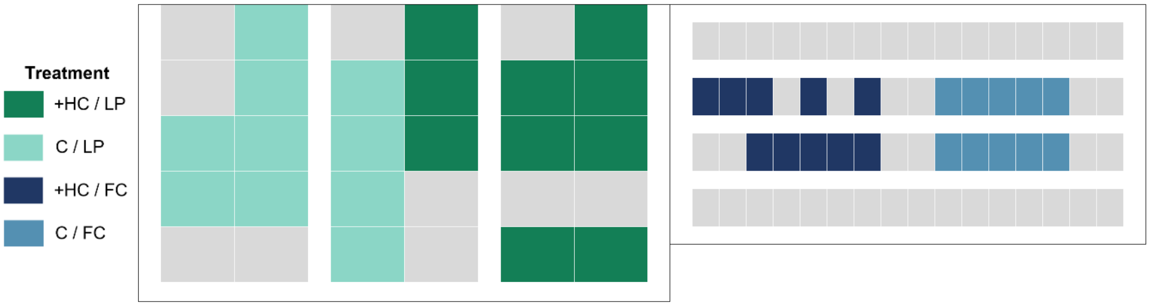
3.3.1.2 Human Contact Treatment during the Lactation Period

Piglets from the C treatment received only routine contact with stockpeople associated with regular husbandry and management. The +HC treatment also involved routine husbandry and management as in the C treatment, in addition to the opportunity to interact with a female experimenter five days per week from 1 d of age until weaning. The +HC treatment involved the experimenter gently patting, stroking and scratching piglets that approached her and piglets sleeping in the creep area for a duration of 3 min. Piglets were patted, stroked and scratched on the back, the abdomen and behind the ears. The +HC treatment was imposed at the litter level, in the morning after sows had been fed (approximately between 7:00 and 9:00 h) mainly by one female experimenter. However, on six days an additional female experimenter assisted in imposing the treatment on half of the +HC litters, as other experimental activities had to be completed so the total time imposing treatment needed to be reduced.

To impose the +HC treatment in farrowing crates, the experimenter slowly entered and crouched down at the back of the crate in front of the creep area (see location marked as "X" in Figure 3.1a). To impose the +HC treatment in loose pens, the experimenter stood outside the pen and secured the sow in the feeding stall before slowly entering the pen and crouching next to the creep area (see location marked as "X" in Figure 3.1b). To minimise distress to sows from being secured in the stalls, and consequently, to minimise disruption to the piglets during +HC imposition, all sows from loose pens were trained to voluntarily enter the feeding stalls. This training began 1 d after sows had been introduced to the pens and involved offering food rewards (5–10 small chocolates in the feeder) after the sow entered the stall, and securing the sow for increasing durations, starting with 30 s on the first day of training and building up to 10 min on the last day. In the event a sow from a +HC pen did not voluntarily enter the stall prior to treatment imposition, the experimenter quietly lifted the creep roof, squatted outside the pen and extended their hand inside the creep area to impose the positive handling treatment. The handling treatment was delivered this way in 20% of instances. Whenever any sow from a loose pen was offered food rewards, either during the training period or occasionally prior to +HC treatment delivery to lure the sow into the feeding stall, all other sows including those from farrowing crates were provided with the same quantity. During imposition of the positive handling treatment sows from C pens were always secured in the feeding stalls for the same duration as those from +HC pens, which was never longer than 10 min.

3.3.1.3 Human Contact Treatment Allocation

C and +HC litters were allocated to opposite ends of each room in order to minimise any carry-over effects of the positive handling treatment. In particular, it was important to minimise the amount of visual exposure to humans that C litters received through the imposition of the +HC treatment, as fear of humans is reduced in pigs that observe positive handling of other pigs (Luna et al., 2021). Thus, in the farrowing crate room where 2 parallel rows of crates were used, non-experimental litters (also from first parity sows) were allocated in between +HC and C litters (Figure 3.2b). In the loose pen room, which contained 30 pens in three blocks of back-to-back pens, 20 experimental litters were positioned in the three blocks as shown in Figure 3.2a with non-experimental litters in the other pens. In this room there was a reduced chance of carry-over effects between +HC and C litters as the loose pens had high walls that restricted piglets' visual contact outside of their own pen.



(a) **(b)**

Figure 3.2. Allocation of positive human contact (+HC) and routine human contact (C) litters: (a) loose pen (LP) room; (b) farrowing crate (FC) room. Grey boxes in both housing systems represent non-experimental litters.

3.3.2 Management of Animals

At least 5 d prior to expected parturition, sows were moved from group gestation pens into one of the two farrowing and lactation housing systems. As per standard practice at the piggery, sows in farrowing crates had a cotton rope suspended in front of the crate from 1 d after entry to the farrowing house until 1 d after parturition, while sows in loose pens were provided with a small amount (approximately two large handfuls) of sawdust in the central area of the pen 2 d prior to expected parturition. No other bedding or enrichment was provided to sows or piglets after farrowing. Sows and piglets from both housing systems had ad libitum access to water. While farrowing crates and loose pens were in separate but adjacent rooms, the animals were managed by the same group of male and female stockpeople. All animals received twice daily health and welfare checks by stockpeople. This involved a stockperson visually inspecting the animals and entering the pen when necessary, for example to assess sow teat function or remove a dead piglet. Sows were hand-fed by stockpeople twice per day, and piglets were hand-fed creep food once per day after 14 d of age. The farrowing spread was 7 days across all treatments and litters were not equalised for size or balanced for equal sex ratios. Cross fostering occurred on a minimal basis, always within the same housing system and within the first 24 h of life. The total number of piglets fostered was 6 in the FC treatment and 7 in the LP treatment. All piglets underwent routine husbandry and management as per standard commercial practice, including processing (intramuscular iron injection, administration of an oral coccidiosis treatment and tail clipping with a gas cautery iron) at 3 d of age, administration of an intramuscular vaccination (porcine circovirus-associated disease) at 3 wk of age and weaning at 22 d of age (SD = 2.4). Weaning age did not differ between treatment groups. At weaning all pigs from the same sex, housing system and human contact treatment were moved to the weaner facility together and then separated into groups of 10 pigs in 3.0 × 1.5 m pens with $^3/_4$ slatted steel flooring (10 mm width between slats). Pens were spread across two adjacent rooms with pigs from the same sex and treatment group generally housed in nearby pens. After weaning, $n_{\text{+HC/FC}} = 11$, $n_{\text{C/FC}} = 8$, $n_{\text{+HC/LP}} = 7$ and $n_{\text{C/LP}} = 9$ single sex pens. The pigs were not individually identifiable, however, prior to weaning all were marked with stock spray which enabled litter identification once pigs had been mixed post-weaning.

3.3.3 Measurements

3.3.3.1 Behavioural and Physiological Responses to Routine Husbandry Procedures

Processing occurred at 3 d of age and was carried out by two stockpeople. One stockperson entered the farrowing crate or loose pen and lifted a piglet, injected an iron supplement intramuscularly and passed the piglet to a second stockperson who administered an oral coccidiosis treatment and placed the piglet into a trolley. In loose pens, a solid stockboard was held to visually separate and protect the stockperson from the sow. Once all piglets from the litter were in the trolley, the first stockperson picked each piglet up and clipped the tail with gas heated cautery clippers before returning the piglet to the home pen. Direct observations were used to record the behavioural responses of all piglets to each of these procedures. The intensity of piglet escape behaviour was scored using the following ordinal scale adapted from Leidig and colleagues (2009): 0—no movement; 1—movement of one or two limbs; 2—movement of multiple limbs and the spine; and 3—movement of multiple limbs and the spine but with high intensity, repeatedly. The same two observers scored each litter, with the first observer responsible for scoring the intensity of escape behaviour in response to capture by the stockperson and iron injection (these events almost occurred simultaneously, so only one score was given), and tail clipping. The second observer was responsible for scoring escape behaviour during administration of the oral treatment. A third observer recorded piglets' vocalisations throughout processing by holding a microphone (Samson Meteor USB microphone) between the two stockpeople conducting processing, so that it was always less than 1 m away from piglets. The microphone was connected to a laptop running Raven Pro sound analysis software (Center for Conservation Bioacoustics, 2014), which was used to record the vocalisations and later determine the total number and duration of vocalisations for each litter. Blood samples for subsequent analysis of cortisol were collected 45 min after processing from 2 males and 2 females selected from each litter (see subsequent section "Sample Collection Details and Assay Characteristics" for further detail). Four piglets from each litter were selected for sampling on the basis of the first piglet alternately sighted in the front and back of the pens.

At 3 wk of age, all piglets received an intramuscular vaccination (porcine circovirus-associated disease) and their behavioural responses to capture and vaccination were scored by one observer using the previously mentioned scale. This observer was one of the people responsible for scoring the behavioural responses of piglets at processing.

3.3.3.2 Behavioural Responses to Novelty and Humans in a Standard Test

At 2 wk of age, the behavioural responses to novel and human stimuli were assessed in 2 male and 2 female piglets selected from each litter. Four piglets from each litter were selected for sampling on the basis of the first piglet alternately sighted in the front and back of the pens. The four piglets from a litter underwent testing together, which involved consecutive 1 min exposures to the following conditions: empty novel arena located adjacent to the home pen; novel object introduced to arena; human hand introduced to arena; unfamiliar human standing inside arena. The 1.8 m × 0.60 m × 0.60 m novel arena was constructed of black wooden board and was portable, allowing testing to take place 1 m away from each FC and LP. Painted lines were used to mark thirds on the floor and a Go-Pro camera was mounted above the arena. Piglets were individually lifted from the home pen and gently placed at one end of the arena by an experienced technician. Once all 4 piglets had been placed inside, they were left in the empty arena for 1 min. A female experimenter, unfamiliar to the piglets, then slowly approached and presented an orange traffic cone at the opposite end of the arena to where piglets had been initially placed. After 1 min, the experimenter approached, removed the traffic cone and squatted side-on outside the arena while extending their hand inside. After a further minute the experimenter stood up and stepped inside the arena, remaining stationary for the final minute of the test. Each piglet was then individually returned to the home pen. Direct observations by one observer were used to record the intensity of escape behaviour of piglets during capture from the home pen prior to testing and capture from the arena at the conclusion of the test, using the scale previously described (see previous section "Responses to Routine Husbandry Procedures"). Using video footage the number of entries each piglet made into different sections of the empty arena were recorded. Additionally, the following behavioural responses to the novel object, human hand and the standing human were recorded: latency to approach within 0.6 m, time spent within 0.6 m, latency to initiate tactile interaction (sniffing, nosing, chewing or stepping on stimulus), and the number of tactile interactions with the stimulus. A maximum response time of 1 min was given if a piglet did not approach or interact with the stimulus.

3.3.3.3 Behavioural and Physiological Responses to Weaning

At weaning, pigs were mixed into group pens of 10 animals of the same sex from the same housing system and human contact treatment. Direct observations were used to record the behaviour of pigs for 30 s at 15 min, 1 h, 2 h, 3 h, 4 h, 24 h, 25 h and 26 h after weaning. One observer stood 2 m away from each pen and recorded the number of pigs upright (either stationary or walking), nosing the pen floor (upwards and downwards movement of snout against the floor), nosing a pen mate (upwards and downwards movement of the snout against another pig's head or body) and vocalising, as well as the number engaging in aggression (knocking, biting or pushing a pen mate), tail biting (mouthing or chewing the tail area of another pig) and play (energetic running or hopping, pivoting on the spot or tossing the head) at each time point. Blood samples were obtained from 4 pigs selected from each pen 1.5 h after weaning for subsequent analysis of plasma cortisol and total neutrophil and lymphocyte cell counts, and 25 h after weaning for subsequent analysis of plasma cortisol and haptoglobin (see subsequent section "Sample Collection and Assay Characteristics" for further detail). Four pigs from each pen were selected for sampling on the basis of the first pig alternately sighted in the front and back of the pens.

3.3.3.4 Sow Reproductive Performance and Piglet Growth, Injuries and Survival

The litters were weighed at 3 d and 18 d of age. To assess piglet injuries, scratches and abrasions were scored at 2 wk of age on the 2 males and 2 females from each litter selected for behaviour testing and 2 d after weaning on 4 pigs selected from each pen. Each selected pig received one score for scratches and abrasions on the body and head using the following scale as first described by Widowski and colleagues (2003): 0—no scratches or skin loss were evident; 1—one to three small (2 cm) scratches or areas of abraded skin were evident; 2—one to three larger (>2 cm) scratches or areas of abraded skin were observed; and 3—more than three scratches (usually >2 cm) or larger areas of superficial skin loss. Sow reproductive performance and piglet survival were assessed through records of the total number of piglets born alive, stillborn and weaned.

3.3.3.5 Sample Collection Details and Assay Characteristics

Blood sampling was carried out by two teams of experienced technicians who collected samples from pigs in adjacent pens simultaneously. In each team, there was one person to pick up and hold the piglet inverted, one person to collect the sample and another to record the time and order of sampling within the pen. Piglets were not individually identifiable and were selected for sampling on the basis of the first pig alternately sighted in the front and back of the pens, although obvious runts were excluded. All samples were obtained within 2 min of picking up the piglet. Blood was collected via jugular venipuncture into 4 mL EDTA-coated tubes (BD Vacutainer, NSW, Australia). Tubes were inverted 8–10 times and placed immediately on ice after collection. Samples obtained after processing were collected between 11:00 and 14:00 h, and samples obtained after weaning were collected between 9:00 and 11:00 h. Samples for analysis of cortisol and haptoglobin were centrifuged for 10 min at 2000× *g* and plasma was transferred to polypropylene tubes and frozen at −20 °C until later analysis. Cortisol was quantified using a commercially available ELISA kit from Enzo Life Sciences (ADI-900-071), with a normal detectable range of 156–10,000 pg/mL. Samples were assayed at 1:16 dilution. Haptoglobin was quantified using a commercially available ELISA kit from Abcam (ab205091) with a normal detectable range of 6.25–400 ng/mL. Samples were assayed at 1:40,000 dilution. The intra- and inter-assay co-efficients of variation were less than 10% for the cortisol and haptoglobin assays. Samples collected for haematology were transported to a commercial laboratory where the absolute numbers of neutrophil and lymphocyte cells were counted using a Sysmex XT-2000i analyser (Sysmex, Kobe, Japan). The ratio of neutrophil to lymphocyte cells was then determined.

3.3.3.6 Statistical Analysis

All analyses were conducted in GenStat for Windows 18th Edition (VSN International, 2018). Unless otherwise stated, data were analysed using linear mixed models with the REML (Restricted Maximum Likelihood) directive, with human contact treatment, housing system and the interaction between the two as fixed effects. Sex was also included as a fixed effect for analysis of injury scores, behaviours observed after weaning, and physiological measurements collected after processing and weaning. For behavioural responses in the behaviour test, the sex of the piglets was not identifiable from video footage, however, 2 males and 2 females were tested from each litter and thus sex was balanced across treatments. For behavioural responses to husbandry procedures, the sex of the piglets was not known during the observation period. Litter and age were included as random effects in the model if they returned a variance component greater than 0. Age was excluded from the model for measurements obtained when all litters were of equal age, including responses to husbandry procedures and litter weights. For measures obtained post-weaning, post-weaning pen was included as an additional random effect. During processing piglet vocalisations were recorded continuously for each litter, and so the total number and duration of vocalisations was divided by the litter size to estimate the number and duration of vocalisations per piglet. Similarly, weights were obtained at 3 d and 18 d of age at the litter-level. The total weight of the litter was divided by the litter size on the day of weighing to estimate the weight of each piglet.

All data were checked for outliers, normality and homoscedasticity via visual inspection of residual plots. Logarithmic (base 10) transformations were applied to the number of vocalisations recorded during processing and to all physiological measurements collected after processing and weaning to correct the skewed distribution of the residuals. Using the GLMM procedure, generalised linear mixed models using a Poisson distribution with a logarithmic link function were used to analyse the number of tactile interactions piglets made with stimuli in the standard test. Generalised linear mixed models using a binomial distribution with a logit link function were used to analyse the number of pigs engaging in key behaviours after weaning. As behaviours after weaning were observed at several time points, time of the observation was added as an additional fixed effect. The behaviour of nosing the pen floor was only observed once on the first day of observations, and thus only data from observations at 24 h, 25 h and 26 h after weaning were included in the analysis of this behaviour. For all behaviours observed after weaning, odds ratios were calculated by exponentiating the difference between treatment group means. The odds ratio represents the odds of a pig from one treatment group partaking in the behaviour over a pig from another treatment group, thus when the odds ratio is 1 the chance of pigs from different treatments partaking in the behaviour measured is equal. Where transformations were applied or where generalised linear mixed models were fitted, back transformed means are presented.

The effects of human contact treatment on the number of piglets born alive and the number of piglets stillborn were not tested as the positive handling treatment only began at 1 d of age (the day after farrowing), and thus only effects of housing system were tested on these measurements. Even after transformations were applied, the residuals were markedly skewed for the number of piglets stillborn and so a non-parametric Mann–Whitney U (Wilcoxon rank-sum) test was used to test for effects of housing system on the number of piglets stillborn.

3.4 Results

3.4.1 Responses to Husbandry Procedures at 3 d and 3 wk of Age

3.4.1.1 Behavioural Responses to Processing and Vaccination

During processing at 3 d of age, +HC piglets showed a lower intensity of escape behaviour to capture and iron injection compared to C piglets (mean score; 1.32 vs. 1.70; $F_{1,36} = 4.43$; $p = 0.042$) and to administration of an oral treatment (1.35 vs. 1.68; $F_{1,36} = 5.41$; $p = 0.026$; Table 3.1). The intensity of escape behaviour to capture and iron injection at processing was also lower in FC piglets compared to LP piglets (1.32 vs. 1.70; $F_{1,36} = 4.41$; $p = 0.043$). There were no significant ($p > 0.05$) human contact treatment or housing system effects on the intensity of escape behaviour during tail clipping. There was a significant effect of human contact treatment on the duration of vocalisations at processing, with C piglets vocalising for longer in comparison to +HC piglets (6.30 vs. 4.47 s; $F_{1,36} = 6.32$; $p = 0.018$). There was also a tendency for C piglets to vocalise more often than +HC piglets (9.53 vs. 7.20; $F_{1,36} = 3.62$; $p = 0.067$). There were no significant ($p > 0.05$) housing system effects on vocalisations during processing, and no significant housing system × human contact interactions on any measurements at processing. During administration of a vaccination at 3 wk of age, +HC piglets showed a lower intensity of escape behaviour in comparison to C piglets (mean score; 1.49 vs. 1.85; $F_{1,36} = 8.96$; $p = 0.005$; Table 3.1). However, there was no significant effect ($p > 0.05$) of housing system as seen during processing earlier in life. There was no significant ($p > 0.05$) housing system × human contact interaction effect on behavioural responses to vaccination at 3 wk of age.

3.4.1.2 Physiological Responses to Processing

There were no significant ($p > 0.05$) effects of human contact treatment, housing system or the interaction between the two on plasma cortisol concentrations 45 min after processing at 3 d of age (Table 3.1).

Table 3.1. Effects of human contact (+HC, positive human contact; C, routine human contact) and lactation housing system (FC, farrowing crate; LP, loose pen) on piglet responses to routine husbandry procedures at 3 d and 3 wk of age. Data represent means (95% confidence intervals). The number of vocalisations during processing and plasma cortisol concentrations 45 min after processing were logarithmically transformed prior to analysis; back-transformed data are presented.

Measurement	**Human Contact**		**Housing System**		***p*-Value**		
	+HC	**C**	**FC**	**LP**	**Human Contact**	**Housing System**	**Contact × Housing**
Processing at 3 d of age							
Escape behaviour score at capture and iron injection	1.32 (1.06, 1.57)	1.70 (1.44, 1.95)	1.32 (1.06, 1.57)	1.70 (1.44, 1.95)	**0.042**	**0.043**	0.691
Escape behaviour score at oral treatment administration	1.35 (1.16, 1.55)	1.68 (1.49, 1.88)	1.45 (1.25, 1.64)	1.59 (1.39, 1.79)	**0.026**	0.337	0.251
Escape behaviour score at tail clipping	1.61 (1.38, 1.84)	1.61 (1.37, 1.84)	1.69 (1.46, 1.91)	1.53 (1.29, 1.76)	0.970	0.340	0.126
Duration of vocalisations (s)	4.47 (3.41, 5.52)	6.30 (5.31, 7.29)	5.68 (4.62, 6.74)	5.08 (4.09, 6.07)	**0.018**	0.407	0.693
Number of vocalisations	7.20 (5.87, 8.84)	9.53 (7.88, 11.5)	7.94 (6.47, 9.75)	8.70 (7.19, 10.5)	0.067	0.577	0.618
Cortisol (ng/mL)	48.8 (40.1, 59.2)	51.2 (42.3, 61.8)	45.6 (37.7, 55.1)	54.6 (44.9, 66.3)	0.711	0.214	0.368
Vaccination at 3 wk of age							
Escape behaviour score at capture and vaccination	1.49 (1.32, 1.65)	1.86 (1.69, 2.03)	1.77 (1.60, 1.93)	1.57 (1.39, 1.74)	**0.005**	0.114	0.107

3.4.2 Responses to Novelty and Humans in a Standard Test at 2 wk of Age

3.4.2.1 Behavioural Responses to Capture before and after Testing

In comparison to C piglets, piglets from +HC litters showed a lower intensity of escape behaviour to capture from the home pen for testing (means score; 1.21 vs. 2.14; $F_{1,36} = 15.2$; $p < 0.001$) and to capture from the arena after testing had been conducted (1.48 vs. 2.13; $F_{1,36} = 16.56$; $p < 0.001$; Table 3.2). There were no significant ($p > 0.05$) housing system effects or housing system × human contact interaction effects on piglet responses to capture before or after the test.

3.4.2.2 Behavioural Responses to Novel and Human Stimuli during the Test

There were no significant ($p > 0.05$) effects of human contact or housing system on the number of entries piglets made into different sections of the empty arena during the first minute of the test, although there was a tendency for FC piglets to enter more sections than LP piglets (5.30 vs. 4.16; $F_{1,36} = 3.44$; $p = 0.072$; Table 3.2). In comparison to LP piglets, FC piglets were faster to approach within 0.6 m of the traffic cone (15.5 vs. 30.8 s; $F_{1,36} = 8.90$; $p = 0.005$) and the human hand (20.9 vs. 34.9 s; $F_{1,36} = 7.69$; $p = 0.009$). Piglets from FC were also faster to physically interact with the traffic cone (22.8 vs. 34.5 s; $F_{1,36} = 5.31$; $p = 0.027$) and the human hand (31.0 vs. 42.9 s; $F_{1,36} = 7.97$; $p = 0.008$). There was also a tendency for FC piglets to be faster than LP piglets in approaching (24.1 vs. 34.8 s; $F_{1,36} = 3.86$; $p = 0.058$) and interacting (31.0 vs. 40.6 s; $F_{1,36} = 3.30$; $p = 0.078$) with the standing human. In comparison to C piglets, +HC piglets were faster to approach within 0.6 m of (24.0 vs. 34.9 s; $F_{1,36} = 4.21$; $p = 0.048$), and physically interact with (30.4 vs. 41.2 s; $F_{1,36} = 4.49$; $p = 0.042$), the standing human. However, there were no significant ($p > 0.05$) effects of human contact on responses to the traffic cone or human hand. There were also no significant ($p > 0.05$) effects of human contact or housing system on the time spent near or the number of tactile interactions with any stimuli, and no significant ($p > 0.05$) housing system × human contact interactions on any variables measured in the test.

Table 3.2. Effects of human contact (+HC, positive human contact; C, routine human contact) and lactation housing system (FC, farrowing crate; LP, loose pen) on the behavioural responses of piglets to novelty and humans at 2 wk of age. Data represent means (95% confidence intervals).

Measurement	**Human Contact**		**Housing System**		***p*-Value**		
	+HC	**C**	**FC**	**LP**	**Human Contact**	**Housing System**	**Contact × Housing**
Response to capture							
Escape behaviour score at capture from home pen pre-test	1.21 (0.881, 1.54)	2.14 (1.81, 2.47)	1.50 (1.17, 1.83)	1.85 (1.52, 2.18)	**<0.001**	0.148	0.911
Escape behaviour score at capture from arena post-test	1.48 (1.28, 1.68)	2.13 (1.93, 2.32)	1.91 (1.71, 2.11)	1.69 (1.49, 1.89)	**<0.001**	0.290	0.172
Response to empty arena							
Number of sections entered	4.53 (3.66, 5.39)	4.94 (4.08, 5.80)	5.30 (4.44, 6.16)	4.16 (3.30, 5.03)	0.517	0.072	0.763
Response to traffic cone							
Latency to approach 0.6 m (s)	20.5 (13.2, 27.8)	25.8 (18.7, 32.9)	15.5 (8.17, 22.8)	30.8 (23.8, 38.0)	0.291	**0.005**	0.107
Latency to interact (s)	27.3 (20.1, 34.6)	30.0 (23.0. 37.1)	22.8 (15.6, 30.1)	34.5 (27.5, 41.6)	0.559	**0.027**	0.105
Number of interactions	2.76 (2.02, 3.78)	2.67 (1.95, 3.66)	3.14 (2.29, 4.30)	2.35 (1.71, 3.22)	0.970	0.202	0.206
Time spent within 0.6 m (s)	20.6 (14.5, 26.7)	20.7 (14.7, 26.7)	22.2 (16.1, 28.3)	19.1 (13.1, 25.1)	0.982	0.448	0.218
Response to human hand							
Latency to approach 0.6 m (s)	26.8 (19.7, 33.9)	29.0 (22.1, 35.9)	20.9 (13.9, 28.0)	34.9 (28.0, 41.8)	0.643	**0.009**	0.534
Latency to interact (s)	34.5 (27.6, 41.2)	39.5 (32.7, 46.2)	31.0 (24.2, 37.8)	42.9 (36.0, 49.8)	0.231	**0.008**	0.799
Number of interactions	1.34 (0.813, 2.21)	1.23 (0.747, 2.03)	1.59 (0.964, 2.64)	1.04 (0.627, 1.71)	0.769	0.132	0.169
Time spent within 0.6 m (s)	14.2 (9.10, 19.4)	13.5 (8.55, 18.5)	15.8 (10.6, 20.9)	12.0 (7.02, 17.0)	0.863	0.326	0.445
Response to standing human							
Latency to approach 0.6 m (s)	24.0 (16.6, 31.4)	34.9 (27.7. 42.0)	24.1 (16.7, 31.4)	34.8 (27.6, 42.0)	**0.048**	0.058	0.420
Latency to interact (s)	30.4 (22.1, 38.7)	41.2 (32.9, 49.4)	31.0 (22.7, 39.3)	40.6 (32.2, 49.0)	**0.042**	0.078	0.546
Number of interactions	2.50 (1.39, 4.49)	1.79 (0.995, 3.21)	2.38 (1.32, 4.30)	1.87 (1.04, 3.38)	0.322	0.444	0.501
Time spent within 0.6 m (s)	20.7 (13.2, 28.1)	16.1 (8.68, 23.6)	20.4 (12.9, 27.8)	16.4 (8.85, 24.0)	0.355	0.423	0.393

3.4.3 Responses to Weaning

3.4.3.1 Piglet Behaviour after Weaning

There were significant effects of housing system on several behaviours recorded from 15 min to 26 h after weaning. Higher proportions of pigs from LP than FC were observed to be upright and stationary (78.3 vs. 59.1%; $F_{1,31}$ = 11.53; p = 0.002), upright and walking (40.3 vs. 32.0%; $F_{1,29.7}$ = 5.63; p = 0.024), vocalising (2.93 vs. 1.93 %; $F_{1,28.7}$ = 9.83; p = 0.004) and nosing a pen mate (22.2 vs. 17.8%; $F_{1,27.7}$ = 4.93; p = 0.035; Table 3.3). There were also more pigs from LP nosing the pen floor at 24 h, 25 h and 26 h after weaning (13.2 vs. 6.75%; $F_{1,30}$ = 6.32; p = 0.038). Housing system had no significant ($p > 0.05$) effects on the number of pigs engaging in play or aggressive behaviour. No tail biting behaviour was observed. There were no significant ($p > 0.05$) human contact effects or housing system × human contact interaction effects on pig behaviour after weaning.

3.4.3.2 Physiological Responses after Weaning

There were no significant ($p > 0.05$) effects of human contact or housing system on plasma cortisol concentrations or on the ratio of neutrophil to lymphocyte cells 1.5 h after pigs were weaned (Table 3.4). However, 25 h post-weaning, there was a significant human contact × housing system interaction effect on plasma cortisol concentrations ($F_{1,28.2}$ = 7.38; p = 0.012). Plasma cortisol concentrations were higher in C/LP pigs (44.3 ng/mL (33.9, 57.8)) compared to +HC/LP pigs (28.2 ng/mL (21.1, 37.6)) and C/FC pigs (28.6 ng/mL (21.7, 37.7)), but not +HC/FC pigs (37.8 ng/mL (29.7, 48.3)). Pigs from +HC litters also had higher plasma haptoglobin concentrations than C pigs 25 h after weaning (1130 vs. 697 µg/mL; $F_{1,28.3}$ = 11.10; p = 0.002).

Table 3.3. Effects of human contact (+HC, positive human contact; C, routine human contact) and lactation housing system (FC, farrowing crate; LP, loose pen) on pig behaviours post-weaning. Data represent the mean percent of pigs engaging in each behaviour at 15 min, 1 h, 2 h, 3 h, 4 h, 24 h, 25 h and 26 h post-weaning. The odds ratio (95% confidence interval) represents the likelihood of a +HC pig partaking in the behaviour over a C pig, or alternatively the likelihood of an LP pig partaking in the behaviour over an FC pig. When the odds ratio is 1, the chance of pigs from different treatments partaking in the behaviour is equal.

Measurement	**Human Contact**			**Housing System**			***p*-Value**		
	+HC	**C**	**Odds Ratio**	**FC**	**LP**	**Odds Ratio**	**Human Contact**	**Housing System**	**Contact × Housing**
Upright, stationary	71.9	67.1	1.25 (0.74, 2.12)	59.1	78.3	2.49 (1.47, 4.22)	0.639	**0.002**	0.365
Upright, walking	34.8	37.3	0.90 (0.67, 1.21)	32.0	40.3	1.43 (1.06, 1.93)	0.300	**0.024**	0.290
Vocalising	2.45	2.31	1.06 (0.81, 1.39)	1.93	2.93	1.53 (1.17, 2.00)	0.947	**0.004**	0.389
Nosing pen mate	19.4	20.4	1.06 (0.83, 1.36)	17.8	22.2	1.32 (1.03, 1.69)	0.461	**0.035**	0.420
Nosing pen floor [1]	8.55	11.4	0.83 (0.43, 1.61)	6.75	13.2	2.03 (1.04, 3.96)	0.580	**0.038**	0.145
Play behaviour	0.621	1.04	0.59 (0.34, 1.04)	0.885	0.729	0.82 (0.47, 1.44)	0.124	0.649	0.299
Aggressive behaviour	1.52	1.24	1.24 (0.54, 2.82)	1.28	1.47	1.15 (0.50, 2.63)	0.684	0.666	0.115
Tail biting	0	0	-	0	0	-	-	-	-

[1] Nosing the pen floor was rarely observed; only data from 24 h, 25 h and 26 h after weaning were included in the analysis.

Table 3.4. Effects of human contact (+HC, positive human contact; C, routine human contact) and lactation housing system (FC, farrowing crate; LP, loose pen) on the physiology of pigs after weaning. Data represent means (95% confidence intervals). All measurements were logarithmically transformed prior to analysis; back transformed data are presented.

Measurement	**Human Contact**		**Housing System**		***p*-Value**		
	+HC	**C**	**FC**	**LP**	**Human Contact**	**Housing System**	**Contact × Housing**
1.5 h post-weaning							
Cortisol (ng/mL)	32.1 (27.0, 38.1)	31.3 (26.8, 36.7)	30.1 (25.7, 35.2)	33.3 (28.1, 39.6)	0.946	0.393	0.988
Neutrophil to lymphocyte ratio	1.15 (0.966, 1.37)	1.12 (0.937, 1.34)	1.11 (0.934, 1.32)	1.16 (0.968, 1.40)	0.803	0.563	0.388
25 h post-weaning							
Cortisol (ng/mL)	33.7 (27.3, 41.4)	36.2 (29.4, 44.6)	34.0 (27.7, 41.6)	35.9 (28.9, 44.6)	0.652	0.597	**0.012**
Haptoglobin (µg/mL)	1130 (937, 1370)	697 (574, 846)	817 (679, 983)	966 (769, 1173)	**0.002**	0.212	0.139

3.4.4 Sow Reproductive Performance and Piglet Growth, Injuries and Survival

3.4.4.1 Injury Scores

LP piglets had higher injury scores than FC piglets at 2 wk of age during the lactation period (mean score; 1.30 vs. 0.850; $F_{1,36} = 9.26$; $p = 0.004$; Table 3.5), but there was no significant ($p > 0.05$) effect of housing system on injury scores obtained 2 d after weaning and mixing with unfamiliar pigs. Human contact had no significant ($p > 0.05$) effect on injuries at 2 wk of age during the lactation phase, however, after weaning and mixing with unfamiliar pigs +HC pigs had fewer injuries than C pigs (0.944 vs. 1.59; $F_{1,30} = 10.68$; $p = 0.003$). There were no significant ($p > 0.05$) housing system × human contact interaction effects on injury scores.

3.4.4.2 Piglet Weights

There were no significant ($p > 0.05$) effects of human contact, housing system or the interaction between the two on piglet weights at 3 d or 18 d of age (Table 3.5).

3.4.4.3 Piglet Survival during Lactation

There were no significant ($p > 0.05$) effects of housing system on the number of piglets born alive or the number of piglets stillborn (Table 3.5). The total number of piglets weaned was higher in FC than LP (9.50 vs. 8.25; $F_{1,36} = 4.79$; $p = 0.035$). There was no significant ($p > 0.05$) human contact effect or human contact × housing system interaction effect on the number of piglets weaned.

Table 3.5. Effects of human contact (+HC, positive human contact; C, routine human contact) and lactation housing system (FC, farrowing crate; LP, loose pen) on piglet injury scores, weights and survival. Data represent means (95% confidence intervals).

Measurement	Human Contact		Housing System		*p*-Value		
	+HC	C	FC	LP	Human Contact	Housing System	Contact × Housing
Injuries							
2 wk of age	1.03 (0.819, 1.23)	1.13 (0.919, 1.33)	0.850 (0.644, 1.06)	1.30 (1.09, 1.51)	0.503	**0.004**	0.185
2 d post-weaning	0.944 (0.675, 1.21)	1.59 (1.32, 1.85)	1.31 (1.05, 1.57)	1.22 (0.939, 1.50)	**0.003**	0.641	0.706
Weights							
3 d of age (kg)	1.55 (1.41, 1.69)	1.69 (1.55, 1.83)	1.60 (1.46, 1.74)	1.64 (1.50, 1.78)	0.162	0.677	0.895
18 d of age (kg)	4.43 (4.07, 4.78)	4.44 (4.08, 4.80)	4.45 (4.09, 4.81)	4.42 (4.06, 4.77)	0.953	0.896	0.827
Piglet survival							
Number born alive [1]	-	-	11.8 (10.8, 12.8)	10.9 (9.88, 11.9)	-	0.226	-
Number stillborn [1]	-	-	0.750 (0.260, 1.24)	1.05 (0.376, 1.72)	-	0.536	-
Number weaned [2]	8.85 (8.06, 9.64)	8.90 (8.11, 9.69)	9.50 (8.71, 10.3)	8.25 (7.46, 9.04)	0.931	**0.035**	0.146

[1] Only effects of housing system were tested as the positive handling treatment began at 1 d of age. [2] Includes fostered piglets.

3.5 Discussion

This experiment studied the effects of farrowing and lactation housing systems and positive human interaction on stress resilience in piglets. Contrary to the expected findings, piglets reared in loose farrowing and lactation pens were slower to approach and interact with novel and human stimuli and were more reactive during capture by a stockperson early in life compared to piglets from farrowing crates. Piglets from loose pens also had higher injury scores during the lactation period and were more frequently observed to be active, vocalising, nosing the pen floor and nosing another pig after weaning. In both housing systems, providing regular opportunities for positive human interaction reduced piglets' vocalisations and escape behaviour during husbandry procedures, and reduced the latency of piglets to approach and interact with a standing unfamiliar human.

Compared to piglets from loose pens, piglets from farrowing crates showed a lower intensity of escape behaviour during capture and iron administration by a stockperson at 3 d of age. While there were no effects of housing system on piglets' behavioural responses to other husbandry procedures or to capture during behaviour testing, piglets from farrowing crates were faster to approach and initiate physical interaction with a novel object and an unfamiliar person at 2 wk of age, suggesting reduced fear of novelty and humans in piglets from farrowing crates compared to loose pens. Piglets from loose pens had increased opportunity for interaction with the sow, greater space and more complexity in their physical environment through pen features such as varied flooring and sloped walls. However, piglets from farrowing crates had substantially more visual, auditory and olfactory contact with stockpeople and adjacent pigs for several reasons. Firstly, the high walls of the loose pens restricted piglets contact outside the pen in general, to the extent that people were only visible to piglets when standing directly in front of the pen. Contact with neighbouring litters was also restricted until 2–3 wk of age when piglets were large enough to reach the pen windows, whereas piglets from farrowing crates were observed interacting over the wall with piglets from adjacent litters earlier in life. Secondly, there was more human traffic in the farrowing crate room as stockpeople required use of the aisles to access neighbouring sheds. Lastly, there were twice as many litters housed in the farrowing crate room, which increased the time stockpeople spent in this area conducting routine inspections and feeding. The design of the farrowing crate and PigSAFE Pen are likely to restrict contact between piglets and sows and contact between piglets and stockpeople conducting routine management, respectively. Further research therefore is required to test whether these restrictions contribute to fear responses of piglets to novelty and humans.

Piglets from farrowing crates may have been less fearful of humans due to increased opportunity for habituation to human presence. However, habituation is a stimulus-specific process (Hinde, 1970), and responses to the novel object suggest piglets from farrowing crates were less fearful in general. Behaviour and physiological stress responses are affected by previous experiences coping with stress (Moberg, 2000). While exposure to severe stress early in life is damaging, repeated exposure to mild stress can assist animals in producing adaptive coping strategies for future stressful situations (Lyons et al., 2009; Lyons et al., 2010; Parker & Maestripieri, 2011). It may be that piglets in farrowing crates were provided with more opportunities to overcome mild stress through frequent and close visual exposure to stockpeople and their equipment and increased interaction with non-littermates. In contrast, piglets from loose pens were raised in a more isolated environment which may have contributed to increased fear of novelty and humans. Furthermore, sows in farrowing crates show fewer interactions with their piglets and reduced responsiveness to piglet vocalisations compared to sows in loose systems (Cronin et al., 1996; Singh et al., 2017; Thodberg et al., 2002b), and these reduced maternal responses may have resulted in the farrowing crate piglets having to be more reliant on learning to deal independently with stress. Whether these results are specific to the PigSAFE pens studied here or reflect piglet behaviour in other loose systems remains unknown. For example, differences in pen layout, room design and management may facilitate more contact with stockpeople and adjacent litters than in the PigSAFE pens in this experiment. While the effects of farrowing and lactation housing systems on piglets' fear responses have not been widely examined, research by Chaloupkova and colleagues (2007) reported similar findings to the present experiment where piglets from farrowing crates were more likely to make physical contact with an unfamiliar human compared to piglets from loose pens enriched with straw. Clearly further research on PigSAFE pens, as well as examination of other loose systems, is required to identify whether they similarly affect the fear responses of piglets to novelty and humans.

During processing at 3 d of age, piglets from the positive handling treatment vocalised for shorter durations and showed a lower intensity of escape behaviour during capture, iron injection and administration of an oral vaccination treatment. Positive human contact had no effect on the behavioural responses of piglets to tail clipping, although tail clipping was the last procedure to take place at processing and likely the most stressful. In a study by Muns and colleagues (2015), piglets were gently touched on the head and snout during at least six suckling bouts on the first day of life. Each litter received at least 36 min of this treatment prior to processing, and Muns and colleagues reported that the treatment reduced escape behaviour in piglets during tail docking. In the present experiment, positive human contact litters had been exposed to the handling treatment for 9 min total prior to processing which may not have been enough to affect responses to painful events such as tail clipping. Nevertheless, both studies demonstrate that relatively small amounts of positive human interaction can improve the stress resilience of piglets to husbandry procedures imposed by stockpeople.

There were no human contact treatment effects on the cortisol response of piglets to processing at 3 d of age. However, effects on behavioural responses at processing were maintained, with positive human contact piglets showing less escape behaviour than routine contact piglets at 2 wk of age during capture for behaviour testing, and at 3 wk of age during capture and intramuscular vaccination. Furthermore, positive human contact piglets at 2 wk of age were faster to approach and physically interact with an unfamiliar human standing stationary. In line with several other studies, these results show that brief bouts of patting and stroking reduce fear of humans in pigs (Hemsworth & Barnett, 1992; Hemsworth et al., 1986a; Tallet et al., 2014; Tanida et al., 1995). While positive human contact reduced piglets' fear of the standing human, there were no effects on piglets' behavioural responses to the same human extending their hand inside the test arena, possibly because the standing human stimulus more closely resembled the imposition of the positive human contact treatment. Additionally, piglets may not have associated the presentation of a hand inside the arena with a human. Aligning with these results, Muns and colleagues (2015) found that positive human contact tended to reduce escape behaviour during capture at 15 d of age but had no effect on whether piglets interacted with a human hand. In the present experiment there were also no effects of human contact on responses to the novel arena or novel object, suggesting that the positive handling treatment reduced fear of humans but did not reduce general fearfulness. Earlier research on pigs (Hemsworth et al., 1986b) and poultry (Jones & Waddington, 1992) showed similar effects.

Previous positive or negative interactions with humans have been shown to affect how pigs cope with weaning (Sommavilla et al., 2011). Pigs from routine human contact pens had more injuries after weaning which may indicate increased aggression or escape attempts from the pen, however, there were no effects of the positive handling treatment on pig behaviours post-weaning. There were however several effects of the farrowing and lactation housing system, with pigs from loose pens more frequently observed to be active, vocalising, nosing a pen mate and nosing the pen floor. This may have been due to pigs from loose pens experiencing a change in flooring after weaning. Pigs from farrowing crates were familiar with the slatted steel flooring in the post-weaning pens as it resembled the flooring in the farrowing crate system, while pigs from loose pens had only been exposed to plastic flooring prior to weaning. It is also possible that the reduced contact with stockpeople and non-littermates in the loose pen environment contributed to impaired stress resilience at weaning. Oostindger and colleagues (2011) suggested that through increased opportunity for interaction with the sow, loose systems assist piglets in adapting to the post-weaning environment. The authors found that piglets from loose pens performed less belly nosing and manipulative behaviour and more play behaviour and food exploration after weaning compared to piglets from farrowing crates. These behaviours were sampled for 12 d post-weaning, whereas we only observed pigs until 26 h after weaning, which is a limitation of our study.

The physiological stress response to weaning in pigs can involve elevations of cortisol (plasma: 2 h post-weaning (Turpin et al., 2017; van der Meulen et al., 2010); serum: 1 d post-weaning (Moeser et al., 2007)) and an increase in the ratio of neutrophil to lymphocyte cells (2 h post-weaning: (Turpin et al., 2017), 1 d after weaning: (Puppe et al., 1997) and 3 d post-weaning: (Turpin et al., 2016)). In this experiment, there were no effects of housing system or human contact treatment on plasma cortisol concentrations or the ratio of neutrophil to lymphocyte cells 1.5 h post-weaning. However, at 25 h post-weaning, pigs reared in loose pens with routine human contact had higher plasma cortisol concentrations than pigs from +HC/LP and C/FC groups. One interpretation of this increased cortisol response at weaning in pigs from the loose pens is less stress resilience, although this effect was only found in pigs from loose pens with routine human contact. The cortisol concentrations of pigs from farrowing crates with positive human contact were intermediate and did not differ from the other treatment combinations.

In addition to the effects on plasma cortisol post-weaning, plasma haptoglobin concentrations 25 h after weaning were higher in pigs from the positive human contact treatment than from the routine handling treatment. Haptoglobin is one of several acute phase proteins (APP) released in response to infection, inflammation and stress (Murata et al., 2004), and has been shown to increase in pigs after weaning (De et al., 2017; Pomorska-Mol et al., 2012; Sauerwein et al., 2005). While the process behind the APP response to stress is not completely known, it is suggested that activation of the hypothalamic–adrenal axis and the release of glucocorticoids contributes to the synthesis of APP (Murata, 2007). It was therefore surprising to find elevated cortisol concentrations in C/LP pigs but elevated haptoglobin concentrations in +HC pigs after weaning, although correlations between cortisol and haptoglobin are not always strong (Ott et al., 2014). Haptoglobin also increases as part of the APP response to inflammation, and thus concentrations may increase due to injuries obtained from fighting with pen mates. However, positive human contact pigs had fewer injuries than routine human contact pigs 2 d after weaning, which suggests the elevated haptoglobin levels of positive human contact pigs were not a result of an inflammatory response. The study of the APP response to stress is somewhat new in pig welfare assessment and as such the effects of positive human contact on haptoglobin are not completely understood. More frequent sampling, as well as baseline measurements obtained prior to weaning, would provide further insight into the effects of housing systems and human contact on the physiological stress response of pigs to weaning.

There were no effects of housing system on the number of injuries pigs had 2 d after weaning. Results from previous studies assessing injuries at similar timepoints after weaning are varied, with pigs from farrowing crates reported to have more (4 d post-weaning: (Kutzer et al., 2009)), less (3 d post-weaning: (Martin et al., 2015)) or similar (6 h post-weaning: (Chaloupková et al., 2007)) injuries compared to pigs from loose pens. While injuries 2 d after weaning were similar between the housing systems in the present experiment, there were more injuries in loose pens than farrowing crates at 2 wk of age during the lactation period. Injuries are commonly sustained from fighting between pen mates (Turner et al., 2009), although they do not always correlate with aggressive behaviour (Widowski et al., 2003) and may also be caused by collisions with the sow or pen fittings; both of which are more likely to occur in loose pens due to free movement of the sow and increased structural complexity of the pen. There were no effects of housing system or human contact treatment on piglet weights, the number of piglets born alive or the number stillborn, however, there were more piglets weaned from farrowing crates than loose pens. Previous experiments at the research site have shown that the number of pigs weaned is comparable between PigSAFE pens and farrowing crates (Morrison et al., 2015; Morrison & Baxter, 2013). However, sows in these experiments were of older parity and at least 2 kg of straw was provided in PigSAFE pens prior to farrowing. Piglet survival is likely to be higher in litters from more experienced sows and with the provision of bedding.

Only litters from primiparous sows were studied in the present experiment since there is evidence that piglet mortality is affected by changing farrowing system across parities (King et al., 2019). Sows are generally more stressed at their first parturition than at subsequent ones (Jarvis et al., 2001; Thodberg et al., 2002a), and stressors such as housing may also affect the sow which in turn may affect the piglets' responses to stressors. However, the limited scientific literature on primiparous sows indicates little or no differences in stress, on the basis of cortisol concentrations, when housed in farrowing crates versus in loose systems (reviewed in: (Hemsworth, 2018)). The authors are presently studying piglets reared in farrowing and lactation housing systems with which their dams are familiar (i.e., systems they have previously farrowed in).

3.6 Conclusion

While sow and piglet welfare are generally considered to be superior in loose farrowing and lactation systems (Baxter et al., 2018; Martin et al., 2015; Oostindjer et al., 2011; Singh et al., 2017), the results from this experiment showed that piglets reared in loose pens displayed a higher intensity of escape behaviour when being captured during processing, and were slower to approach and physically interact with a novel object and an unfamiliar human compared to piglets from farrowing crates. Furthermore, piglets reared in loose pens had more injuries during lactation and were more likely to be upright, vocalising, nosing a pen mate and nosing the floor after weaning. While there were no effects of housing system on stress physiology 45 min after processing or 1.5 h after weaning, these results raise questions that require further research on the ability of piglets reared in loose pens to cope with stressors such as exposure to humans, novelty, husbandry procedures and weaning. Whether these effects are specific to the PigSAFE system studied here or are reflective of other loose systems is unknown. The PigSAFE system offers greater space and structural complexity and increased opportunities for interaction with the sow, however, in this experiment the PigSAFE environment was more restrictive in terms of piglets' contact with humans and non-littermates. For piglets in farrowing crates, the increased visual, auditory and olfactory contact with stockpeople and other pigs may be adaptive and contribute to improved stress resilience by allowing piglets more opportunities to overcome mild stress. This experiment also provides evidence that regular positive human interaction reduces pigs' fear of humans and husbandry procedures imposed by stockpeople. However, more research is clearly necessary to understand the effects of positive human contact on the injuries and stress physiology of pigs after weaning. The effects on stress physiology in particular highlight the need for a multifaceted approach to the welfare assessment of pigs. Additionally, whether the effects of the positive human contact treatment and those of the early housing system are sustained long-term requires further investigation.

3.7 References

Backus, B. L., & McGlone, J. J. (2018). Evaluating environmental enrichment as a method to alleviate pain after castration and tail docking in pigs. *Applied Animal Behaviour Science, 204*, 37-42.

Baxter, E. M., Andersen, I. L., & Edwards, S. A. (2018). Sow welfare in the farrowing crate and alternatives. In M. Špinka (Ed.), *Advances in pig welfare* (pp. 27-72). Duxford, United Kingdom: Woodhead Publishing.

Baxter, E. M., Lawrence, A. B., & Edwards, S. A. (2011). Alternative farrowing systems: Design criteria for farrowing systems based on the biological needs of sows and piglets. *Animal, 5*(4), 580-600.

Brajon, S., Laforest, J.-P., Bergeron, R., Tallet, C., Hötzel, M.-J., & Devillers, N. (2015). Persistency of the piglet's reactivity to the handler following a previous positive or negative experience. *Applied Animal Behaviour Science, 162*, 9-19.

Büttner, K., Czycholl, I., Basler, H., & Krieter, J. (2018). Effects of an intensified human–animal interaction on tail biting in pigs during the rearing period. *Journal of Agricultural Science, 156*(8), 1039-1046.

Center for Conservation Bioacoustics. (2014). Raven pro: Interactive sound analysis software (version 1.5). Ithaca, NY: The Cornell Lab of Ornithology.

Chaloupková, H., Illmann, G., Bartoš, L., & Špinka, M. (2007). The effect of pre-weaning housing on the play and agonistic behaviour of domestic pigs. *Applied Animal Behaviour Science, 103*(1), 25-34.

Chaloupkova, H., Illmann, G., Neuhauserova, K., Tomanek, M., & Valis, L. (2007). Preweaning housing effects on behavior and physiological measures in pigs during the suckling and fattening periods. *Journal of Animal Science*, 1741-1749.

Cronin, G. M., Simpson, G. J., & Hemsworth, P. H. (1996). The effects of the gestation and farrowing environments on sow and piglet behaviour and piglet survival and growth in early lactation. *Applied Animal Behaviour Science, 46*(3), 175-192.

De Jonge, F. H., Bokkers, E. A. M., Schouten, W. G. P., & Helmond, F. A. (1996). Rearing piglets in a poor environment: Developmental aspects of social stress in pigs. *Physiology & Behavior, 60*(2), 389-396.

de Oliveira, D., Keeling, L. J., & Paranhos da Costa, M. J. R. (2019). Individual variation over time in piglet´s reactions to early handling and its association to weight gain. *Applied Animal Behaviour Science, 215*, 7-12.

de Oliveira, D., Paranhos da Costa, M. J. R., Zupan, M., Rehn, T., & Keeling, L. J. (2015). Early human handling in non-weaned piglets: Effects on behaviour and body weight. *Applied Animal Behaviour Science, 164*, 56-63.

De, U. K., Nandi, S., Mukherjee, R., Gaur, G. K., & Verma, M. R. (2017). Identification of some plasma biomarkers associated with early weaning stress in crossbred piglets. *Comparative Clinical Pathology, 26*(2), 343-349.

Hayes, M. E., Hemsworth, L. M., Morrison, R. S., Butler, K. L., Rice, M., Rault, J.-L., & Hemsworth, P. H. (2021). Effects of positive human contact during gestation on the behaviour, physiology and reproductive performance of sows. *Animals, 11*(1), 214.

Hemsworth, P. H. (2018). Key determinants of pig welfare: Implications of animal management and housing design on livestock welfare. *Animal Production Science, 58*(8), 1375-1386.

Hemsworth, P. H., & Barnett, J. L. (1992). The effects of early contact with humans on the subsequent level of fear of humans in pigs. *Applied Animal Behaviour Science, 35*, 83-90.

Hemsworth, P. H., Barnett, J. L., & Hansen, C. (1986a). The influence of handling by humans on the behaviour, reproduction and corticosteroids of male and female pigs. *Applied Animal Behaviour Science, 15*(4), 303.

Hemsworth, P. H., Barnett, J. L., Hansen, C., & Gonyou, H. W. (1986b). The influence of early contact with humans on subsequent behavioural response of pigs to humans. *Applied Animal Behaviour Science, 15*, 55-63.

Hemsworth, P. H., & Coleman, G. J. (2011). *Human-livestock interactions: The stockperson and the productivity and welfare of intensively farmed animals* (2nd ed.). Wallingford, United Kingdom: CABI Publishing.

Hinde, R. A. (1970). *Animal behaviour; a synthesis of ethology and comparative psychology* (2nd ed.). New York, United States of America: McGraw-Hill.

Iacoviella, B. M., & Charney, D. S. (2019). Cognitive and behavioral components of resilience to stress. In A. Chen (Ed.), *Stress resilience: Molecular and behavioral aspects* (pp. 23). London, England: Academic Press.

Jarvis, S., Van der Vegt, B. J., Lawrence, A. B., McLean, K. A., Deans, L. A., Chirnside, J., & Calvert, S. K. (2001). The effect of parity and environmental restriction on behavioural and physiological responses of pre-parturient pigs. *Applied Animal Behaviour Science, 71*(3), 203-216.

Jones, R. B., & Waddington, D. (1992). Modification of fear in domestic chicks, gallus gallus domesticus, via regular handling and early environmental enrichment. *Animal Behaviour, 43*(pt.6), 1021-1033.

King, R. L., Baxter, E. M., Matheson, S. M., & Edwards, S. A. (2019). Consistency is key: Interactions of current and previous farrowing system on litter size and piglet mortality. *Animal, 13*(1), 180-188.

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. *Applied Animal Behaviour Science, 121*(1), 16-24.

Leidig, M. S., Hertrampf, B., Failing, K., Schumann, A., & Reiner, G. (2009). Pain and discomfort in male piglets during surgical castration with and without local anaesthesia as determined by vocalisation and defence behaviour. *Applied Animal Behaviour Science, 116*(2), 174-178.

Luna, D., González, C., Byrd, C. J., Palomo, R., Huenul, E., & Figueroa, J. (2021). Do domestic pigs acquire a positive perception of humans through observational social learning? *Animals, 11*(1), 127.

Lyons, D. M., Parker, K. J., Katz, M., & Schatzberg, A. F. (2009). Developmental cascades linking stress inoculation, arousal regulation, and resilience. *Frontiers in Behavioral Neuroscience, 3*(32).

Lyons, D. M., Parker, K. J., & Schatzberg, A. F. (2010). Animal models of early life stress: Implications for understanding resilience. *Developmental Psychobiology, 52*(5), 402-410.

Martin, J. E., Ison, S. H., & Baxter, E. M. (2015). The influence of neonatal environment on piglet play behaviour and post-weaning social and cognitive development. *Applied Animal Behaviour Science, 163*, 69-79.

Moberg, G. P. (2000). Biological response to stress: Implications for animal welfare. In G. P. Moberg & J. A. Mench (Eds.), *The biology of animal stress: Basic principles and implications for animal welfare* (pp. 1-21). Wallingford, United Kingdom: CABI Publishing.

Moeser, A. J., Vander Klok, C., Ryan, K. A., Wooten, J. G., Little, D., Cook, V. L., & Blikslager, A. T. (2007). Stress signaling pathways activated by weaning mediate intestinal dysfunction in the pig. *American Journal of Physiology: Gastrointestinal and Liver Physiology, 292*(1), G173-G181.

Morrison, R., Athorn, R., & McDonald, E. (2015). Developing commercially viable, confinement free farrowing and lactation systems. Part 2: Utilising confinement free systems to maximise economic performance. ‘Two stage’ farrowing and lactation system. *Final report prepared for the Co-operative Research Centre for High Integrity Australian Pork, 1A-105*.

Morrison, R., & Baxter, E. (2013). Developing commercially viable, confinement free farrowing and lactation systems. Part 1: Pigsafe system. *Final report for the Co-operative Research Centre for High Integrity Australian Pork, 1A-105*.

Muns, R., Rault, J. L., & Hemsworth, P. (2015). Positive human contact on the first day of life alters the piglet's behavioural response to humans and husbandry practices. *Physiology & Behavior, 151*, 162-167.

Murata, H. (2007). Stress and acute phase protein response: An inconspicuous but essential linkage. *Veterinary Journal, 173*(3), 473-474.

Murata, H., Shimada, N., & Yoshioka, M. (2004). Current research on acute phase proteins in veterinary diagnosis: An overview. *Veterinary Journal, 168*(1), 28-40.

Oostindjer, M., Bolhuis, J. E., Mendl, M., Held, S., Gerrits, W., van den Brand, H., & Kemp, B. (2010). Effects of environmental enrichment and loose housing of lactating sows on piglet performance before and after weaning. *Journal of Animal Science, 88*(11), 3554-3562.

Oostindjer, M., van den Brand, H., Kemp, B., & Bolhuis, J. E. (2011). Effects of environmental enrichment and loose housing of lactating sows on piglet behaviour before and after weaning. *Applied Animal Behaviour Science, 134*(1), 31-41.

Ott, S., Soler, L., Moons, C. P., Kashiha, M. A., Bahr, C., Vandermeulen, J., Janssens, S., Gutiérrez, A. M., Escribano, D., Cerón, J. J., Berckmans, D., Tuyttens, F. A., & Niewold, T. A. (2014). Different stressors elicit different responses in the salivary biomarkers cortisol, haptoglobin, and chromogranin a in pigs. *Research in Veterinary Science, 97*(1), 124-128.

Parker, K. J., & Maestripieri, D. (2011). Identifying key features of early stressful experiences that produce stress vulnerability and resilience in primates. *Neuroscience & Biobehavioral Reviews, 35*(7), 1466-1483.

Piñeiro, M., Piñeiro, C., Carpintero, R., Morales, J., Campbell, F. M., Eckersall, P. D., Toussaint, M. J. M., & Lampreave, F. (2007). Characterisation of the pig acute phase protein response to road transport. *Veterinary Journal, 173*(3), 669-674.

Pomorska-Mol, M., Kwit, K., & Markowska-Daniel, I. (2012). Major acute phase proteins in pigs from birth until slaughter. *Bulletin of the Veterinary Institute in Pulaway, 56*(4), 553-557.

Puppe, B., Tuchscherer, M., & Tuchscherer, A. (1997). The effect of housing conditions and social environment immediately after weaning on the agonistic behaviour, neutrophil/lymphocyte ratio, and plasma glucose level in pigs. *Livestock Production Science, 48*(2), 157-164.

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*(1), 110-115.

Sauerwein, H., Schmitz, S., & Hiss, S. (2005). The acute phase protein haptoglobin and its relation to oxidative status in piglets undergoing weaning-induced stress. *Redox Report, 10*(6), 295-302.

Singh, C., Verdon, M., Cronin, G. M., & Hemsworth, P. H. (2017). The behaviour and welfare of sows and piglets in farrowing crates or lactation pens. *Animal, 11*(7), 1210-1221.

Sommavilla, R., Hötzel, M. J., & Dalla Costa, O. A. (2011). Piglets' weaning behavioural response is influenced by quality of human–animal interactions during suckling. *Animal, 5*(9), 1426-1431.

Tallet, C., Sy, K., Prunier, A., Nowak, R., Boissy, A., & Boivin, X. (2014). Behavioural and physiological reactions of piglets to gentle tactile interactions vary according to their previous experience with humans. *Livestock Science, 167*, 331-341.

Tanida, H., Miura, A., Tanaka, T., & Yoshimoto, T. (1995). Behavioural response to humans in individually handled weanling pigs. *Applied Animal Behaviour Science, 42*, 249-259.

Thodberg, K., Jensen, K. H., & Herskin, M. S. (2002a). Nest building and farrowing in sows: Relation to the reaction pattern during stress, farrowing environment and experience. *Applied Animal Behaviour Science, 77*(1), 21-42.

Thodberg, K., Jensen, K. H., & Herskin, M. S. (2002b). Nursing behaviour, postpartum activity and reactivity in sows: Effects of farrowing environment, previous experience and temperament. *Applied Animal Behaviour Science, 77*(1), 53-76.

Turner, S. P., Roehe, R., D'Eath, R. B., Ison, S. H., Farish, M., Jack, M. C., Lundeheim, N., Rydhmer, L., & Lawrence, A. B. (2009). Genetic validation of postmixing skin injuries in pigs as an indicator of aggressiveness and the relationship with injuries under more stable social conditions. *Journal of Animal Science, 87*(10), 3076-3082.

Turpin, D. L., Langendijk, P., Sharp, C., & Pluske, J. R. (2017). Improving welfare and production in the peri-weaning period: Effects of co-mingling and intermittent suckling on the stress response, performance, behaviour, and gastrointestinal tract carbohydrate absorption in young pigs. *Livestock Science, 203*, 82-91.

Turpin, D. L., Langendijk, P., Tai-Yuan, C., Lines, D., & Pluske, J. R. (2016). Intermittent suckling causes a transient increase in cortisol that does not appear to compromise selected measures of piglet welfare and stress. *Animals, 6*(3), 24.

van der Meulen, J., Koopmans, S. J., Dekker, R. A., & Hoogendoorn, A. (2010). Increasing weaning age of piglets from 4 to 7 weeks reduces stress, increases post-weaning feed intake but does not improve intestinal functionality. *Animal, 4*, 1653-1661.

Villain, A. S., Lanthony, M., Guérin, C., Noûs, C., & Tallet, C. (2020). Manipulable object and human contact: Preferences and modulation of emotional states in weaned piglets. *Frontiers in Veterinary Science, 7*.

VSN International. (2018). Genstat for windows 19th edition. Hemel Hempstead, United Kingdom: VSN International.

Widowski, T. M., Cottrell, T., Dewey, C. E., & Friendship, R. M. (2003). Observations of piglet-directed behavior patterns and skin lesions in eleven commercial swine herds. *Journal of Swine Health and Production, 11*(4), 181-185.

Yonezawa, T., Takahashi, A., Imai, S., Okitsu, A., Komiyama, S., Irimajiri, M., Matsuura, A., Yamazaki, A., & Hodate, K. (2012). Effects of outdoor housing of piglets on behavior, stress reaction and meat characteristics. *Asian-Australasian Journal of Animal Sciences, 25*(6), 886-894.

Zupan, M., Rehn, T., de Oliveira, D., & Keeling, L. J. (2016). Promoting positive states: The effect of early human handling on play and exploratory behaviour in pigs. *Animal, 10*(1), 135-141.

Chapter 4

Effects of Early Human Contact and Housing on Lifetime Stress Resilience in Pigs

4.1 Abstract

Early life experiences such as contact with humans, maternal care and the physical environment can impact the development of stress coping mechanisms in animals. This experiment examined the effects of the early housing environment and early positive interactions with humans on stress resilience in pigs until 21 wk of age. Using a 2 × 2 factorial arrangement, 48 litters of pigs were reared in either a standard farrowing crate (FC) with a confined sow or a loose pen (LP) which was larger, more physically complex and allowed the sow to move freely throughout the farrowing and lactation period. Piglets were provided with either routine contact from stockpeople (C), or, routine contact plus regular opportunities for positive human contact (+HC) during the lactation period. The +HC treatment involved 5 min of scratching, patting and stroking and was imposed at a litter level from 1 day of age until weaning. Measures of stress resilience included behavioural and physiological responses to stressors such as routine husbandry and management practices, isolation, novelty and humans, basal behavioural and physiological measurements that reflect how pigs cope with their general environment, as well as survival during the lactation period and lifetime growth.

Overall, there was evidence of improved stress resilience in positively handled pigs and in pigs from the farrowing crate treatment. Positive human contact from 0-4 wk of age was extremely effective at reducing fear of humans during the lactation period, based on increased approach and reduced proximity to an unfamiliar human in +HC piglets than C piglets when tested at 3 wk of age. The +HC treatment also reduced piglets' fear of a novel object at the same age. There was evidence of +HC pigs showing less fear of humans in tests at 6, 9 and 14 wk of age, indicating that some reduction in fear of humans lasted well beyond the application of the positive handling treatment. The +HC pigs vocalised less and showed less escape behaviour during husbandry practices imposed during the lactation period and had lower cortisol concentrations 2 d after weaning as well as after isolation at 7 wk of age. Additionally, at 4 wk of age they had higher concentrations of brain-derived neurotrophic factor (BDNF; a neurotrophin linked with stress resilience) and fewer injuries, with limited evidence also suggesting these same effects much later in life at 17 wk of age. There was no evidence that early positive handling of piglets affected sow maternal behaviour, piglet survival during the lactation period or growth to 18 wk of age. Furthermore, there was little evidence that the +HC treatment affected the time budgets of behaviour of pigs or basal measurements of cortisol and IgA.

Compared to pigs reared in the loose farrowing and lactation treatment, pigs reared in farrowing crates showed less fear of humans and novelty at 3 wk of age, less escape behaviour when captured by a stockperson for husbandry practices at 4 d of age, and a lower cortisol response 2 h post weaning and

mixing. After weaning when all pigs were in identical housing, there was some indication of better stress resilience in FC pigs, as shown by lower levels of fear towards humans at 4 and 6 wk of age, less vocalising and lower cortisol concentrations in response to isolation at 7 wk of age, and fewer baulks when moved out of the home pen at 21 wk of age. Sows in loose farrowing and lactation pens showed improved maternal behaviour compared to sows in farrowing crates, based on more posture changes and piglet directed behaviour in response to unfamiliar piglet vocalisations in the second week of lactation, but piglet mortality during the lactation period was higher in the loose system. Growth during the lactation period was higher in LP litters, however there were no differences in growth after weaning to 18 wk of age. During the lactation period, LP piglets displayed more play behaviour and interactions with the sow and less repetitive nosing directed to pen mates. Additionally, they had higher concentrations of BDNF at 4 wk of age, but this effect was not sustained over time.

These results show that positive human interactions early in life, such as patting, stroking and scratching, reduced pigs' fear of humans, but also appeared to have broader benefits, based on fewer injuries, higher BDNF concentrations and reduced responses to stressors such as novelty, weaning, isolation and routine husbandry practices. Reduced fear of humans may have mediated these broader effects, and/or close human contact early in life may have provided a minor challenge for pigs to overcome that led to improved coping in general. These results also suggest the welfare of LP piglets in their undisturbed home pens may have been superior to that of FC piglets, based on more play behaviour and interactions initiated with the sow, higher BDNF concentrations and less repetitive nosing of pen mates during the lactation period. However, the LP pigs were clearly less able to cope with additional stressors such as routine husbandry practices, weaning, isolation, and exposure to novelty and humans. The LP pigs were reared in a more isolated environment than FC pigs, with fewer opportunities early in life to learn to cope with challenges such as frequent and/or close contact with stockpeople and other pigs. This may have contributed to the greater stress responses of LP pigs. While some effects of the early human contact and housing treatments may have weakened over time with subsequent handling and housing, this experiment still showed that early life experiences can give rise to differences in pigs' flexibility to some challenges later in life, highlighting the importance of the early environment in the development of stress coping mechanisms.

4.2 Introduction

Pigs in intensive production systems typically encounter several stressful situations such as abrupt weaning, painful husbandry procedures, intense contact with stockpeople and exposure to novel social and physical environments. How well pigs cope with these routine stressors may be affected by experiences with stress that occur early in life. Extensive research on rodents and non-human primates has shown that exposure to stress early in life that is 'challenging but not overwhelming' leads to stress resilience by allowing animals to learn adaptive coping strategies for future stressful situations (Lyons et al., 2009; Lyons et al., 2010; Lyons & Schatzberg, 2019; Parker & Maestripieri, 2011). The hypothalamic-pituitary-adrenal (HPA) axis is thought to be involved in this 'programming' of stress resilience, whereby brief but regular stress during early life increases the sensitivity of the hippocampus to glucocorticoids, which enhances negative feedback on HPA axis activity resulting in reduced behavioural and physiological responses to stressors (Meaney et al., 1988; Meaney et al., 1993). In contrast, exposure to prolonged or intense stress dysregulates the HPA axis, leading to stress vulnerability and an impaired ability to cope with stress in the future (Gunnar & Quevedo, 2007; Parker & Maestripieri, 2011; Rincón-Cortés & Sullivan, 2014). A lack of stress exposure altogether may also lead to stress vulnerability as it leaves too few opportunities for animals to learn to cope with stress effectively (Parker & Maestripieri, 2011). Early experiences that are likely to affect the development of lifetime stress resilience and vulnerability in pigs include maternal behaviour of the sow, the physical environment, and interactions with humans.

The farrowing and lactation housing system can determine how complex the early physical environment is for piglets and can also restrict or facilitate the amount of maternal care they receive from the sow. Compared to sows in farrowing crates, sows in loose lactation housing systems show better maternal behaviour and increased interactions with their piglets (Chidgey et al., 2016; Cronin et al., 1996; Singh et al., 2017; Thodberg et al., 2002), which have been suggested to contribute to piglets from loose systems being better able to cope with stress (Oostindjer et al., 2011). The stress of the sow induced by the farrowing and lactation housing system may also have an indirect effect on the stress of her piglets (Morgan et al., 2021). Indeed, there is evidence that piglets from housing systems where the sow is loose during lactation show improved coping with stress. Oostindjer and colleagues (2011) found that after weaning, pigs from loose lactation pens performed less belly nosing and manipulative behaviour and more food exploration compared to pigs from farrowing crates. Additionally, Chaloupkova and colleagues (2007) found that cortisol responses to transport at 6 mo of age were lower in pigs reared in straw-enriched loose pens than pigs reared in farrowing crates. Furthermore, play behaviour, which may indicate low levels of stress and may also contribute to resilience by providing opportunities for animals to recover from challenge (Spinka et al., 2001), has been reported to occur more frequently in loose systems compared to farrowing crates (Clarkson et al., 2021; Martin et al., 2015; Singh et al., 2017). Increased occurrence of play behaviour in loose systems may be attributed to non-restricted sow-piglet interactions contributing to normal socio-cognitive development, a larger space facilitating play behaviours that require more room (e.g., running/gamboling), and/or in some cases, increased complexity of the physical environment. A more complex physical environment may improve stress resilience by promoting exploration, providing agency for animals to learn about aspects of their environment, and providing more behavioural options to cope with stressors, such as hiding in response to threats (Carlstead & Shepherdson, 2000; Crofton et al., 2015).

In contradiction to the notion that loose systems better prepare pigs to cope with challenge, recently we showed that piglets reared in loose farrowing and lactation pens (PigSAFE pens) that offered greater space, more physical complexity, and more opportunity for interaction with the sow, showed impaired coping with stress compared to piglets from farrowing crates where the sow was confined throughout farrowing and lactation (Hayes et al., 2021b). Piglets from the loose system were more reactive during capture by a stockperson, showed more avoidance of novelty and humans, and were more frequently observed to be active, vocalising, nosing the pen floor and nosing other pigs after weaning (Hayes et al., 2021b). One explanation for these effects is that piglets from the loose system were reared in a more isolated environment than piglets in farrowing crates, and that reduced contact with people and other pigs in the loose system contributed to increased stress vulnerability. With increasing interest in reducing the use of farrowing crates (Baxter et al., 2018; Hemsworth, 2018), there is a clear need to better understand the effects of rearing pigs in alternative farrowing and lactation housing systems on their resilience to stress. In particular, whether the effects of the early housing environment on stress responses are sustained throughout the pigs' life, and what impact the early housing environment has on pigs' fear of humans.

The majority of interactions between pigs and stockpeople are inadvertently negative for pigs and can increase their fear of humans (Hemsworth & Coleman, 2011). High levels of fear towards humans can induce chronic stress which may compromise animal welfare and productivity (Hemsworth & Coleman, 2011). We and others have shown that positive interactions such as patting, stroking and talking softly to pigs decreases fear of humans (Hemsworth & Coleman, 2011; Tallet et al., 2014; Tanida et al., 1995) and can reduce the aversiveness of routine stressors involving humans. For example, regular patting and scratching reduced the avoidance of sows to stockpeople imposing pregnancy testing and vaccination (Hayes et al., 2021a), and reduced the vocalisations (Hayes et al., 2021b) and escape behaviour (Hayes et al., 2021b; Muns et al., 2015) of piglets during husbandry procedures conducted during the lactation period. Pigs may be more sensitive to the effects of handling early in life, since patting and stroking pigs from 0-3 wk of age has been shown to be more effective in reducing subsequent fear responses to humans at 18 wk of age, compared to positive handling imposed from 3-6 or 6-9 wk of age (Hemsworth & Barnett, 1992). This suggests that the period from 0-3 wk of age may represent a sensitive period of development during which positive handling can produce the most benefit in terms of ameliorating the biological cost of stressors involving humans.

In addition to modifying fear levels, positive interactions with humans may confer broader stress resilience in pigs. For example, talking to and patting sows daily alleviated the cortisol response of sows to tether housing (Pederson et al., 1998), and the presence of a familiar person after social isolation reduced weaner pigs' vocalisations (Villain et al., 2020), suggesting that familiar people can buffer stress associated with challenging situations. Additionally, piglets that were held and stroked regularly were more willing to accept tactile contact by familiar and unfamiliar people, but also demonstrated more play behaviour and vocalised less in a novel arena, indicating that the handling treatment reduced fear responses to both humans and novel situations (de Oliveira et al., 2015; Zupan et al., 2016). It is possible that in addition to habituating pigs to humans, brief and regular close human contact may provide a minor challenge, that when overcome, leads to improved coping with other types of challenges faced in the future.

The aim of this experiment was to examine the effects of the early farrowing and lactation housing environment and early positive interactions with humans on lifetime stress resilience and vulnerability in pigs. Stress resilience is characterized by both resistance to adverse effects of stressors and fast recovery to normal functioning after stress exposure, while stress vulnerability is considered the inverse (Chen, 2019). Therefore, stress resilience and vulnerability were measured by examining the responses that pigs typically exhibit when exposed to challenging situations, including behavioural, neuroendocrine and immunological responses to stressors such as routine husbandry and management practices, isolation, novelty and humans, as well as basal behavioural, physiological and fitness measurements that reflect how pigs cope with their environment in general.

4.3 Materials and Methods

This experiment was conducted at the Rivalea Australia Research and Innovation Unit, Corowa, NSW, Australia. All animal procedures were conducted with prior institutional ethical approval under the requirements of the New South Wales Prevention of Cruelty to Animals Act 1985 in accordance with the National Health and Medical Research Council/Commonwealth Scientific and Industrial Research Organization/Australian Animal Commission Code of Practice for the Care and Use of Animals for Scientific Purposes (Rivalea Animal Ethics Committee #19B018C).

4.3.1 Animals and Treatments

Twelve litters of Landrace × Large White piglets received each combination of a 2 housing system × 2 human contact treatment factorial arrangement during the lactation period (48 litters total). The housing systems were either a farrowing crate (FC) where the sow was confined throughout the farrowing and lactation period, or a larger and more structurally complex loose pen (LP), where the sow was free throughout the farrowing and lactation period. All litters were born from second parity sows that previously farrowed in a similar housing system, either farrowing crates or loose housing, at their first parity. The two human contact treatments were routine contact (C), which involved interaction with stockpeople only associated with regular husbandry and management, or positive human contact (+HC), which involved routine contact (as in C) plus regular opportunities to be patted, stroked and scratched by an experimenter during the lactation period. Further details on the housing systems and human contact treatments are described in subsequent sections. The farrowing spread was 7 d across all treatments and litters were not equalised for size or balanced for equal sex ratios. Cross fostering occurred on a minimal basis, always within the same housing system and within the first 24 h of life.

4.3.1.1 Housing Systems during the Farrowing and Lactation Period

The layouts of the farrowing crate and loose pen housing systems are depicted in Figure 4.1. The two housing systems were in separate but adjacent rooms managed by the same stockpeople. No bedding or enrichment was provided to sows or piglets in either housing system. Each farrowing crate contained a 2.3 × 1.7 m area for the piglets, and a 2.3 × 0.60 m internal space for the sow which allowed the sow to stand or lay down, but not turn around. The flooring was slatted steel (10 mm width between slats) with the exception of a 1.1 × 0.41 m solid creep mat that was heated by an overhead lamp. The walls were solid and 0.51 m high, allowing sows and piglets to have visual contact with people in the aisles.

The design of the loose pen system was PigSAFE (Piglet and Sow Alternative Farrowing Environment). Loose pens were 3.6 × 2.4 m and allowed free movement of the sow during farrowing and the entire lactation period. Each pen contained a triangular creep area only accessible to piglets, which was heated by an overhead lamp and covered by a removable solid roof. The sows' feeder was located within a stalled area where the sow could be confined temporarily if stockpeople required access to the pen or piglets, although, stockpeople very rarely used the feeding stall for this purpose. The flooring in the central area of the pen, including the creep, was solid plastic, while the flooring in the back of the pen and the feeding stall was slatted plastic (MIK Rubin flooring, 10 mm width between slats). The walls were solid and 1.2 m high, which restricted piglets' visual contact with people in the room unless they were standing directly in front of the pen. The wall in front of the creep area was 0.8 m. The walls in the central and back areas of the pen contained sloped sides to reduce the incidence of piglets being crushed by the sow (see light grey partitions depicted in Figure 4.1a). There were barred windows in the back of the pen which allowed interaction between adjacent sows, and interaction between adjacent piglets once piglets could reach the windows at 2-3 wk of age. At the back of each pen due to recent design modifications, there was a 2.4 × 0.40 m area of space which was not accessible to sows or piglets (pictured in Figure 4.1a, not included in the length of the pen).

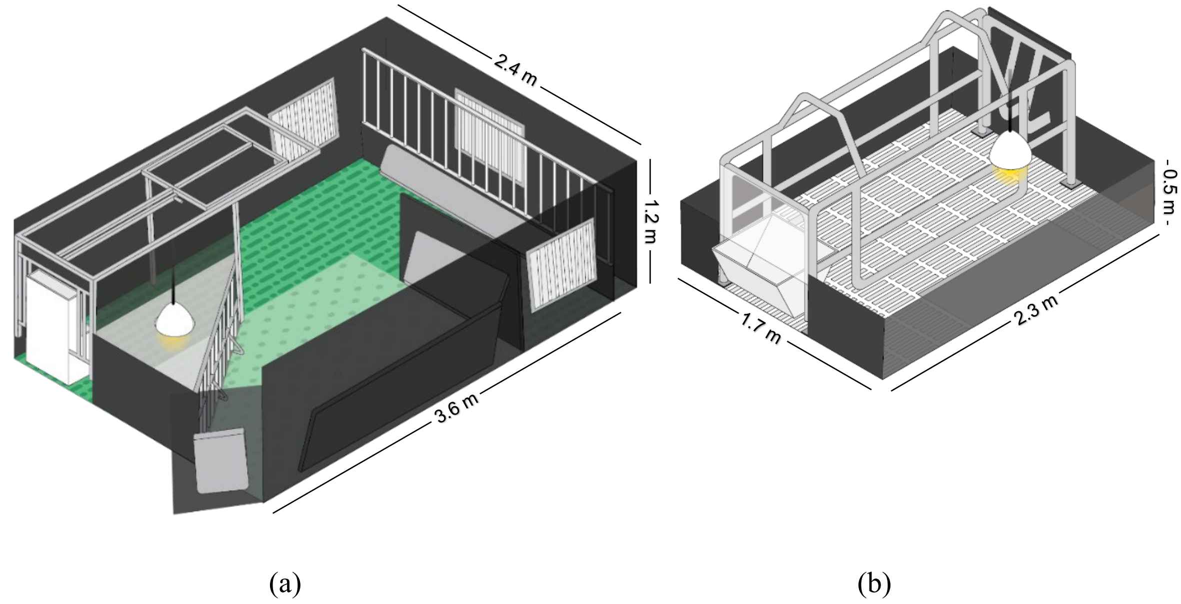
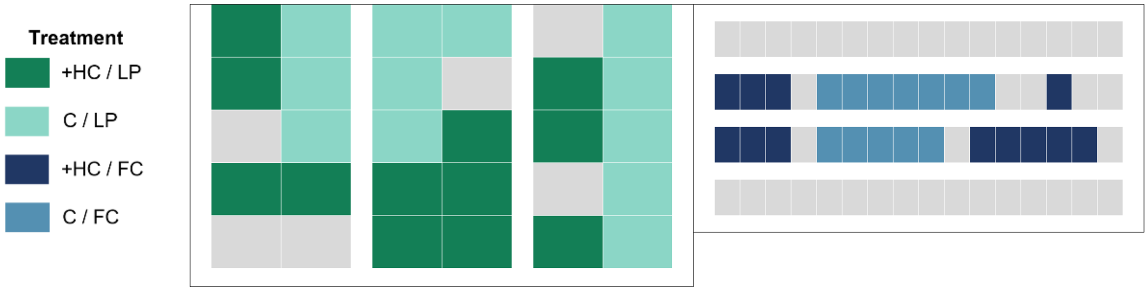


Figure 4.1. Diagrams of the two housing systems: (a) loose pen (LP); (b) farrowing crate (FC).

4.3.1.2 Human Contact Treatment during the Lactation Period

Piglets from the C treatment received only routine contact with stockpeople associated with regular husbandry and management. This involved twice daily health and welfare checks by a stockperson who visually inspected the animals and entered the crate or pen when necessary, for example to assess sow teat function or remove a dead piglet. Additionally, piglets received routine visual contact with humans when sows were hand-fed by stockpeople twice per day, and when creep food was provided to piglets once per day after 14 d of age. The +HC treatment also involved routine husbandry and management as in the C treatment, in addition to the opportunity for piglets to interact with an experimenter five days per week from 1 d of age until weaning. The +HC treatment involved the experimenter gently patting, stroking and scratching piglets that approached and piglets sleeping in the creep area for a duration of 5 min. Piglets were patted, stroked and scratched on the back, the abdomen and behind the ears. The +HC treatment was imposed at the litter level, in the morning after sows had been fed (approximately between 7:00 and 10:00 h) by one of two experimenters (one male and one female). To impose the +HC treatment in farrowing crates, the experimenter slowly entered and crouched down at the back of the crate in front of the creep area. To impose the +HC treatment in loose pens, the experimenter quietly lifted the creep roof, squatted outside the pen and extended their hand inside the creep area to interact with piglets. Therefore, +HC piglets in farrowing crates had more opportunity for visual and physical contact with the experimenter during treatment imposition compared to +HC piglets in loose pens.

As fear of humans is reduced in pigs that observe positive handling of other pigs (Luna et al., 2021), an important consideration when allocating positions of C and +HC litters in the rooms was minimising the amount of visual exposure to humans that C litters received through imposition of the +HC treatment. Thus, C litters were allocated to areas of both rooms where the experimenter could avoid walking past during imposition of the positive handling treatment (Figure 4.2). Additionally in the farrowing crate room, non-experimental litters were allocated in between +HC and C litters to reduce any carry-over effects of the handling treatment. In the loose pen room, there was a reduced chance of carry-over effects between +HC and C litters as the loose pens had high walls that restricted piglets' visual contact outside of their own pen.



(a) (b)

Figure 4.2. Allocation of positive human contact (+HC) and routine human contact (C) litters in (a): the loose pen (LP) room and (b): the farrowing crate (FC) room. Grey boxes in both housing systems represent non-experimental litters.

4.3.1.3 Housing and Management during the Weaner and Grower/Finisher Periods

Weaning occurred at 4 weeks of age (mean age = 27 days; SD = 1.5; no difference between treatments). Before weaning, two litters of the same farrowing and lactation housing system and human contact treatment were paired to be mixed after weaning. After weaning, each pen contained 8 same sex pigs from a pair of litters (n = 24 pairs), with males and females from the same pair housed in adjacent pens (Figure 4.3). Litters were selected to be paired on the basis of there being enough pigs from each sex to make up one pen of 8 males and one pen of 8 females after weaning. Mixing of the two litters in each pair occurred in two phases, such that half of the pigs from each pair were mixed on the day of weaning, and the other half were mixed 48 h after weaning. The process for weaning and mixing was as follows: sows were removed from the farrowing rooms. Four same sex pigs from each pair of litters, referred to as cohort 1, were mixed in 1.8 × 0.80 m pens containing slatted plastic flooring (MIK Rubin flooring, 10 mm width between slats) in an adjacent shed. Cohort 1 stayed in these pens for two days while the remaining pigs, cohort 2, stayed in the home pens in the farrowing rooms. Two days after weaning, cohort 1 was moved to the weaner shed and mixed with an additional 4 same sex pigs of the same pair from cohort 2. Thus, in the weaner shed there were 48 pens each made up of 8 pigs of the same sex (four from each cohort) from two litters of the same farrowing and lactation housing system and human contact treatment. All pigs were transported between sheds in trolleys by experienced handlers unfamiliar to the pigs. Pigs were individually identifiable via a unique ear tag number which allowed pigs from each litter to be selected for cohorts 1 and 2 prior to weaning using a number generator, although obvious runts were excluded. After the 8 male and 8 female pigs from each pair had been moved to the weaner shed, the remaining pigs from cohort 2 were removed from the experiment. The pens in the weaner shed were 3.0 × 1.5 m and contained ¾ slatted steel flooring and ¼ solid concrete flooring. At 10 weeks of age, pigs remained in the same groups but were moved to the grower/finisher shed where they stayed until the conclusion of the experiment. Pigs were moved to the grower/finisher shed by an unfamiliar experienced handler using a solid stockboard. The pens in the grower shed were 3.7 × 2.6 m and contained ¾ slatted concrete flooring and ¼ solid concrete flooring. Pens in both the weaner and grower/finisher periods had four open barred sides, with the exception of pens located on the two outer rows of the room which contained solid sided back walls. During both the weaner and grower/finisher periods, pigs were provided with ad libitum access to water and food and received daily health and welfare checks by stockpeople. No enrichment was provided.

During the weaner and grower/finisher periods, treatments were allocated to six blocks, each containing eight pens that were distributed in two rows as show in Figure 4.3. A six-replicate split plot design was used with housing allocated to main plots and human contact treatment allocated to sub plots. Each sub plot consisted of a pen of males and a pen of females (one pair of litters), and each main plot consisted of two male and two female pens in the same row (two pairs of litters).

Figure 4.3. Allocation of treatments in the weaner and grower/finisher housing. +HC: positive human contact; C: routine human contact; LP: loose pen; FC: farrowing crate. Pens of male pigs are denoted by ♂ and pens of female pigs are denoted by ♀.

4.3.2 Measurements

Measurements of stress resilience in pigs were collected from birth to slaughter and included behavioural and physiological responses to routine stressors, behavioural and physiological responses to emotionality tests, time budgets of behaviour, basal physiological measures and assessments of tear staining, injuries, growth and survival (Figure 4.4). Additionally, the maternal responsiveness of sows was measured during the lactation period.

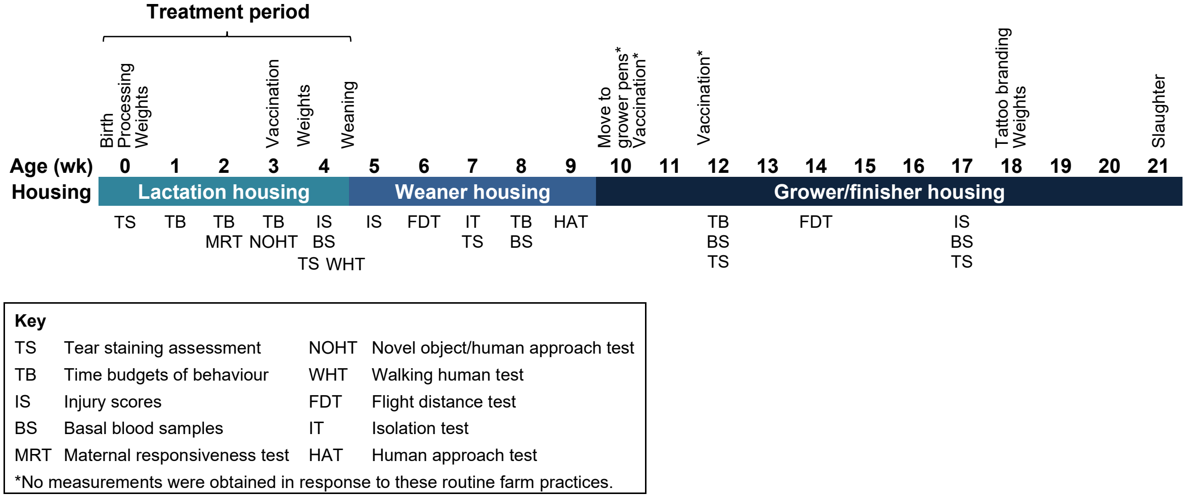


Figure 4.4. Timeline of the experiment and overview of measurements collected.

4.3.2.1 Maternal Responsiveness Test

In wk 2 of lactation, the maternal behaviour of all sows was assessed in response to an audio recording of an unfamiliar piglet squealing. Following the protocol adapted from Singh and colleagues (2017), an experimenter wearing a portable stereo unit, broadcasting a pre-recorded sound of an unfamiliar squealing piglet (80 decibels at a distance of 1 m), walked through the farrowing rooms. The experimenter completed 4 laps of the farrowing rooms in succession, stopping in front of each farrowing crate and loose pen for 3 s in each lap. Using video footage, the sows' behavioural responses were assessed by one observer during each 3 s bout of the audio broadcasting when the experimenter was standing stationary in front of each crate or pen. Maternal responsiveness in the test was measured through changes in posture (changed from lying or sitting to standing), and the occurrence of sows vocalising, becoming alert (turned head to orient to experimenter) and displaying behaviours directed towards their piglets (turned head to orient to, sniff or nose piglets).

4.3.2.2 Time Budgets of Behaviour and Basal Physiological Measurements

4.3.2.2.1 Time Budgets of Behaviour at 1, 2, 3, 8 and 12 wk of Age

The time budgets of behaviour of all pigs were recorded at 1, 2, and 3 wk of age during the lactation period, at 8 wk of age during the weaner period and at 12 wk of age during the grower/finisher period. Observations were conducted from video footage obtained on one day from approximately 10:00 – 17:00 in wk 1, 2 and 3, from 12:00 – 16:00 in wk 8 and from 8:00 – 12:00 in wk 12. These times were chosen as they were periods of the day outside of routine feeding and management by stockpeople. Instantaneous scan sampling at 2 min intervals was used to record the number of pigs performing each of the behaviours described in the ethogram in Table 4.1. One observer conducted observations for wk 1, 2 and 3, while a separate observer conducted observations for wk 8 and 12. Behaviours involving the sow were only recorded in wk 1, 2 and 3. In wk 2 and 3 of the lactation period piglets were offered solid feed on the floor in the creep area, however, from the video footage it was not possible to tell if there was feed left on the floor. No distinguishment could be made between piglets eating and interacting with the pen floor, therefore, eating behaviour was likely captured by recordings of piglets interacting with the pen at 2 and 3 wk of age. The video cameras covered between 90-100% of the pen and behaviours were only recorded from pigs that had at least half of the front of the body visible in the camera's field of view. Behaviours were expressed as the number of pigs in the field of view displaying the behaviour at each sampling point.

Table 4.1. Ethogram of pig behaviours.

Behaviour	Description
Social	
Play	Pig performs one of more of the following behaviours using bouncy, jerky movements (descriptions adapted from Martin and colleagues (2015)): *Scampering* - Sequence of at least two forward hops in rapid succession *Gamboling* - Running forward energetically *Pivoting* - Turning on the spot on a horizontal plane *Tossing head* - Circular, vertical or horizontal movement of the head *Flopping* - Rapid drop of the body from the upright position to recumbency *Hopping* - Two or all four feet off the floor in an energetic upwards movement *Rolling* - Lying on back while swaying entire body left to right
Aggression	Pig performs one or more of the following behaviours using fast, rigid movements, resulting in avoidance or retaliation by the receiver (excluding aggression during suckling bouts in the lactation period): *Knocking* - Vigorously thrusting the head against another pig *Biting* - Rapid opening and closing of the mouth on the body of another pig *Pushing* - Using the head or shoulders to press against the body of another pig
Nosing pen mate	Repetitive movement of the snout up and down on the body of another pig
Investigating pen mate	Gentle tactile contact with another pig using the snout
Tail biting	Mouthing or chewing the tail of another pig
Ear biting	Mouthing or chewing the ear of another pig
Sow interaction	
Climbing on sow	Piglet uses feet to elevate itself onto the body or head of the sow
Investigating sow	Gentle contact with the sow using the snout, excluding nosing around the mammary area
Activity	
Interacting with pen	Sniffing, nosing or chewing physical components of the pen including the floor
Walking	Slow locomotion around the pen, not engaged in another activity
Standing	Standing stationary on all four legs, not engaged in another activity
Sitting/lying	Sitting or lying, not engaged in another activity. During the lactation period, piglets were recorded as either lying/sitting with the sow (lying or sitting next to or on top of the sow, including lying at the mammary area when no suckling is occurring) or lying/sitting away from the sow (lying or sitting with no tactile contact between piglet and sow)

4.3.2.2.2 Basal Physiology at 4, 8, 12 and 17 wk of Age

Blood samples were collected from the 4 pigs from each pen from cohort 1 for subsequent analysis of serum cortisol, immunoglobulin A (IgA) and brain-derived neurotrophic factor (BDNF; a neurotrophin associated with neuronal growth, learning and memory (Cunha et al., 2010; Huang & Reichardt, 2001; Miranda et al., 2019), hypothesised to mediate stress resilience by regulating HPA axis activity early in life (Taliaz et al., 2011)) at 4 wk of age during the lactation period, at 8 wk of age during the weaner period and at 12 and 17 wk of age during the grower/finisher period. For further detail see the subsequent section “Sample Collection Details and Assay Characteristics”.

4.3.2.2.3 Tear Staining Assessments at 1, 4, 7, 12 and 17 wk of Age

Tear staining, which refers to the red/brown accumulation under the inner corner of the eye, has been used as an indicator of stress in rodents (Mason et al., 2004) and more recently, pigs (DeBoer et al., 2015; Larsen et al., 2019; Telkänranta et al., 2016). Left-eye staining may be a more sensitive indicator than right-eye staining (Marchant-Forde & Marchant-Forde, 2014), therefore, tear staining assessments were conducted on photographs of the left eyes of pigs that were obtained at 1, 4, 7, 12 and 17 wk of age. In wk 1 and 4 respectively, photographs of all pigs were obtained at 4 d of age after processing and at 22 d of age after weighing. In wk 7, 12 and 17, photographs of the 4 pigs from each pen from cohort 1 were obtained after blood sampling. The severity of tear staining under the eye was scored by one experienced observer using an adapted version of the DeBoer-Marchant-Forde scale (DeBoer et al., 2015): 0 – no signs of staining or area stained is < 1 % of total eye area; 1 – staining is barely detectable and area stained does not extend below the eyelid, area stained is approximately 1-10% of total eye area; 2 – staining is obvious and area stained is approximately 10-50 % of total eye area; 3 – staining is obvious and area stained is approximately 50-100 % of total eye area; 4 – staining is severe, area stained does not extend below the mouth line, area stained is approximately 100-250 % of total eye area, and; 5 – staining is severe, area stained extends below the mouth line, area stained is > 250 % of total eye area. In addition to the descriptive score, the total area and perimeter of tear staining were calculated using the image processing software ImageJ (Schneider et al., 2012), by using the pigs' ear tag as a known reference distance.

4.3.2.3 Behavioural and Physiological Responses to Routine Stressors

4.3.2.3.1 Piglet Processing at 4 d of Age

Processing occurred at 4 d of age and was carried out by two stockpeople. In loose pens, the sow was secured in the feeding stall during processing. One stockperson entered the farrowing crate or loose pen, lifted a piglet and passed it to a second stockperson who placed the piglet into a trolley. Once all piglets from the litter were in the trolley, the first stockperson lifted a piglet, injected an iron supplement intramuscularly and passed the piglet to the second stockperson who administered an oral coccidiosis vaccination and returned the piglet to the trolley. After all piglets from the litter had been administered iron and the oral vaccination, the first stockperson lifted a piglet and held it inverted while the second stockperson docked the tail with gas heated cautery clippers before returning the piglet to the home pen. The behavioural responses of piglets to capture, oral vaccination and tail docking were assessed by one observer using video footage. The intensity of piglet escape behaviour was scored using the following scale from Leidig and colleagues (2009): 0 – no movement; 1 – movement of one limb; 2 – movement of more than one limb; 3 – participation of the vertebral column; 4 – pattern as in 3 but with high intensity. Piglet vocalisations were recorded throughout processing on a microphone (Samson Meteor USB microphone) held less than 1 m away from piglets. The microphone was connected to a laptop running Raven Pro sound analysis software (Center for

Conservation Bioacoustics, 2014) which was used to later determine the duration, number and peak frequency of vocalisations. Blood samples for subsequent analysis of serum cortisol were collected 1 h after processing from 2 males and 2 females from each litter (see subsequent section "Sample Collection Details and Assay Characteristics" for further detail). The samples were collected from the first 2 piglets of each sex that were caught, prior to all piglets being weighed and receiving an ear tag for identification.

4.3.2.3.2 Vaccination at 3 wk of Age

At 3 wk of age, all piglets were administered an intramuscular vaccination against porcine circovirus associated disease and additionally male piglets were administered an immunization against boar taint (Improvac; immunocastration vaccination). Vaccination was carried out by 4 stockpeople in loose pens and 5 stockpeople in farrowing crates. In loose pens, the sow was secured in the feeding crate and the piglets were moved and confined to the back of the pen by one stockperson with a solid stockboard. Two stockpeople each picked a piglet up simultaneously and held them horizontally while another stockperson administered vaccination. In farrowing crates, two stockpeople each picked a piglet up simultaneously and immediately passed the piglets to another two stockpeople that were standing inside the farrowing crate. In farrowing crates, female piglets were held horizontally by the stockperson and male piglets were held horizontally inside a plastic piglet cradle while a fifth stockperson administered vaccination. Using the previously mentioned scale, the behavioural responses of piglets to capture were assessed from video footage by the same observer that assessed the responses of piglets to processing at 4 d of age. Additionally, as with processing, piglet vocalisations were recorded throughout vaccination and later analysed in Raven Pro (Center for Conservation Bioacoustics, 2014).

4.3.2.3.3 Weaning and Mixing at 4 wk of Age

Weaning occurred at 4 wk of age following the procedure outlined in Figure 4.5. Further detail on the process of weaning and mixing is described in the previous section "Housing and Management during the Weaner and Grower/Finisher Periods". Behavioural observations were conducted by one observer using video footage of pigs from cohort 1 at 0-1 and 3-4 h after weaning and mixing, and of all pigs when cohorts 1 and 2 were mixed at 48-49 h after weaning. Instantaneous scan sampling at 1 min intervals was used to record behaviours, excluding those involving the sow, listed in the ethogram in Table 4.1. In addition to the behaviours listed in Table 4.1, 'escape attempt', was added to the ethogram for post-weaning observations. An escape attempt was defined as a pig lifting at least two legs from the floor simultaneously, either in an attempt to jump or climb out of the pen. The video cameras covered between 90-100% of the pen and behaviours were only recorded from pigs in the camera's field of view. A pig was recorded as being in view if at least half of the front of the body was visible. Behaviours were expressed based on the number of pigs in the field of view at each sampling point. Blood samples were collected from the 4 pigs from each pen from cohort 1 at 2 h after weaning and mixing for subsequent analysis of neutrophil and lymphocyte counts and serum cortisol, and at 49 h after weaning and mixing for subsequent analysis of serum cortisol and haptoglobin (see subsequent section "Sample Collection Details and Assay Characteristics" for further detail).

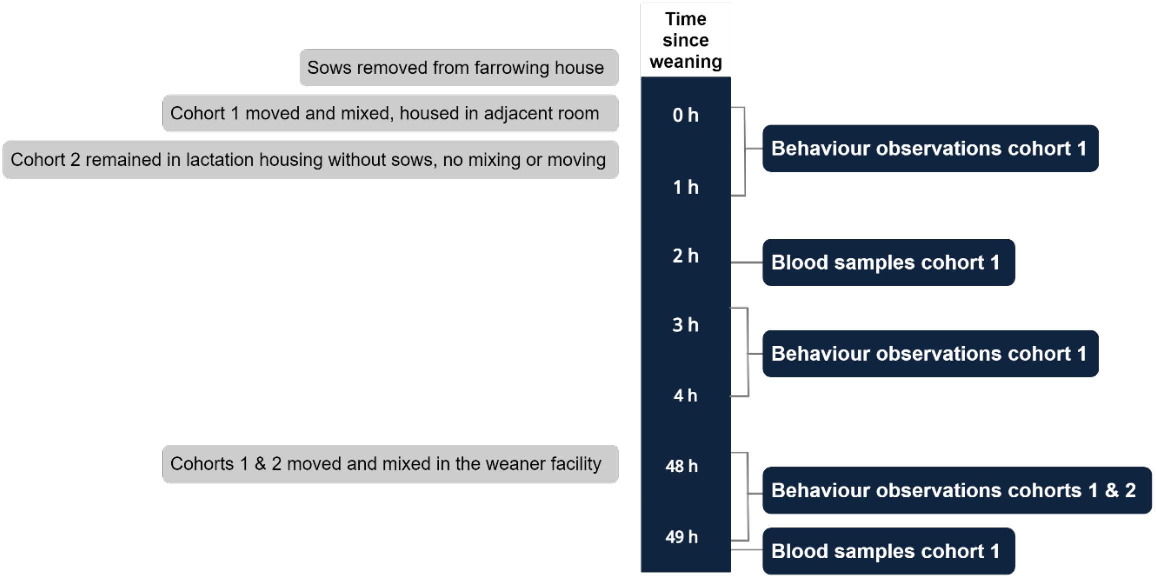


Figure 4.5. Timeline and overview of measurements collected at weaning and mixing at 4 wk of age.

4.3.2.3.4 Tattoo Branding at 18 wk of Age

At 18 wk of age, pigs were individually weighed and tattooed for identification at the abattoir. The tattoo was administered after the pig walked out of the weigh crate. Using video footage, the behavioural responses of the first 4 pigs in each pen that were weighed and tattooed were recorded by one observer. The occurrence of each pig vocalising (squealing), jumping (two or more legs lifted from the floor) and running (fast movement away from the person administering tattoo) were recorded within 1 s of the tattoo being imposed.

4.3.2.3.5 Moving out of Pens at 21 wk of Age

At 21 wk of age, pigs were moved out of the home pen to be loaded for slaughter. One stockperson opened the gate and walked in a clockwise direction around the pen with a solid stockboard to encourage pigs to move forward out of the pen. The stockperson was instructed to only use additional interventions, such as slapping or pushing pigs with their hand or hitting the stockboard to the ground, when necessary to move pigs forward. Using video footage, the time taken to move all pigs out of the pen and the total number of times the stockperson used additional interventions to move pigs forward was assessed. Additionally, the occurrence of pigs baulking (moving backwards rather than forwards out of the pen) was assessed.

4.3.2.4 Behavioural and Physiological Responses to Emotionality Tests

4.3.2.4.1 Novel Object and Human Approach Test at 3 wk of Age

At 3 wk of age, the behavioural responses to novel and human stimuli were assessed in 2 male and 2 female piglets selected from each litter prior using a number generator. The four piglets from each litter were tested in a group. Following the protocol described by Hayes and colleagues (2021b), testing involved consecutive 1 min exposures to the following conditions: empty novel arena located adjacent to the home pen; novel object introduced to arena; human hand introduced to arena; unfamiliar human standing inside arena. The 1.8 m × 0.60 m × 0.60 m novel arena was constructed of black wooden board and was portable, allowing testing to take place 1 m away from each FC and LP. Painted lines were used to mark thirds on the arena floor. Piglets were lifted two at a time from the home pen and gently placed at one end of the arena (along a short wall) by an experienced handler. Once all 4 piglets had been placed inside, they were left in the empty arena for 1 min, without visual contact of people and pigs in the room. A female experimenter, unfamiliar to the piglets, then slowly approached and presented an orange traffic cone at the opposite end of the arena to where piglets had been initially placed. After 1 min, the experimenter approached, removed the traffic cone and squatted side on outside the arena while extending their hand inside. After a further minute, the experimenter stood up and stepped inside the arena, remaining stationary for the final minute of the test. The piglets were then individually returned to the home pen. Using video footage, the number of entries each piglet made into sections of the empty arena was recorded. Additionally, the following behavioural responses to the novel object, human hand and the standing human were recorded: latency to approach within 0.6 m, time spent within 0.6 m, latency to initiate physical interaction (sniffing, nosing, chewing or stepping on stimulus), and the number of physical interactions with the stimulus. A maximum response time of 60 s was given if a piglet did not approach or interact with the stimulus.

4.3.2.4.2 Walking Human Test at 4 wk of Age

One day after weaning and mixing at 4 wk of age, the behavioural responses to an unfamiliar human walking past the pen were assessed in all pigs from cohort 1. A female experimenter entered the shed and completed six laps of the room in succession, walking at a pace of 1 step per/s and stopping in front of each pen for 3 s. Using video footage, the position of each pig in the pen (front, middle or back of the pen) was measured three times in each lap as follows. The first recording was taken when the experimenter started moving forward from the previous pen, the second when the experimenter reached the pen, and the third when the experimenter started moving away from the pen. The position of each pig was determined based on the pig's two front legs being in either the front, middle or back third of the pen.

4.3.2.4.3 Flight Distance Test at 6 wk of Age

At 6 wk of age, the behavioural responses to an unfamiliar human walking inside the home pen were assessed in all pigs. A female experimenter slowly entered the pen and stood stationary in front of the gate for 5 s, before completing one lap of the pen walking in a clockwise direction at a pace of 1 step per/s. After reaching the starting point in front of the gate, the experimenter stood stationary for an additional 30 s before slowly exiting and moving on to test the adjacent pen. Using video footage, instantaneous point sampling was used to record the proximity of each pig to the experimenter. During the beginning and end phases of the test when the experimenter was stationary, the proximity of each pig was recorded at 5 s intervals. During the middle of the test, the proximity of each pig was recorded at every step the experimenter took around the pen. The pens contained ten rows of flooring that were each 30 cm wide. The rows of flooring were used to record proximity, such that if a pig was recorded as being 1 row away from the experimenter, there was at least 30 cm between the pig and the experimenter. Pigs in the same row of the experimenter were recorded as being 0 rows away. The position of each pig was determined based on which row the pig's two front legs were in.

4.3.2.4.4 Isolation Test at 7 wk of Age

At 7 wk of age, the behavioural and physiological responses to isolation were assessed in the 4 pigs from each pen from cohort 1. Pigs were tested individually in a portable 0.7 × 0.7 × 0.7 m arena positioned in the aisle adjacent to the home pen. Painted lines were used to mark quadrants on the arena floor. The arena was constructed of black wooden board and was elevated 0.9 m off the floor, secured to a trolley. Pigs in the arena had no visual contact with the experimenters or with other pigs. To conduct the isolation test, an experimenter lifted a test pig from the home pen and gently placed the pig in the centre of the arena, in the middle of the intersection of the quadrants. The 2 min test commenced once the experimenter moved away from the arena and at the end of the test, the pig was lifted up and gently placed in the home pen and the next pig from the pen was tested. Using video footage, the number of entries each pig made into sections of the arena were recorded, in addition to the latency of the pig to vocalise and attempt to escape and the total number of vocalisations and escape attempts. An escape attempt was defined as the pig lifting at least two legs from the floor simultaneously, either in an attempt to jump or climb out of the arena. A maximum response time of 120 s was given if a pig did not vocalise or make an escape attempt. Blood samples for subsequent analysis of serum cortisol and IgA were collected from all test pigs 45 min after isolation testing (see subsequent section "Sample Collection Details and Assay Characteristics" for further detail).

4.3.2.4.5 Human Approach Test at 9 wk of Age

At 9 wk of age, the behavioural responses to a stationary unfamiliar human were assessed in 4 pigs from each pen. Pigs were tested individually in a 3.0 × 1.5 × 1.5 m arena constructed of black wooden boarding inside an empty pen in the same room where the pigs were housed. The arena contained a solid 1.5 m high gate on one of the 1.5 m long walls. Painted lines were used to mark quadrants on the arena floor. Two teams of experimenters conducted testing simultaneously in identical arenas located on opposite sides of the room. In each team, one experimenter (a male experimenter in one team, a female in the other) moved the pig from the home pen to the test arena with the assistance of a solid stockboard. Pigs were selected for testing on the basis of the first pig sighted at the front of the pen when the experimenter opened the pen gate, therefore a mix of cohort 1 and 2 pigs were tested. Once the pig was inside the arena an observer recorded the number of entries the pig made into sections of the empty arena. After 2 min, the experimenter slowly entered the arena and stood stationary in front of the gate for 3 min and verbally relayed the pig's behaviour to the observer. The following behavioural responses to the stationary experimenter in the arena were recorded: latency of the pig to approach within 0.5 m, time spent within 0.5 m, latency to initiate physical interaction (sniffing, nosing, chewing or stepping on experimenter), and the number of physical interactions with the experimenter. A maximum response time of 180 s was given if a pig did not approach or interact with the experimenter. At the end of the test the pig was walked back to the home pen and the next pig from the pen was tested.

4.3.2.4.6 Flight Distance Test at 14 wk of Age

At 14 wk of age, the behavioural responses to an unfamiliar human walking inside the home pen were assessed in all pigs. A female experimenter slowly entered the pen and stood stationary in front of the gate for 5 s, before completing one lap of the pen walking in a clockwise direction at a pace of 1 step per/s. After reaching the starting point in front of the gate, the experimenter stood stationary for an additional 30 s before slowly exiting and moving on to test the adjacent pen. Using video footage, instantaneous scan sampling at 5 s intervals was used to record the number of pigs within 1 m of the experimenter at each phase of the test.

4.3.2.4.1 Growth at 4 d, 22 d and 18 wk of Age

Individual weights of all pigs were obtained at 4 and 22 d of age during the lactation period and at 18 wk of age during the grower/finisher period.

4.3.2.5 Growth, Injuries and Survival

4.3.2.5.1 Piglet Survival until Weaning at 4 wk of Age

Piglet survival was assessed through records of litter counts at 1 d of age and at weaning at 4 wk of age.

4.3.2.5.2 Injuries at 4, 5 and 17 wk of Age

Injuries were assessed in the 4 pigs from each pen from cohort 1 at 4, 5 and 17 wk of age. In wk 4 and 5 respectively, the assessments took place 2 d prior and 1 wk post weaning. Each pig received two injury scores; one for scratches and abrasions on the head and one for scratches and abrasions on the rest of the body. Injuries were scored by one experimenter using the following scale from Widowski and colleagues (2003): 0 – no scratches or skin loss were evident; 1 – one to three small (2 cm) scratches or areas of abraded skin were evident; 2 – one to three larger (> 2 cm) scratches or areas of abraded skin were observed; 3 – more than three scratches (usually > 2 cm) or larger areas of superficial skin loss.

4.3.3 Sample Collection Details and Assay Characteristics

Blood sampling was carried out by two teams of experienced technicians that collected samples via jugular venipuncture from pigs in adjacent pens simultaneously. In each team, there was one person to secure the pig and one person to collect the sample. Samples were collected within 2 min of securing the pig. During the lactation and weaner periods pigs were held inverted for sample collection and during the grower/finisher period pigs were secured with a snout snare. Samples obtained 2 h post weaning for haematology were collected into EDTA coated tubes (BD Vacutainer, New South Wales, Australia) and transported to a commercial laboratory where the absolute numbers of neutrophil and lymphocyte cells were counted using a Sysmex XT-2000i analyser (Sysmex, Japan). All other samples were collected into serum tubes (BD Vacutainer, New South Wales, Australia), inverted 5-6 times and left to clot for at least 1 h before being centrifuged for 10 min at 1300 × *g*. After centrifugation serum was transferred to polypropylene tubes and stored in a –20 ºC freezer before being moved to a –80 ºC freezer. All samples were assayed in duplicate. Serum concentrations of cortisol were determined using a commercial radioimmunoassay kit (Cortisol Coated Tube RIA Kit, MP Biomedicals Australia Pty Ltd, Seven Hills, New South Wales, Australia). The intra-assay coefficients of variation for samples containing 20.2 and 53.2 ng/L were 6.9% and 8.0% and the inter-assay coefficients of variation were 8.7% and 9.2% respectively. Serum concentrations of haptoglobin were determined using a pig haptoglobin ELISA kit (#CSB-E13424p, Cusabio, Houston, Texas, USA). The samples were diluted 1:50 in serum diluent as recommended by the manufacturer. The intra-assay coefficient of variation was 4.8% and the inter-assay coefficient of variation was 7.4%. Serum concentrations of IgA were determined using a pig immunoglobulin A ELISA kit (#CSB-E13234p, Cusabio, Houston, Texas, USA). The samples were diluted 1:1500 or 1:1000 in serum diluent as recommended by the manufacturer. The intra-assay coefficient of variation was 6.4% and the inter-assay coefficient of variation was 8.5%. Serum concentrations of BDNF were determined using a BDNF ELISA kit (#BEK-2211, Biosensis, Thebarton, South Australia, Australia). The samples were diluted 1:5 or 1:10 in serum diluent. The intra-assay coefficient of variation was 3.3% and the inter-assay coefficient of variation was 13.5%.

4.3.4 Observer reliability

Observers conducting video observations, tear staining assessments and injury assessments were blind to treatment, with the exception of measurements collected in the home pens during the lactation period where it was impossible to be blind to the housing treatment. Reliability was assessed for all video observations using intraclass correlation coefficient estimates based on single measure, absolute agreement, two-way mixed effects models. Intraclass correlation coefficient estimates were all above 0.89, with 95% confidence intervals for the estimates between 0.82 and 1, indicating good to excellent reliability.

4.3.5 Statistical Analysis

The unit of analysis for statistical analyses of measurements collected during the lactation period was a single litter of piglets. The unit of analysis for statistical analyses of measurements collected during the weaner and grower/finisher periods was a pair of litters. Unless otherwise stated, all measurements were analysed using analysis of variance with one of structures presented in Table 4.2. To allow for separate residual variance between housing treatments, Restricted Maximum Likelihood mixed models were used to analyse the number of interactions with the standing human in the human approach test at 3 wk of age, the number of piglets weaned and the proportion of mortalities from 1 d of age until weaning. Restricted Maximum Likelihood mixed models were also used to analyse the number of baulks and the proportion of pigs that baulked when being moved out of the home pen at 21 wk of age, to look at treatment effects after adjusting for the number of interventions used by the stockperson, since stockperson behaviour may affect pig behaviour.

Prior to analyses, the following transformations were conducted so that the amount of residual variation did not increase as the mean increased and/or so that the residuals did not have a markedly skewed distribution: logarithmic transformations – all physiological measurements, the number of vocalisations during processing and vaccination; square root transformations – the number of interactions with each stimulus in the novel object test and in the human approach tests, the number of escape attempts in the isolation test; angular transformations – all behavioural time budget measurements, all behavioural measurements after weaning, latency to approach and interact and time spent near each stimulus in the novel object test and in the human approach tests, proportion of pigs in the front, middle and back of the pen in the walking human test, proportion of pigs that made an escape attempt and vocalised in the isolation test.

Non-parametric permutation tests were used to calculate *p*-values for the following variables: proportion of pigs playing at 1, 2, 3 and 12 wk of age and at 0-1 and 3-4 h after weaning, proportion of pigs climbing on the sow at 1 wk of age, proportion of pigs nosing pen mates at 12 wk of age and at all time periods after weaning, proportion of pigs attempting escape, ear biting and tail biting at all time periods after weaning, proportion of pigs engaging in aggression at 3-4 h after weaning, all sow behaviours in the maternal responsiveness test, the number of escape attempts, latency to make an escape attempt and the proportion of pigs that made an escape attempt and vocalised in the isolation test, proportion of pigs that jumped, ran and vocalised in response to tattoo branding. In all these cases there were many pens with no pigs partaking in the behaviour, or, there were discreteness in the data. These permutation tests were calculated using the usual F statistic obtained from the analysis of variance in Table 4.2, but with the permutation distribution implied from the structure of this analysis of variance.

Due to equipment failures (camera and microphone malfunctions), there were no tear staining measurements for one litter in wk 1 and two litters in wk 4, no vocalisation measurements for two litters at processing and one litter at vaccination, and no behaviour measurements for three pairs in response to moving out of the home pens at 21 wk of age. Due to blood samples being haemolysed or returning results outside the normal detectable range of the assay, there were no BDNF concentration measurements for one pen in wk 4. Analyses for the number of piglets weaned and the proportion of mortalities during the lactation period included foster piglets.

Analyses were carried out using the ANOVA directive, the REML directive and the APERMTEST procedure of Genstat for Windows 19th edition (VSN International, 2018).

Table 4.2. Analysis of variance structures for statistical analysis of measurements collected during different periods of the experiment. Refer to Section 4.3.1.3 and Figure 4.3 to see allocation of treatments within blocks and rows in the weaner and grower/finisher housing.

Measurements collected during the lactation period	
Source of variation	**Degrees of freedom**
Housing system	1
Human contact treatment	1
Housing system by human contact treatment interaction	1
Residual	44
Measurements collected during the weaner and grower/finisher periods	
Source of variation	**Degrees of freedom**
Block stratum	5
Row within block stratum	
Housing system	1
Residual	5
Pair within row stratum	
Human contact treatment	1
Housing system by human contact treatment interaction	1
Residual	10

4.4 Results

4.4.1 Maternal Responsiveness Test

In response to an audio recording of unfamiliar piglet squeals during the maternal responsiveness test in wk 2 of lactation, there were higher proportions of LP sows than FC sows that changed posture from resting to standing (13 vs. 2.1 % of sows; $p = 0.034$) and showed behaviour directed towards their piglets (15 vs. 2.1 % of sows; $p = 0.027$; Table 4.3). There were no significant effects ($p > 0.05$) of housing system on the proportion of sows that were alert or vocalised during the test. There were also no significant effects ($p > 0.05$) of human contact treatment and no significant ($p > 0.05$) housing system × human contact treatment interactions on the behaviour of sows in the maternal responsiveness test.

Table 4.3. Effects of early housing and human contact for piglets on the behavioural responses of sows to an audio recording of unfamiliar piglet vocalisations in the maternal responsiveness test in wk 2 of lactation. FC, farrowing crate; LP, loose pen; C, routine human contact; +HC, positive human contact.

	Housing System			**Human Contact**			***p*-Value**		
	FC	**LP**	**S.e.d.**	**C**	**+HC**	**S.e.d.**	**Housing System**	**Human Contact**	**Housing × Contact**
Behaviour (% sows)									
Vocalised	13	15	7.42	14	14	7.42	0.80 [a]	1.00 [a]	0.32 [a]
Alert (directed head towards recording)	32	40	10.1	35	37	10.1	0.53 [a]	0.97 [a]	0.97 [a]
Changed posture (lying or sitting to standing)	2.1	13	4.76	5.2	9.4	4.76	**0.034** [a]	0.32 [a]	0.32 [a]
Showed behaviour towards piglets	2.1	15	5.65	10	6.2	5.65	**0.027** [a]	0.42 [a]	0.42 [a]

[a] *p*-values calculated using permutation tests.

4.4.2 Time Budgets of Behaviour and Basal Physiological Measurements

4.4.2.1 Time Budgets of Behaviour at 1, 2, 3, 8 and 12 wk of Age

At 3 wk of age, there were more +HC piglets than C piglets nosing pen mates (0.31 vs. 0.19 %; $p = 0.031$), however there were no other significant effects ($p > 0.05$) of human contact treatment on the behavioural time budgets of pigs at 1, 2, 3, 8 and 12 wk of age (Table 4.4).

There were more FC piglets than LP piglets nosing pen mates at 1 wk (0.22 vs. 0.069 %; $p < 0.001$) and 3 wk (0.48 vs. 0.094 %; $p < 0.001$) of age. There were more LP piglets than FC piglets engaging in aggressive (0.42 vs. 0.18 %; $p = 0.034$) and play behaviour (0.13 vs. 0.015 %; $p = 0.0061$) at 2 wk of age, and tendencies for more LP pigs engaging in play behaviour at 3 wk (0.18 vs. 0.071 %; p = 0.079) and 8 wk (0.47 vs. 0.29 %; p = 0.071) of age.

There were more FC piglets than LP piglets climbing on the sow at 2 wk (0.19 vs. 0.045 %; p = 0.0017) and 3 wk (0.23 vs. 0.079 %; p = 0.0022) of age, and a similar tendency at 1 wk of age (0.058 vs. 0.021 %; p = 0.073). There were more LP piglets than FC piglets investigating the sow at 2 wk (0.82 vs. 0.43 %; p = 0.0063) and 3 wk (0.92 vs. 0.65 %; p = 0.046) of age. There were more LP piglets than FC piglets sitting or lying away from the sow at 3 wk of age (55 vs. 47 %; p = 0.025), and tendencies for fewer LP piglets sitting or lying with the sow at 2 wk (22 vs. 28 %; p = 0.053) and 3 wk (20 vs. 25 %; p = 0.065) of age.

There were more LP piglets than FC piglets walking (2.4 vs. 1.2 %; $p < 0.001$) and standing (2.3 vs. 1.1 %; $p < 0.001$) at 2 wk of age. There were more LP pigs than FC pigs interacting with the pen (25 vs. 20 %; p = 0.039) and fewer LP pigs sitting or lying (49 vs. 55 %; p = 0.034) at 8 wk of age. There were no other significant effects ($p > 0.05$) of housing system on the behavioural time budgets of pigs at 1, 2, 3, 8 and 12 wk of age. There was a significant housing system × human contact treatment interaction on the number of pigs investigating pen mates at 8 wk of age (back-transformed means, +HC/FC: 2.0 %, C/FC: 2.2 %, +HC/LP: 2.2 %, C/LP: 1.7 %; angularly-transformed means, +HC/FC: 8.1, C/FC: 8.6, +HC/LP: 8.6, C/LP: 7.5; s.e.d. = 0.495 – 0.959; p = 0.042), but no other significant ($p > 0.05$) interactions effects on behavioural time budgets.

Table 4.4. Effects of early housing and human contact on the time budgets of behaviour of pigs at 1, 2, 3, 8 and 12 wk of age. All data were angularly transformed prior to analysis; back-transformed means are presented in parentheses. FC, farrowing crate; LP, loose pen; C, routine human contact; +HC, positive human contact.

	Housing System			**Human Contact**			***p*-Value**		
	FC	**LP**	**S.e.d.**	**C**	**+HC**	**S.e.d.**	**Housing System**	**Human Contact**	**Housing × Contact**
Behaviour 1 wk, lactation period (% pigs)									
Playing	1.6 (0.080)	1.8 (0.098)	0.492	1.6 (0.082)	1.8 (0.096)	0.492	0.73 [a]	0.77 [a]	0.75 [a]
Aggression	2.4 (0.17)	2.9 (0.25)	0.467	2.3 (0.17)	2.9 (0.26)	0.467	0.30	0.23	0.37
Nosing pen mate	2.7 (0.22)	1.5 (0.069)	0.328	2.0 (0.12)	2.2 (0.15)	0.328	**0.00094**	0.54	0.56
Investigating pen mate	3.5 (0.37)	3.6 (0.39)	0.390	3.2 (0.31)	3.9 (0.46)	0.390	0.85	0.077	0.83
Tail biting	0 (0)	0 (0)	-	0 (0)	0 (0)	-	1.0	1.0	1.0
Ear biting	0 (0)	0 (0)	-	0 (0)	0 (0)	-	1.0	1.0	1.0
Climbing on sow	1.4 (0.058)	0.83 (0.021)	0.301	1.0 (0.031)	1.2 (0.044)	0.301	0.073 [a]	0.53 [a]	0.052 [a]
Investigating sow	4.0 (0.48)	4.5 (0.62)	0.423	4.5 (0.61)	4.0 (0.49)	0.423	0.19	0.31	0.65
Interacting with pen	9.0 (2.5)	8.3 (2.1)	0.576	8.6 (2.2)	8.8 (2.3)	0.576	0.20	0.72	0.78
Walking	8.3 (2.1)	9.1 (2.5)	0.495	8.7 (2.3)	8.8 (2.4)	0.495	0.12	0.72	0.67
Standing	9.3 (2.6)	10 (3.0)	0.517	9.7 (2.9)	9.5 (2.8)	0.517	0.20	0.70	0.96
Sitting/lying, away from sow	47 (53)	47 (54)	2.02	47 (54)	17 (8.2)	2.02	0.85	0.81	0.82
Sitting/lying, with sow	28 (23)	27 (21)	2.29	28 (21)	28 (22)	2.29	0.58	0.84	0.69
Behaviour 2 wk, lactation period (% pigs)									
Playing	0.69 (0.015)	2.1 (0.13)	0.480	1.3 (0.049)	1.5 (0.069)	0.480	**0.0061** [a]	0.64 [a]	0.32 [a]
Aggression	2.4 (0.18)	3.1 (0.42)	0.595	2.8 (0.24)	3.3 (0.34)	0.595	**0.034**	0.35	0.78
Nosing pen mate	2.5 (0.18)	2.0 (0.12)	0.351	2.1 (0.13)	2.4 (0.18)	0.351	0.20	0.35	0.67
Investigating pen mate	3.7 (0.41)	4.3 (0.55)	0.423	3.9 (0.47)	4.0 (0.49)	0.432	0.19	0.89	0.80
Tail biting	0 (0)	0 (0)	-	0 (0)	0 (0)	-	1.0	1.0	1.0
Ear biting	0 (0)	0 (0)	-	0 (0)	0 (0)	-	1.0	1.0	1.0
Climbing on sow	2.5 (0.19)	1.2 (0.045)	0.382	1.9 (0.11)	1.8 (0.10)	0.382	**0.0017**	0.90	0.33
Investigating sow	3.8 (0.423)	5.2 (0.82)	0.506	4.5 (0.62)	4.4 (0.60)	0.506	**0.0063**	0.86	0.69
Interacting with pen	9.2 (2.6)	9.7 (2.9)	0.693	9.7 (2.9)	9.2 (2.6)	0.693	0.44	0.43	0.12
Walking	6.4 (1.2)	9.0 (2.4)	0.649	7.3 (1.6)	8.0 (2.0)	0.649	**0.00023**	0.27	0.14
Standing	6.0 (1.1)	8.6 (2.3)	0.703	7.2 (1.6)	7.4 (1.7)	0.703	**0.00048**	0.76	0.58
Sitting/lying, away from sow	46 (51)	46 (52)	2.34	47 (54)	44 (49)	2.34	0.98	0.23	0.44
Sitting/lying, with sow	32 (28)	28 (22)	2.09	29 (24)	31 (26)	2.09	0.053	0.53	0.33

	FC	LP	S.e.d.	C	+HC	S.e.d.	Housing System	Human Contact	Housing × Contact
Behaviour 3 wk, lactation period (% pigs)									
Playing	1.5 (0.071)	2.4 (0.18)	0.492	1.8 (0.099)	2.2 (0.14)	0.492	0.079 [a]	0.47 [a]	0.54 [a]
Aggression	4.0 (0.49)	4.3 (0.55)	0.612	3.8 (0.43)	4.5 (0.63)	0.612	0.69	0.21	0.57
Nosing pen mate	4.0 (0.48)	1.8 (0.094)	0.323	2.5 (0.19)	3.2 (0.31)	0.323	$\mathbf{2.4 \times 10^{-8}}$	**0.031**	0.57
Investigating pen mate	5.1 (0.78)	4.6 (0.65)	0.430	4.1 (0.62)	5.2 (0.82)	0.430	0.32	0.11	0.47
Tail biting	0 (0)	0 (0)	-	0 (0)	0 (0)	-	1.0	1.0	1.0
Ear biting	0 (0)	0 (0)	-	0 (0)	0 (0)	-	1.0	1.0	1.0
Climbing on sow	2.8 (0.23)	1.6 (0.079)	0.353	1.9 (0.11)	2.5 (0.18)	0.353	**0.0022**	0.13	0.88
Investigating sow	4.6 (0.65)	5.5 (0.92)	0.419	4.9 (0.73)	5.2 (0.83)	0.419	**0.046**	0.46	0.71
Interacting with pen	11 (3.4)	11 (3.6)	0.770	10 (3.3)	11 (3.7)	0.770	0.54	0.28	0.89
Walking	8.4 (2.1)	9.0 (2.4)	0.596	8.6 (2.2)	8.8 (2.3)	0.596	0.34	0.75	0.53
Standing	9.6 (2.8)	9.4 (2.7)	0.669	9.6 (2.8)	9.4 (2.7)	0.669	0.81	0.68	0.35
Sitting/lying, away from sow	43 (47)	48 (55)	1.87	46 (52)	45 (49)	1.87	**0.025**	0.39	0.53
Sitting/lying, with sow	30 (25)	26 (20)	1.91	27 (21)	29 (23)	1.91	0.065	0.50	0.61
Behaviour 8 wk, weaner period (% pigs)									
Playing	3.1 (0.29)	3.9 (0.47)	0.368	3.6 (0.39)	3.5 (0.36)	0.528	0.071	0.78	0.46
Aggression	11 (3.6)	12 (4.4)	0.937	12 (4.3)	11 (3.8)	0.998	0.30	0.55	0.87
Nosing pen mate	3.3 (0.34)	4.5 (0.62)	0.598	4.3 (0.56)	3.5 (0.38)	0.823	0.11	0.38	0.23
Investigating pen mate	8.3 (2.1)	8.0 (2.0)	0.892	8.1 (2.0)	8.3 (2.1)	0.350	0.75	0.47	**0.042**
Tail biting	3.6 (0.39)	4.1 (0.52)	0.230	4.1 (0.50)	3.6 (0.40)	0.656	0.061	0.54	0.43
Ear biting	6.7 (1.4)	7.0 (1.5)	0.477	7.2 (1.6)	6.5 (1.3)	0.858	0.63	0.49	0.92
Interacting with pen	27 (20)	30 (25)	1.17	27 (21)	29 (24)	2.25	**0.039**	0.44	0.81
Walking	11 (3.6)	12 (4.4)	0.351	12 (4.3)	11 (3.8)	0.642	0.11	0.90	0.79
Standing	8.5 (2.2)	9.9 (2.9)	0.801	9.2 (2.6)	9.2 (2.6)	0.623	0.15	0.99	0.73
Sitting/lying	48 (55)	45 (49)	1.01	47 (53)	46 (51)	2.66	**0.034**	0.73	0.90
Behaviour 12 wk, grower/finisher period (% pigs)									
Playing	0.96 (0.028)	0.72 (0.016)	0.383	0.93 (0.026)	0.75 (0.017)	0.611	0.56 [a]	0.78 [a]	0.79 [a]
Aggression	8.0 (1.9)	7.6 (1.8)	0.367	8.0 (1.9)	7.6 (1.8)	0.388	0.41	0.38	0.33
Nosing pen mate	1.0 (0.031)	1.4 (0.058)	0.519	0.87 (0.023)	1.5 (0.069)	0.557	0.57 [a]	0.29 [a]	0.56 [a]
Investigating pen mate	6.7 (1.4)	7.5 (1.7)	0.474	7.3 (1.6)	6.9 (1.4)	0.611	0.16	0.53	0.45
Tail biting	2.5 (0.19)	1.9 (0.11)	0.385	2.3 (0.16)	2.1 (0.13)	0.448	0.16	0.68	0.56
Ear biting	3.7 (0.42)	4.1 (0.50)	0.438	4.2 (0.53)	3.6 (0.39)	0.449	0.44	0.23	0.60
Interacting with pen	27 (21)	29 (23)	1.04	29 (23)	27 (20)	1.32	0.18	0.20	0.47
Walking	9.3 (2.6)	9.5 (2.7)	0.383	9.3 (2.6)	9.5 (2.7)	0.301	0.68	0.69	0.64
Standing	14 (5.6)	13 (4.9)	1.17	14 (5.5)	15 (6.6)	0.690	0.45	0.42	0.99
Sitting/lying	51 (60)	49 (57)	1.62	49 (57)	51 (60)	1.55	0.30	0.27	0.48

[a] *p*-values calculated using permutation tests.

4.4.2.2 Basal Physiology at 4, 8, 12 and 17 wk of Age

At 12 wk of age, +HC pigs had lower serum cortisol concentrations than C pigs (29 vs. 36 ng/ml; *p* = 0.040; Table 4.5). There were no significant effects ($p > 0.05$) of human contact treatment on cortisol concentrations at 4, 8 and 17 wk of age, and no significant effects ($p > 0.05$) of housing system at any weeks. At 4 wk of age, +HC piglets had higher serum BDNF concentrations than C piglets (1500 vs. 560 pg/ml; *p* = 0.020). There were no significant effects ($p > 0.05$) of human contact treatment on BDNF concentrations at 8 and 12 wk of age, but +HC pigs had higher BDNF concentrations than C pigs at 17 wk of age (54 vs. 42 pg/ml; *p* = 0.036). At 4 wk of age, LP piglets had higher serum BDNF concentrations than FC piglets (1500 vs. 550 pg/ml; *p* = 0.014), but there were no significant effects ($p > 0.05$) of housing system on concentrations at 8, 12 or 17 wk of age. There were no significant effects ($p > 0.05$) of housing system on serum IgA concentrations at 4, 8 or 12 wk of age, however at 17 wk of age, LP pigs had lower concentrations than FC pigs (1100 vs. 2000 µg/ml; *p* = 0.021). There were no significant effects ($p > 0.05$) of human contact treatment on IgA concentrations at any weeks, and no significant ($p > 0.05$) housing system × human contact treatment interactions on basal cortisol, IgA or BDNF concentrations at any weeks.

Table 4.5. Effects of early housing and human contact on basal concentrations of serum cortisol, brain-derived neurotrophic factor (BDNF) and immunoglobulin A (IgA) in pigs at 4, 8, 12 and 17 wk of age. All measurements were logarithmically transformed prior to analysis; back-transformed means are presented in parentheses. FC, farrowing crate; LP, loose pen; C, routine human contact; +HC, positive human contact.

	Housing System			**Human Contact**			***p*-Value**		
	FC	**LP**	**S.e.d.**	**C**	**+HC**	**S.e.d.**	**Housing System**	**Human Contact**	**Housing × Contact**
Cortisol (ng/ml)									
4 wk	1.5 (33)	1.5 (30)	0.0573	1.5 (32)	1.5 (31)	0.0573	0.44	0.77	0.36
8 wk	1.6 (41)	1.7 (52)	0.0497	1.7 (48)	1.6 (44)	0.0315	0.088	0.19	0.81
12 wk	1.5 (34)	1.5 (31)	0.0315	1.6 (36)	1.5 (29)	0.0359	0.31	**0.040**	0.29
17 wk	1.6 (36)	1.5 (34)	0.0225	1.6 (39)	1.5 (34)	0.0282	0.35	0.19	0.35
BDNF (pg/ml)									
4 wk	2.7 (550)	3.2 (1500)	0.176	2.7 (560)	3.2 (1500)	0.176	**0.014**	**0.020**	0.089
8 wk	2.7 (490)	2.6 (440)	0.178	2.6 (420)	2.7 (520)	0.235	0.78	0.68	0.52
12 wk	2.0 (91)	1.8 (66)	0.109	1.8 (68)	1.9 (88)	0.0935	0.25	0.27	0.27
17 wk	1.7 (48)	1.7 (48)	0.0912	1.6 (42)	1.7 (54)	0.0460	0.98	**0.036**	0.14
IgA (µg/ml)									
4 wk	2.9 (720)	3.0 (910)	0.0655	2.9 (790)	2.9 (830)	0.0655	0.13	0.74	0.76
8 wk	3.6 (4100)	3.6 (4000)	0.0404	3.7 (4500)	3.6 (3700)	0.0503	0.73	0.17	0.36
12 wk	3.2 (1600)	3.2 (1600)	0.162	3.1 (1400)	3.3 (1800)	0.0849	0.95	0.19	0.30
17 wk	3.3 (2000)	3.1 (1100)	0.0701	3.2 (1600)	3.2 (1400)	0.0943	**0.021**	0.54	0.32

4.4.2.3 Tear Staining Assessments at 1, 4, 7, 12 and 17 wk of Age

Compared to FC piglets, LP piglets had higher tear staining scores (1.4 vs. 1.0; $p < 0.001$) and larger areas (14 vs. 8.4 mm^2; $p < 0.001$) and perimeters (21 vs. 14 mm; $p < 0.001$) of tear staining at 4 wk of age (Table 4.6). There were no significant effects ($p > 0.05$) of housing system on tear staining at 1, 7, 12 or 17 wk of age, although there were tendencies for LP pigs to have higher scores than FC pigs at 1 wk of age (1.0 vs. 0.87; $p = 0.060$), and greater areas of staining at 7 wk of age (22 vs. 13 mm^2; $p = 0.082$). There were no significant effects ($p > 0.05$) of human contact treatment on tear staining at any weeks, although at 12 wk of age there were tendencies for C pigs to have higher scores (2.0 vs. 1.7; $p = 0.057$) and larger perimeters (44 vs. 36 mm; $p = 0.082$) of staining compared to +HC pigs. There were no significant ($p > 0.05$) housing system × human contact treatment interactions on tear staining at any weeks.

Table 4.6. Effects of early housing and human contact on the severity of tear staining in pigs at 1, 4, 7, 12 and 17 wk of age. FC, farrowing crate; LP, loose pen; C, routine human contact; +HC, positive human contact.

	Housing System			**Human Contact**			***p*-Value**		
	FC	**LP**	**S.e.d.**	**C**	**+HC**	**S.e.d.**	**Housing System**	**Human Contact**	**Housing × Contact**
Tear staining 1 wk									
Area (mm^2)	4.6	5.5	0.685	5.4	4.7	0.685	0.20	0.28	0.74
Perimeter (mm)	9.1	11	1.25	11	9.5	1.25	0.11	0.28	0.77
Score	0.87	1.0	0.0814	0.99	0.90	0.0814	0.060	0.25	0.95
Tear staining 4 wk									
Area (mm^2)	8.4	14	1.40	11	11	1.40	**0.00055**	0.90	0.89
Perimeter (mm)	14	21	1.77	18	17	1.77	**0.00036**	0.71	0.36
Score	1.0	1.4	0.0975	1.3	1.2	0.0975	**0.000078**	0.22	0.65
Tear staining 7 wk									
Area (mm^2)	13	22	4.07	17	17	3.20	0.082	0.99	0.52
Perimeter (mm)	17	20	3.50	19	19	2.16	0.45	0.93	0.62
Score	1.0	1.3	0.126	1.2	1.1	0.132	0.14	0.65	0.32
Tear staining 12 wk									
Area (mm^2)	98	110	22.0	110	90	18.5	0.70	0.32	0.19
Perimeter (mm)	39	41	5.09	44	36	3.68	0.61	0.082	0.19
Score	1.9	1.8	0.244	2.0	1.7	0.129	0.88	0.057	0.43
Tear staining 17 wk									
Area (mm^2)	160	210	39.6	210	160	26.2	0.26	0.12	0.097
Perimeter (mm)	58	61	7.86	64	54	5.61	0.68	0.10	0.14
Score	2.3	2.4	0.237	2.5	2.2	0.182	0.52	0.12	0.10

4.4.3 Behavioural and Physiological Responses to Routine Stressors

4.4.3.1 Piglet Processing at 4 d of Age

During processing at 4 d of age, +HC piglets showed a lower intensity of escape behaviour during capture by the stockperson compared to C piglets (mean score, 1.6 vs. 2.1; $p = 0.010$; Table 4.7). FC piglets also showed a lower intensity of escape behaviour during capture by the stockperson compared to LP piglets (1.7 vs. 2.1; $p = 0.041$). There were no significant effects ($p > 0.05$) of housing system or human contact treatment on the intensity of escape behaviour by piglets during oral vaccination or tail docking. Compared to C pigs, +HC piglets vocalised for shorter durations (4.8 vs. 6.7 s; $p = 0.028$) and had a shorter average vocalisation length (0.43 vs. 0.54 s; $p < 0.001$) during processing. There were no significant effects ($p > 0.05$) of human contact treatment on the number or peak frequency of vocalisations. There were also no significant effects ($p > 0.05$) of housing system on vocalisations during processing, although there were tendencies for FC piglets to vocalise more often (12 vs. 8.7; $p = 0.057$) and at a higher frequency (3000 vs. 2800 Hz; $p = 0.052$) than LP piglets. There were no significant effects ($p > 0.05$) of housing system or human contact treatment on serum cortisol concentrations 1 h after processing, and no significant ($p > 0.05$) housing system × human contact treatment interactions on any measurements during processing at 4 d of age.

4.4.3.2 Vaccination at 3 wk of Age

During vaccination at 3 wk of age, +HC piglets showed a lower intensity of escape behaviour compared to C piglets during capture by the stockperson (mean score, 0.90 vs. 1.1; $p = 0.040$; Table 4.8). There were no significant effects ($p > 0.05$) of human contact treatment on piglet vocalisations during vaccination. There were also no significant effects ($p > 0.05$) of housing system, and no significant ($p > 0.05$) housing system × human contact treatment interactions on piglets' escape behaviour and vocalisations during vaccination at 3 wk of age.

Table 4.7. Effects of early housing and human contact on the behavioural and physiological responses of piglets to processing at 4 d of age. The number of calls during processing and serum cortisol concentrations 1 h after processing were logarithmically transformed prior to analysis; back-transformed means are presented in parentheses. FC, farrowing crate; LP, loose pen; C, routine human contact; +HC, positive human contact.

	Housing System			**Human Contact**			***p*-Value**		
	FC	**LP**	**S.e.d.**	**C**	**+HC**	**S.e.d.**	**Housing System**	**Human Contact**	**Housing × Contact**
Escape behaviour									
Capture	1.7	2.1	0.174	2.1	1.6	0.174	**0.041**	**0.010**	0.52
Oral vaccination	2.2	2.0	0.170	2.2	2.0	0.170	0.18	0.13	0.27
Tail docking	1.7	1.9	0.163	1.9	1.7	0.163	0.22	0.35	0.78
Vocalisations									
Duration of calls (s)	6.3	5.1	0.834	6.7	4.8	0.833	0.16	**0.028**	0.47
Average call length (s)	0.48	0.49	0.0315	0.54	0.43	0.0315	0.91	**0.00096**	0.69
Number of calls	1.1 (12)	0.94 (8.7)	0.0784	1.1 (11)	0.97 (9.3)	0.0783	0.057	0.41	0.79
Peak frequency of calls (Hz)	3000	2800	84.9	2900	2900	84.8	0.052	0.85	0.57
Physiology									
Cortisol 1 h after processing (ng/ml)	2.5 (340)	2.5 (280)	0.0655	2.5 (320)	2.5 (290)	0.0655	0.21	0.50	0.084

Table 4.8. Effects of early housing and human contact on the behavioural responses of piglets to vaccination at 3 wk of age. The number of calls during vaccination was logarithmically transformed prior to analysis; back-transformed means are presented in parentheses. FC, farrowing crate; LP, loose pen; C, routine human contact; +HC, positive human contact.

	Housing System			**Human Contact**			***p*-Value**		
	FC	**LP**	**S.e.d.**	**C**	**+HC**	**S.e.d.**	**Housing System**	**Human Contact**	**Housing × Contact**
Escape behaviour									
Capture	1.0	1.0	0.101	1.1	0.90	0.101	0.84	**0.040**	0.060
Vocalisations									
Duration of calls (s)	2.9	2.7	0.310	2.7	2.9	0.310	0.44	0.43	0.91
Average call length (s)	0.63	0.61	0.0299	0.61	0.62	0.0299	0.57	0.74	0.18
Number of calls	0.64 (4.4)	0.62 (4.2)	0.0382	0.62 (4.2)	0.64 (4.4)	0.0382	0.70	0.60	0.28
Peak frequency of calls (Hz)	2900	2900	96.8	2900	3000	96.8	0.76	0.27	0.80

4.4.3.3 Weaning and Mixing at 4 wk of Age

There were significant housing system × human contact treatment interactions on the number of pigs nosing pen mates, investigating pen mates, interacting with the pen, and attempting to escape from the pen during the period from 0-1 h after weaning and mixing (Table 4.9). Only +HC/LP pigs were observed nosing pen mates (back-transformed means, +HC/FC: 0 %, C/FC: 0 %, +HC/LP: 0.47 %, C/LP: 0 %; angularly transformed means, +HC/FC: 0, C/FC: 0, +HC/LP: 3.9, C/LP: 0; s.e.d. = 0.968; p = 0.0055). +HC/LP pigs were also most frequently observed investigating pen mates (back-transformed means, +HC/FC: 1.6 %, C/FC: 1.5 %, +HC/LP: 5.6 %, C/LP: 1.2 %; angularly transformed means, +HC/FC: 7.3, C/FC: 7.0, +HC/LP: 14, C/LP: 6.4; s.e.d. = 1.97; p = 0.020). C/LP pigs were most frequently observed attempting escape from the pen (back-transformed means, +HC/FC: 0.013 %, C/FC: 0 %, +HC/LP: 0 %, C/LP: 0.32 %; angularly transformed means, +HC/FC: 0.64, C/FC: 0, +HC/LP: 0, C/LP: 3.3; s.e.d. = 0.894; p = 0.0041). +HC/FC pigs were most frequently observed interacting with the pen (back-transformed means, +HC/FC: 56 %, C/FC: 51 %, +HC/LP: 37 %, C/LP: 41 %; angularly transformed means, +HC/FC: 48, C/FC: 40, +HC/LP: 37, C/LP: 40; s.e.d. = 3.24; p = 0.024). There were no other significant (p > 0.05) housing system × human contact treatment interactions on pig behaviours at 0-1, 3-4 and 48-49 h after weaning. There were however significant main effects on several behaviours at 0-1 after weaning, with more +HC pigs than C pigs nosing pen mates (0.12 vs. 0 %; p = 0.0055), investigating pen mates (3.3 vs. 1.4 %; p = 0.013) and engaging in aggressive behaviour (2.1 vs. 1.1 %; p = 0.040), and fewer +HC pigs sitting or lying (16 vs. 26 %; p = 0.050) and attempting escape (0.0031 vs. 0.080 %; p = 0.045). Additionally, at 0-1 after weaning there were more LP pigs than FC pigs nosing pen mates (0.12 vs. 0 %; p = 0.0055), attempting escape (0.080 vs. 0.0031 %; p = 0.042), standing (15 vs. 11 %; p = 0.018) and walking (9.7 vs. 7.1 %; p = 0.0092) and fewer LP pigs interacting with the pen (39 vs. 49 %; p = 0.025).

In the period from 3-4 h after weaning, which coincided with the period from 1-2 h after blood sampling, there were more +HC pigs than C pigs walking (3.4 vs. 2.1 %; p = 0.034; Table 4.9). There were no other significant (p > 0.05) human contact treatment effects, and no significant (p > 0.05) housing system effects on pig behaviours at 3-4 h after weaning.

In the period from 48-49 h after weaning, which coincided with the period from 0-1 h after cohorts 1 and 2 were mixed, there were more +HC pigs than C pigs interacting with the pen (50 vs. 40 %; p = 0.0015; Table 4.9) and fewer +HC pigs sitting or lying (2.0 vs. 5.6 %; p = 0.024). Additionally, at 48-49 h after weaning there were more LP pigs than FC pigs walking (13 vs. 12 %; p = 0.0049) and fewer LP pigs sitting or lying (1.9 vs. 5.7 %; $p = 0.030$). There were no other significant ($p > 0.05$) housing system or human contact treatment effects on pig behaviours at 48-49 h after weaning.

Compared to FC pigs, LP pigs had higher serum cortisol concentrations 2 h after weaning and mixing (83 vs 57 ng/ml; $p = 0.032$; Table 4.10). There was no significant effect ($p > 0.05$) of housing system on serum cortisol concentrations 49 h after weaning. Compared to +HC pigs, C pigs had higher serum cortisol concentrations 49 h after weaning (37 vs. 25 ng/ml; $p = 0.015$). There was no significant effect ($p > 0.05$) of human contact treatment on serum cortisol concentrations 2 h after weaning and mixing. There were no significant effects ($p > 0.05$) of human contact treatment or housing system on the neutrophil to lymphocyte cell ratio 2 h after weaning or serum haptoglobin concentrations 49 h after weaning. There were also no significant ($p > 0.05$) housing system × human contact treatment interactions on physiology after weaning.

4.4.3.4 Tattoo Branding at 18 wk of Age

There were no significant effects ($p > 0.05$) of housing system, human contact treatment or the interaction between the two on the behavioural responses of pigs to tattoo branding at 18 wk of age (Table 4.11).

4.4.3.1 Moving out of Pens at 21 wk of Age

When being moved out of the home pen at 21 wk of age, there was a tendency for LP pigs to baulk more compared to FC pigs, and after adjusting for the number of interventions used by stockpeople to move pigs out of then pen, this effect was significant (0.34 vs 0.22 baulks per pig; $p = 0.034$; Table 4.12). There were no significant effects ($p > 0.05$) of housing system on the proportion of pigs that baulked, the number of stockperson interventions used to move pigs, or the time taken to move pigs out of the pen. There were also no significant effects ($p > 0.05$) of human contact treatment and no significant ($p > 0.05$) housing system × human contact treatment interactions on the behavioural responses of pigs to moving out of the home pens at 21 wk of age.

Table 4.9. Effects of early housing and human contact on the behaviour of pigs at 0-1, 3-4 and 48-49 h after weaning. Observations at 3-4 h after weaning coincided with the period from 1-2 h after blood sampling. Observations at 48-49 h after weaning coincided with the period from 0-1 h after cohorts 1 and 2 were mixed. All data were angularly transformed prior to analysis; back-transformed means are presented in parentheses. FC, farrowing crate; LP, loose pen; C, routine human contact; +HC, positive human contact.

	Housing System			**Human Contact**			***p*-Value**		
	FC	**LP**	**S.e.d.**	**C**	**+HC**	**S.e.d.**	**Housing System**	**Human Contact**	**Housing × Contact**
Behaviour 0-1 h after weaning (% pigs)									
Playing	2.2 (0.14)	0.77 (0.018)	0.777	0.97 (0.029)	2.0 (0.12)	0.777	0.079 [a]	0.24 [a]	0.48 [a]
Aggression	7.9 (1.9)	6.5 (1.3)	1.09	6.0 (1.1)	8.4 (2.1)	1.09	0.22	**0.040**	0.58
Nosing pen mate	0 (0)	2.0 (0.12)	0.684	0 (0)	2.0 (0.12)	0.684	**0.0055** [a]	**0.0055** [a]	**0.0055** [a]
Investigating pen mate	7.1 (1.5)	10 (3.0)	1.39	6.7 (1.4)	11 (3.3)	1.39	0.051	**0.013**	**0.020**
Tail biting	0.55 (0.0092)	0.55 (0.0092)	0.527	0.81 (0.020)	0.29 (0.0026)	0.527	0.93 [a]	0.52 [a]	0.26 [a]
Ear biting	2.5 (0.19)	3.2 (0.32)	0.948	3.1 (0.29)	2.6 (0.21)	0.948	0.43 [a]	0.60 [a]	0.65 [a]
Attempting escape	0.32 (0.0031)	1.6 (0.080)	0.632	1.6 (0.080)	0.32 (0.0031)	0.632	**0.042** [a]	**0.045** [a]	**0.0041** [a]
Interacting with pen	44 (49)	39 (39)	2.29	40 (41)	43 (46)	2.29	**0.025**	0.23	**0.024**
Walking	15 (7.1)	18 (9.7)	0.944	16 (7.9)	17 (8.7)	0.944	**0.0092**	0.38	0.81
Standing	19 (11)	23 (15)	1.52	22 (13)	21 (13)	1.52	**0.018**	0.61	0.80
Sitting/lying	27 (21)	27 (20)	3.44	31 (26)	23 (16)	3.44	0.91	**0.050**	0.54
Behaviour 3-4 h after weaning (% pigs)									
Playing	0 (0)	0 (0)	-	0 (0)	0 (0)	-	1.0 [a]	1.0 [a]	1.0 [a]
Aggression	2.2 (0.15)	2.5 (0.19)	1.21	2.5 (0.18)	2.2 (0.15)	1.21	0.83 [a]	0.85 [a]	0.55 [a]
Nosing pen mate	0 (0)	0.26 (0.0021)	0.264	0 (0)	0.26 (0.0021)	0.264	1.0 [a]	1.0 [a]	1.0 [a]
Investigating pen mate	3.5 (0.37)	3.8 (0.43)	0.890	3.0 (0.27)	4.3 (0.55)	0.890	0.77	0.16	0.91
Tail biting	0 (0)	0.54 (0.0090)	0.378	0.27 (0.0022)	0.26 (0.0021)	0.378	0.48 [a]	1.0 [a]	1.0 [a]
Ear biting	1.6 (0.074)	0.98 (0.029)	0.875	0.65 (0.013)	1.9 (0.11)	0.875	0.51 [a]	0.18 [a]	0.70 [a]
Attempting escape	0.99 (0.030)	2.0 (0.12)	1.04	1.1 (0.036)	1.9 (0.11)	1.04	0.39 [a]	0.47 [a]	0.94 [a]
Interacting with pen	19 (10)	17 (8.6)	2.25	17 (8.1)	19 (11)	2.25	0.47	0.24	0.47
Walking	8.7 (2.3)	10 (3.1)	0.942	8.4 (2.1)	11 (3.4)	0.942	0.12	**0.034**	0.61
Standing	15 (6.3)	18 (9.1)	1.90	15 (6.6)	17 (8.7)	1.90	0.12	0.24	0.94
Sitting/lying	62 (78)	61 (77)	3.23	64 (81)	59 (74)	3.23	0.72	0.17	0.75

	FC	LP	S.e.d.	C	+HC	S.e.d.	Housing System	Human Contact	Housing × Contact
Behaviour 48-49 h after weaning (% pigs)									
Playing	3.8 (0.44)	3.7 (0.43)	1.41	3.1 (0.29)	4.5 (0.61)	1.02	0.96	0.21	0.33
Aggression	11 (3.6)	13 (4.8)	1.03	13 (4.8)	11 (3.6)	1.29	0.14	0.20	0.36
Nosing pen mate	0.29 (0.0026)	0 (0)	0.291	0 (0)	0.29 (0.0026)	0.291	1.0 [a]	1.0 [a]	1.0 [a]
Investigating pen mate	6.1 (1.1)	6.4 (1.2)	1.42	6.1 (1.1)	6.4 (1.2)	0.925	0.84	0.74	0.55
Tail biting	0.68 (0.014)	2.1 (0.13)	0.354	1.1 (0.034)	1.7 (0.087)	0.759	0.052 [a]	0.40 [a]	0.36 [a]
Ear biting	3.0 (0.27)	1.4 (0.056)	0.776	2.3 (0.16)	2.0 (0.12)	0.878	0.091 [a]	0.76 [a]	0.93 [a]
Attempting escape	0 (0)	0 (0)	-	0 (0)	0 (0)	-	1.0 [a]	1.0 [a]	1.0 [a]
Interacting with pen	42 (44)	43 (46)	1.29	39 (40)	45 (50)	1.38	0.45	**0.0015**	0.84
Walking	20 (12)	21 (13)	0.219	22 (13)	21 (13)	1.28	**0.0049**	0.89	0.46
Standing	24 (16)	22 (14)	1.05	25 (17)	21 (13)	1.87	0.17	0.088	0.17
Sitting/lying	14 (5.7)	8.0 (1.9)	1.94	14 (5.6)	8.1 (2.0)	2.12	**0.030**	**0.024**	0.095

[a] *p*-values calculated using permutation tests.

Table 4.10. Effects of early housing and human contact on the physiology of pigs 2 h and 49 h after weaning. All data were logarithmically transformed prior to analysis; back-transformed means are presented in parentheses. FC, farrowing crate; LP, loose pen; C, routine human contact; +HC, positive human contact.

	Housing System			**Human Contact**			***p*-Value**		
	FC	**LP**	**S.e.d.**	**C**	**+HC**	**S.e.d.**	**Housing System**	**Human Contact**	**Housing × Contact**
Physiology 2 h after weaning									
Cortisol (ng/ml)	1.8 (57)	1.9 (83)	0.0710	1.8 (68)	1.8 (70)	0.0710	**0.032**	0.88	0.41
Neutrophil to lymphocyte ratio	-0.061 (0.87)	-0.070 (0.85)	0.0466	-0.045 (0.90)	-0.087 (0.82)	0.0466	0.85	0.38	0.92
Physiology 49 h after weaning									
Cortisol (ng/ml)	1.5 (32)	1.5 (29)	0.0907	1.6 (37)	1.4 (25)	0.0907	0.62	**0.015**	0.15
Haptoglobin (µg/ml)	3.0 (900)	2.8 (690)	0.0821	2.9 (770)	2.9 (800)	0.0507	0.23	0.81	0.81

Table 4.11. Effects of early housing and human contact on behavioural responses of pigs to tattoo branding at 18 wk of age. FC, farrowing crate; LP, loose pen; C, routine human contact; +HC, positive human contact.

	Housing System			Human Contact			*p*-Value		
	FC	LP	S.e.d.	C	+HC	S.e.d.	Housing System	Human Contact	Housing × Contact
Behaviour (% pigs)									
Jumped after tattoo branding	53	47	4.56	43	57	10.5	0.34 [a]	0.20 [a]	0.67 [a]
Ran after tattoo branding	79	66	7.29	68	77	7.05	0.19 [a]	0.22 [a]	0.46 [a]
Vocalised after tattoo branding	47	45	6.59	47	45	8.56	0.88 [a]	0.85 [a]	0.70 [a]

[a] *p*-values calculated using permutation tests.

Table 4.12. Effects of early housing and human contact on behavioural responses of pigs to being moved out of the home pen at 21 wk of age. FC, farrowing crate; LP, loose pen; C, routine human contact; +HC, positive human contact.

	Housing System			Human Contact			*p*-Value		
	FC	LP	S.e.d.	C	+HC	S.e.d.	Housing System	Human Contact	Housing × Contact
Number of stockperson interventions (per pig) [a]	1.0	1.0	0.0530	0.94	1.1	0.281	0.74	0.63	0.084
Time to move out of pen (s/pig)	3.8	3.6	0.352	3.7	3.7	0.658	0.62	0.99	0.41
Number of baulks (per pig)	0.20	0.32	0.0483	0.27	0.25	0.0723	0.052	0.86	0.75
Adjusted for number of stockperson interventions [a]	0.22	0.34	0.0360	0.29	0.27	0.0501	**0.034**	0.71	0.12
Number of pigs that baulked (%)	15	23	4.50	19	20	4.98	0.15	0.81	0.68
Adjusted for number of stockperson interventions [a]	16	23	4.36	20	20	4.97	0.18	0.97	0.40

[a] Number of times the stockperson hit the stockboard to the ground or used tactile contact to encourage pigs to move forward.

4.4.4 Behavioural and Physiological Responses to Emotionality Tests

4.4.4.1 Novel Object and Human Approach Test at 3 wk of Age

There were significant effects of housing system and human contact treatment on most variables measured in the novel object and human approach test at 3 wk of age (Table 4.13). Compared to LP piglets, FC piglets entered more sections of the empty arena during the first minute of the test (3.7 vs. 2.7; $p = 0.0066$). In response to the traffic cone, FC piglets were faster to approach within 0.6 m (20 vs. 44 s; $p < 0.001$), were faster to physically interact (26 vs. 47 s; $p < 0.001$), initiated more interactions (4.5 vs. 2.0; $p < 0.001$), and spent more time near the traffic cone (29 vs. 12 s; $p < 0.001$) compared to LP piglets. In response to the human hand, FC piglets were faster to approach (20 vs. 41 s; $p = 0.0018$), initiated more interactions (1.2 vs. 0.47; $p = 0.016$) and spent more time near the hand (16 vs. 8.0 s; $p = 0.038$) compared to LP piglets. There was also a tendency for FC piglets to be faster to physically interact with the human hand compared to LP piglets (44 vs. 53 s; $p = 0.061$). In response to the standing human, FC piglets were faster to approach (21 vs. 41 s; $p < 0.001$), were faster to physically interact (23 vs. 47 s; $p < 0.001$), initiated more interactions (4.1 vs. 2.0; $p = 0.0015$), and spent more time near the human (28 vs. 12 s; $p = 0.0032$) compared to LP piglets. There was no significant effect ($p > 0.05$) of human contact treatment on the number of entries piglets made into sections of the empty arena. In response to the traffic cone, +HC piglets were faster to approach within 0.6 m (25 vs. 38 s; $p = 0.0041$), were faster to physically interact (30 vs. 44 s; $p = 0.0018$), initiated more interactions (4.6 vs. 1.9; $p < 0.001$), and spent more time near the traffic cone (27 vs. 13 s; $p < 0.001$) compared to C piglets. In response to the human hand, +HC piglets were faster to approach (21 vs. 40 s; $p = 0.0052$), were faster to physically interact (40 vs. 55 s; $p = 0.0037$), initiated more interactions (1.6 vs. 0.29; $p < 0.001$), and spent more time near the hand (17 vs. 7.1 s; $p = 0.0091$) compared to C piglets. In response to the standing human, +HC piglets were faster to approach (22 vs. 41 s; $p = 0.0017$), were faster to physically interact (26 vs. 41 s; $p < 0.001$), initiated more interactions (5.1 vs. 2.2; $p < 0.001$), and spent more time near the human (28 vs. 12 s; $p = 0.0033$) compared to C piglets. There were no significant ($p > 0.05$) housing system × human contact treatment interactions on the behaviour of piglets in the test.

Table 4.13. Effects of early housing and human contact on the behavioural responses of piglets to novelty and humans at 3 wk of age (Novel Object and Human Approach Test). The latency to approach within 0.6 m, the latency to interact and the time spent within 0.6 m of each stimulus were angularly transformed, and the number of interactions with each stimulus were square root transformed prior to analysis; back-transformed means are presented in parentheses. FC, farrowing crate; LP, loose pen; C, routine human contact; +HC, positive human contact.

	Housing System			**Human Contact**			***p*-Value**		
	FC	**LP**	**S.e.d.**	**C**	**+HC**	**S.e.d.**	**Housing System**	**Human Contact**	**Housing × Contact**
Empty arena									
Number of entries	3.7	2.7	0.355	3.0	3.4	0.355	**0.0066**	0.26	0.24
Traffic cone									
Latency to approach (s)	34 (19)	59 (44)	4.09	53 (38)	40 (25)	4.09	**3.4×10^{-7}**	**0.0041**	0.58
Latency to interact (s)	42 (26)	62 (47)	4.36	59 (44)	45 (30)	4.36	**0.000024**	**0.0018**	0.91
Number of interactions	2.1 (4.5)	1.4 (2.0)	0.183	1.4 (1.9)	2.1 (4.6)	0.183	**0.00040**	**0.00019**	0.18
Time within 0.6 m (s)	44 (29)	27 (12)	3.57	28 (13)	42 (27)	3.57	**0.000024**	**0.000025**	0.43
Human hand									
Latency to approach (s)	35 (20)	56 (41)	6.22	54 (40)	36 (21)	6.22	**0.0018**	**0.0052**	0.13
Latency to interact (s)	58 (44)	69 (53)	5.67	73 (55)	55 (40)	5.67	0.061	**0.0037**	0.67
Number of interactions	1.1 (1.2)	0.69 (0.47)	0.166	0.53 (0.29)	1.3 (1.6)	0.166	**0.016**	**0.000084**	0.72
Time within 0.6 m (s)	31 (16)	21 (8.0)	4.55	20 (7.1)	33 (17)	4.55	**0.038**	**0.0091**	0.31
Standing human									
Latency to approach (s)	36 (21)	56 (41)	5.52	55 (41)	37 (22)	5.52	**0.00064**	**0.0017**	0.58
Latency to interact (s)	38 (23)	63 (47)	5.35	60 (45)	40 (26)	5.35	**0.000042**	**0.00059**	0.52
Number of interactions	2.3 (4.1)	1.4 (2.0)	0.254	1.5 (2.2)	2.3 (5.1)	0.210	**0.0015**	**0.00089**	0.70
Time within 0.6 m (s)	43 (28)	27 (12)	5.34	27 (12)	43 (28)	5.34	**0.0032**	**0.0033**	0.80

4.4.4.2 Walking Human Test at 4 wk of Age

In response to an unfamiliar human stopping in front of the pen during the walking human test at 4 wk of age, there were more FC pigs than LP pigs in the front third of the pen in closer proximity to the experimenter (22 vs. 7.3 %; $p = 0.014$; Table 4.14). Consequently, there were also fewer FC pigs than LP pigs in the back third of the pen (63 vs. 81 %; $p = 0.016$). There was no significant effect ($p > 0.05$) of housing system on the proportion of pigs in the middle of the pen. There were also no significant effects ($p > 0.05$) of human contact treatment, and no significant ($p > 0.05$) housing system × human contact treatment interactions on the position of pigs in the pen during the walking human test.

Table 4.14. Effects of early housing and human contact on the behavioural responses of pigs to an unfamiliar human experimenter walking by and stopping in front of the pen, 1 d after weaning and mixing at 4 wk of age (Walking Human Test). All data were angularly transformed prior to analysis; back-transformed means are presented in parentheses. FC, farrowing crate; LP, loose pen; C, routine human contact; +HC, positive human contact.

	Housing System			**Human Contact**			***p*-Value**		
	FC	**LP**	**S.e.d.**	**C**	**+HC**	**S.e.d.**	**Housing System**	**Human Contact**	**Housing × Contact**
Position in pen									
Front (% pigs)	28 (22)	16 (7.3)	4.41	24 (17)	19 (11)	4.41	**0.014**	0.24	0.28
Middle (% pigs)	22 (15)	19 (10)	2.33	22 (13)	20 (11)	2.33	0.13	0.44	0.50
Back (% pigs)	52 (63)	64 (81)	4.42	55 (68)	61 (77)	4.42	**0.016**	0.21	0.31

4.4.4.3 Flight Distance Test at 6 wk of Age

There were significant effects of housing system and human contact treatment on the proximity of pigs to an unfamiliar human inside the home pen during the flight distance test at 6 wk of age (Table 4.15). FC pigs were in closer proximity to the experimenter compared to LP pigs at both the start (distance in rows of flooring, 2.9 vs. 5.9; $p < 0.001$) and end of the test (3.1 vs. 5.8; $p < 0.001$) when the experimenter was stationary, and in the middle of the test (3.2 vs. 4.0; $p < 0.001$) when the experimenter was walking inside the pen. +HC pigs were in closer proximity to the experimenter compared to C pigs at both the start (3.5 vs. 5.2; $p = 0.013$) and end of the test (3.9 vs. 5.0; $p = 0.040$) when the experimenter was stationary, and in the middle of the test (3.4 vs. 3.8; $p = 0.039$) when the experimenter was walking inside the pen. There were no significant ($p > 0.05$) housing system × human contact treatment interactions on the proximity of pigs to the experimenter at any phase of the test.

Table 4.15. Effects of early housing and human contact on the behavioural responses of pigs to an unfamiliar human experimenter inside the home pen at 6 wk of age (Flight Distance Test). The proximity of pigs to the experimenter represents the number of rows of flooring (1 row = 30 cm) that pigs were away from the experimenter at each phase of the test. FC, farrowing crate; LP, loose pen; C, routine human contact; +HC, positive human contact.

	Housing System			**Human Contact**			***p*-Value**		
	FC	**LP**	**S.e.d.**	**C**	**+HC**	**S.e.d.**	**Housing System**	**Human Contact**	**Housing × Contact**
Phase of test									
Start – proximity of pigs to stationary experimenter	2.9	5.9	0.435	5.2	3.5	0.576	**0.00098**	**0.013**	0.76
Middle - proximity of pigs to moving experimenter	3.2	4.0	0.108	3.8	3.4	0.164	**0.00076**	**0.039**	0.51
End - proximity of pigs to stationary experimenter	3.1	5.8	0.362	5.0	3.9	0.499	**0.00064**	**0.040**	0.70

4.4.4.4 Isolation Test at 7 wk of Age

In response to isolation at 7 wk of age, there were more FC pigs than LP pigs that vocalised less than 10 times (44 vs. 13 %; $p = 0.029$), and consequently there tended to be fewer FC pigs than LP pigs that vocalised more than 10 times (57 vs. 88 %; $p = 0.057$; Table 4.16). There was also a tendency for LP pigs to vocalise sooner than FC pigs (23 vs 41 s; $p = 0.055$). There were no significant effects ($p > 0.05$) of housing system on the number of entries pigs made into different sections of the arena, escape attempts or the latency of pigs to make an escape attempt during the isolation test. There were no significant effects ($p > 0.05$) of human contact treatment on the behavioural responses of pigs in the isolation test. There was a tendency for +HC pigs to have a longer latency to make an escape attempt compared to C pigs (120 vs. 110 s; $p = 0.072$), however most pigs were given the maximum response time of 120 s as there were only low numbers of pigs that did attempt escape. There were no significant ($p > 0.05$) housing system × human contact treatment interactions on the behavioural responses of pigs in the isolation test, although, there tended to be fewer +HC/FC pigs attempting escape from the arena (back-transformed means, +HC/FC: 0.088 %, C/FC: 2.3 %, +HC/LP: 6.1 %, C/LP: 4.0 %; $p = 0.073$). There was a significant interaction effect on serum cortisol concentrations 45 min after testing, with +HC/FC pigs having lower concentrations than pigs from the other treatment groups (back-transformed means, +HC/FC: 17 ng/ml, C/FC: 23 ng/ml, +HC/LP: 32 ng/ml, C/LP: 30 ng/ml; log-transformed means, +HC/FC: 1.2, C/FC: 1.4, +HC/LP: 1.5, C/LP: 1.5; s.e.d. = 0.0333 – 0.0557; $p = 0.0063$). There were also significant main effects of housing system and human contact treatment on serum cortisol concentrations 45 after testing, with concentrations being lower in FC pigs than LP pigs (20 vs. 31 ng/ml; $p = 0.014$), and lower in +HC pigs than C pigs (23 vs. 26 ng/ml; $p = 0.046$). There were no significant ($p > 0.05$) effects of human contact treatment, housing system or the interaction between the two on serum IgA concentrations 45 min after isolation.

Table 4.16. Effects of early housing and human contact on the behavioural and physiological responses of pigs to isolation at 7 wk of age (Isolation Test). The number of escape attempts was square root transformed, all data reported as % of pigs were angularly transformed and serum cortisol and IgA concentrations 45 min after testing were logarithmically transformed prior to analysis; back-transformed means are presented in parentheses. FC, farrowing crate; LP, loose pen; C, routine human contact; +HC, positive human contact.

	Housing System			**Human Contact**			***p*-Value**		
	FC	**LP**	**S.e.d.**	**C**	**+HC**	**S.e.d.**	**Housing System**	**Human Contact**	**Housing × Contact**
Behaviour									
Number of entries	18	21	1.42	19	20	0.476	0.10	0.63	0.60
Attempted escape (% pigs)	5.2 (0.82)	13 (5.0)	3.69	10 (3.1)	8.0 (1.9)	2.43	0.099 [a]	0.39 [a]	0.073 [a]
Latency to escape attempt (s)	120	120	2.22	110	120	1.84	0.62 [a]	0.072 [a]	0.64 [a]
Number of escape attempts	0.36 (0.13)	0.47 (0.22)	0.187	0.53 (0.28)	0.29 (0.086)	0.148	0.59 [a]	0.14 [a]	0.86 [a]
Latency to vocalise (s)	41	23	7.34	35	28	6.10	0.055	0.32	0.43
Vocalised 0-10 times (% pigs)	41 (44)	21 (13)	4.48	32 (29)	29 (24)	3.04	**0.029** [a]	0.34 [a]	0.13 [a]
Vocalised > 10 times (% pigs)	49 (57)	69 (88)	4.48	58 (71)	61 (76)	3.04	0.057 [a]	0.34 [a]	0.14 [a]
Physiology									
Cortisol 45 min after testing (ng/ml)	1.3 (20)	1.5 (31)	0.0505	1.4 (26)	1.4 (23)	0.0235	**0.014**	**0.046**	**0.0063**
IgA 45 min after testing (μg/ml)	3.3 (2200)	3.3 (2200)	0.0523	3.3 (2200)	3.3 (2200)	0.0715	0.88	0.99	0.23

[a] *p*-values calculated using permutation tests.

Table 4.17. Effects of early housing and human contact on the behavioural responses of pigs to an unfamiliar human experimenter in an arena at 9 wk of age (Human Approach Test). The latency to approach, the latency to interact and the time spent within 0.5 m of the experimenter were angularly transformed, and the number of interactions with the experimenter was square root transformed prior to analysis; back-transformed means are presented in parentheses. FC, farrowing crate; LP, loose pen; C, routine human contact; +HC, positive human contact.

	Housing System			**Human Contact**			***p*-Value**		
	FC	**LP**	**S.e.d.**	**C**	**+HC**	**S.e.d.**	**Housing System**	**Human Contact**	**Housing × Contact**
Empty arena									
Number of entries	17	17	1.62	17	17	0.904	0.77	0.68	**0.041**
Stationary human									
Latency to approach (s)	24 (29)	28 (40)	4.04	30 (45)	22 (25)	2.69	0.33	**0.013**	0.55
Latency to interact (s)	40 (74)	42 (79)	3.25	44 (85)	38 (68)	5.16	0.61	0.30	0.99
Number of interactions	3.0 (9.2)	2.9 (8.4)	0.338	2.8 (7.6)	3.2 (10)	0.342	0.67	0.25	0.81
Time within 0.5 m (s)	41 (78)	39 (70)	3.86	37 (64)	43 (84)	3.73	0.54	0.12	0.98

4.4.4.5 Human Approach Test at 9 wk of Age

In response to an unfamiliar stationary experimenter in the human approach test at 9 wk of age, +HC pigs were faster than C pigs to approach within 0.5 m of the experimenter (45 vs. 25 s; $p = 0.013$; Table 4.17). There were no significant effects ($p > 0.05$) of human contact treatment on the latency of pigs to physically interact with the experimenter, the number of physical interactions or on the time spent within 0.5 m of the experimenter. There were also no significant effects ($p > 0.05$) of housing system, and no significant ($p > 0.05$) housing system × human contact treatment interactions, on the behavioural responses of pigs to the stationary experimenter. However, there was a significant housing system × human contact treatment interaction on the number of entries pigs made in the empty area prior to the stationary human entering (+HC/FC: 15, C/FC: 18, +HC/LP: 18, C/LP: 16; s.e.d. = 1.28 – 1.86; $p = 0.041$).

4.4.4.6 Flight Distance Test at 14 wk of Age

In response to an unfamiliar human inside the home pen at 14 wk of age, there were more +HC pigs than C pigs within 1 m of the experimenter at the end of the test when the experimenter was stationary for 30 s (82 vs. 69 %; $p = 0.037$; Table 4.18). There were no significant effects ($p > 0.05$) of human contact treatment on the number of pigs within 1 m of the experimenter at the start (experimenter stationary for 5 s) or in the middle (experimenter walking inside pen) phases of the test. There were also no significant effects ($p > 0.05$) of housing system, and no significant ($p > 0.05$) housing system × human contact treatment interactions on the number of pigs within 1 m of the experimenter at any phase of the test.

Table 4.18. Effects of early housing and human contact on the behavioural responses of pigs to an unfamiliar human experimenter inside the home pen at 14 wk of age (Flight Distance Test). FC, farrowing crate; LP, loose pen; C, routine human contact; +HC, positive human contact.

	Housing System			**Human Contact**			***p*-Value**		
	FC	**LP**	**S.e.d.**	**C**	**+HC**	**S.e.d.**	**Housing System**	**Human Contact**	**Housing × Contact**
Phase of test									
Start – % pigs within 1 m of stationary experimenter	67	67	4.43	63	71	6.72	0.90	0.27	0.34
Middle - % pigs within 1 m of moving experimenter	62	62	3.98	58	67	6.51	0.78	0.20	0.71
End - % pigs within 1 m of stationary experimenter	75	76	5.42	69	82	5.69	0.88	**0.037**	0.43

4.4.5 Growth, Injuries and Survival

4.4.5.1 Piglet Survival until Weaning at 4 wk of Age

There were more pigs in FC litters than LP litters at 1 d of age (13 vs. 12; $p = 0.042$) and at weaning at 4 wk of age (11 vs. 8.4; $p < 0.001$), and a higher rate of mortalities in LP litters during the lactation period (26 % vs. 14 %; $p = 0.019$; Table 4.19). There were no significant ($p > 0.05$) effects of human contact treatment and no significant ($p > 0.05$) housing system × human contact treatment interactions on piglet survival during lactation.

Table 4.19. Effects of early housing and human contact on piglet survival until weaning at 4 wk of age. FC, farrowing crate; LP, loose pen; C, routine human contact; +HC, positive human contact.

	Housing System			**Human Contact**			***p*-Value**		
	FC	**LP**	**S.e.d.**	**C**	**+HC**	**S.e.d.**	**Housing System**	**Human Contact**	**Housing × Contact**
No. pigs 1 d	13	12	0.750	12	12	0.750	**0.042**	0.91	0.83
No. pigs at weaning	11	8.4	0.507	10	9.6	0.507	**4.6×10^{-6}**	0.58	0.57
Mortality 1 d of age until weaning (%)	14	26	4.59	19	21	4.59	**0.019**	0.93	0.50

4.4.5.2 Growth at 4 d, 22 d and 18 wk of Age

There was no significant effect of housing system on the weight of pigs at 4 d of age, however at 22 d of age LP pigs were heavier than FC pigs (6.4 vs. 5.8 kg; $p = 0.029$; Table 4.20). This was accompanied by a higher daily gain from 4 until 22 d of age in LP pigs compared to FC pigs (230 vs. 190 g/day; $p = 0.005$). There were no significant effects of housing system on the weight or daily gain of pigs at 18 wk of age. There were also no significant ($p > 0.05$) effects of human contact treatment and no significant ($p > 0.05$) housing system × human contact treatment interactions on the growth of pigs at any age.

Table 4.20. Effects of early housing and human contact on growth in pigs. FC, farrowing crate; LP, loose pen; C, routine human contact; +HC, positive human contact.

	Housing System			**Human Contact**			***p*-Value**		
	FC	**LP**	**S.e.d.**	**C**	**+HC**	**S.e.d.**	**Housing System**	**Human Contact**	**Housing × Contact**
Weight 4 d (kg/pig)	2.3	2.2	0.129	2.3	2.2	0.129	0.62	0.52	0.63
Weight 22 d (kg/pig)	5.8	6.4	0.259	6.2	6.0	0.259	**0.029**	0.56	0.79
Gain 4 d - 22 d (g/day)	190	230	12.3	210	210	12.3	**0.0051**	0.75	0.55
Weight 18 wk (kg/pig)	76	76	1.80	75	77	1.65	0.87	0.37	0.64
Gain 22 d - 18 wk (kg/wk)	5.3	5.3	0.131	5.3	5.4	0.115	0.81	0.34	0.66

4.4.5.3 Injuries at 4, 5 and 17 wk of Age

Compared to C pigs, +HC pigs had lower injury scores on the head (1.3 vs. 1.7; $p = 0.030$) and the rest of the body (mean score; 0.64 vs. 0.93; $p = 0.045$) at 4 wk of age during the lactation period (Table 4.21). There were no significant ($p > 0.05$) effects of human contact treatment on injuries at 5 wk of age 1 wk after weaning. At 17 wk of age, +HC pigs had fewer injuries on the head (1.3 vs. 1.6; $p = 0.042$), however there was no significant effect ($p > 0.05$) of human contact treatment on injuries on the rest of the body. There were no significant ($p > 0.05$) effects of housing system and no significant ($p > 0.05$) housing system × human contact treatment interactions on injury scores at any weeks.

Table 4.21. Effects of early housing and human contact on injury scores of pigs at 4 wk of age 2 d prior to weaning, at 5 wk of age 1 wk after weaning, and at 17 wk of age. FC, farrowing crate; LP, loose pen; C, routine human contact; +HC, positive human contact.

	Housing System			**Human Contact**			***p*-Value**		
	FC	**LP**	**S.e.d.**	**C**	**+HC**	**S.e.d.**	**Housing System**	**Human Contact**	**Housing × Contact**
Injuries 4 wk									
Head	1.4	1.5	0.175	1.7	1.3	0.175	0.60	**0.030**	0.98
Rest of body	0.78	0.79	0.139	0.93	0.64	0.139	0.97	**0.045**	0.44
Injuries 5 wk									
Head	2.0	1.6	0.167	2.0	1.7	0.172	0.055	0.12	0.42
Rest of body	1.3	1.5	0.116	1.6	1.3	0.170	0.19	0.058	0.68
Injuries 17 wk									
Head	1.6	1.3	0.160	1.6	1.3	0.134	0.25	**0.042**	0.76
Rest of body	1.7	1.5	0.0920	1.6	1.6	0.202	0.15	0.80	0.73

4.5 Discussion

4.5.1 Overview

This experiment examined the effects of the farrowing and lactation housing environment and early interactions with humans on lifetime stress resilience in pigs. Overall, there was evidence that positive human contact early in life and rearing in farrowing crates rather than loose housing improved stress resilience in pigs.

Providing regular opportunities for positive interactions with humans from 0-4 wk of age was highly effective in reducing piglets' fear of humans, based on the approach and proximity of piglets to an unfamiliar human at 3 wk of age. The positive handling treatment was also effective at reducing pigs' fear of a novel object at 3 wk of age. There was evidence that some reduction in fear of humans lasted well beyond the period of application of the treatment (lactation), with all measures of fear of humans in tests at 6, 9 and 14 wk of age indicating less fearful responses in +HC pigs, with considerably more of these measures being statistically significant than would be expected by chance (5 out of 10 measures with $p < 0.05$). Positive handling reduced piglets' escape behaviour and vocalisations during husbandry practices imposed during the lactation period (4 out of 12 measures with $p < 0.05$), and reduced the cortisol response of pigs 2 d after weaning as well as after isolation at 7 wk of age. Furthermore, +HC pigs had higher brain-derived neurotrophic factor concentrations and fewer injuries at 4 wk of age, with limited evidence also suggesting similar effects on these measurements at 17 wk of age. A summary of main effects of the handling treatment is shown in Figure 4.6.

When compared to piglets reared in loose farrowing and lactation pens that offered more space, physical complexity and opportunity for sow interaction, rearing in farrowing crates reduced pigs' fear of humans and novelty at 3 wk of age, escape behaviour during capture by a stockperson for husbandry practices at 4 d of age and during from 0-1 h after weaning, and cortisol concentrations 2 h after weaning. There was evidence of some maintenance of better stress resilience in FC pigs than LP pigs after weaning when all pigs were in identical housing, as evidenced by FC pigs showing less fear of humans in tests at 4 and 6 wk of age, less vocalisation and a lower cortisol response to isolation at 7 wk of age, and lower frequencies of baulking when being moved out of the home pen at 21 wk of age. A summary of main effects of the housing treatment is shown in Figure 4.7.

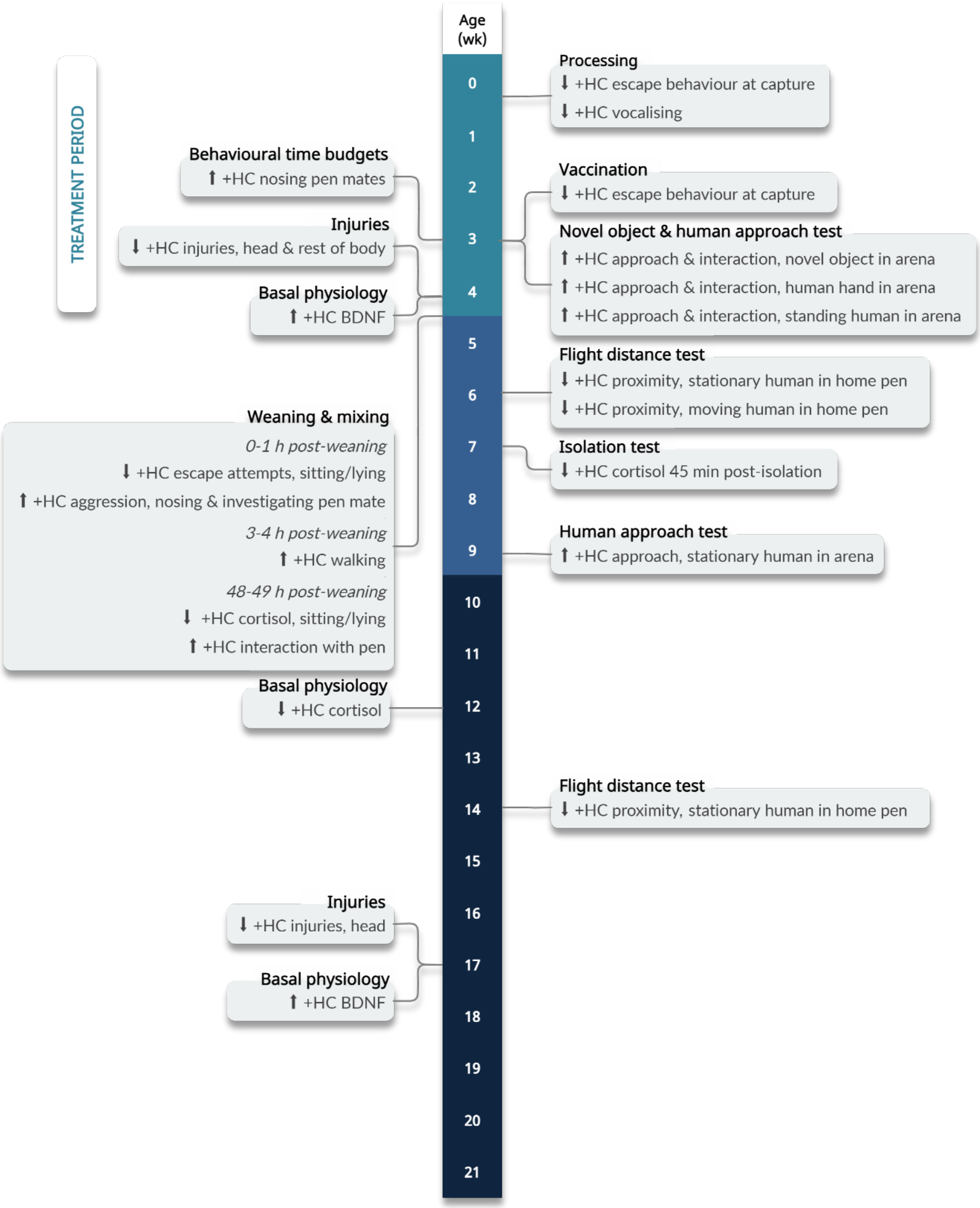


Figure 4.6. Summary of main effects of handling treatment (positive or routine human contact) during lactation on measurements of stress resilience in pigs from birth until 21 wk of age. Arrows indicate effects of handling treatment with *p*-values < 0.05. BDNF, Brain-derived neurotrophic factor; +HC, positive human contact, 5 min of patting, stroking, and scratching imposed 5 times/wk from 0-4 wk of age.

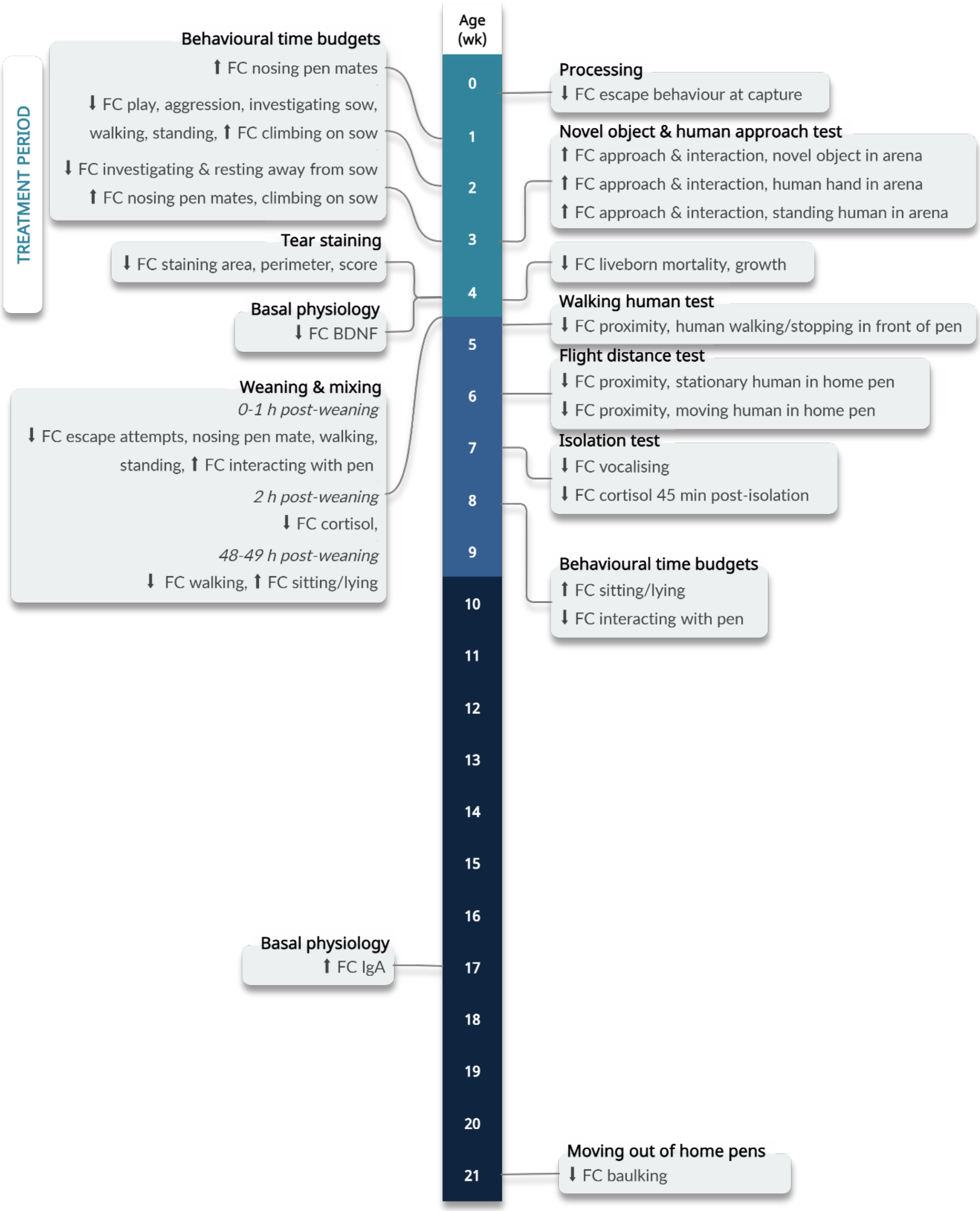


Figure 4.7. Summary of main effects of housing treatment (farrowing crates or loose farrowing and lactation pens) during lactation on measurements of stress resilience in pigs from birth until 21 wk of age. Arrows indicate effects of housing treatment with p-values < 0.05. BDNF, Brain-derived neurotrophic factor; IgA, Immunoglobulin A; FC, farrowing crate, pigs reared in conventional farrowing crates from 0-4 wk of age.

4.5.2 Behavioural and Physiological Responses to Emotionality Tests

Compared to pigs that received routine contact from stockpeople, pigs that received regular positive human contact during the lactation period were less fearful of humans, and consequently showed more exploration of humans, as evident by increased approach and interaction and reduced proximity to an unfamiliar human in tests at 3, 6, 9 and 14 wk of age. There were several methodological differences between each of these tests, including the location where testing was conducted (home pen or novel arena), the posture of the human (walking, standing stationary or crouching and extending hand) and whether pigs were tested individually or in a group. Although there were no human contact treatment effects on the behavioural response of pigs to an unfamiliar human in the walking human test after weaning at 4 wk of age, the results from other behaviour tests in the experiment indicate that the +HC pigs generalized their response to different people and a range of contexts. These results also show that early interactions with humans can have a prolonged effect on the fear responses of pigs to humans.

Hemsworth and Barnett (1992) found that pigs that were patted and stroked individually in an arena from 0-3 wk of age were faster to approach and interact and spent more time in close proximity to an unfamiliar human at 18 wk of age compared to pigs that received routine human contact. However, the authors found no effects of early handling on the responses of pigs to a human at 20, 22 or 24 wk of age, and suggested that subsequent human contact may have weakened the effects of the early handling treatment over time. Subsequent human contact may have also weakened the effects of the early handling treatment on fear of humans in the present experiment since the effects on fear responses at 9 and 14 wk of age were not as strong as at 3 and 6 wk of age. Differences in the sensitivity of the different tests to measure fear behaviour may have also played a role as well as subsequent human contact eventually overriding the effects of early positive interactions with humans. However, the positive handling treatment in this experiment still reduced fear of humans for most of the study period.

In a previous experiment, we found that the effect of 3 min of regular patting, stroking and scratching on the latency of pigs to approach and interact with a standing human, human hand and traffic cone at 2 wk of age, led to smaller reductions in fear responses than in the present experiment (Hayes et al., 2021b). In fact, in the previous experiment no effect was observed to the introduction of a human hand or traffic cone. In the present experiment, +HC pigs that received 5 min of regular patting, stroking, and scratching were faster than C pigs to approach and interact with a standing human, a human hand and a traffic cone at 3 wk of age. Differences in duration of the positive handling treatment as well as in the age at which piglets were tested may be responsible for the stronger responses observed in the present experiment. Additionally, there were differences in the way the +HC treatment was applied in the loose system between the two experiments, whereby in our previous experiment, the experimenter imposed the +HC treatment from inside the pen, while in the present experiment, the experimenter kneeled outside and extended their hand over the pen wall.

In addition to reduced fear of novelty and humans, for piglets that had been reared in farrowing crates, positive handling reduced the physiological stress response to isolation at 7 wk of age, based on lower serum cortisol concentrations 45 min after isolation. Earlier research on pigs (Hemsworth et al., 1986b) and poultry (Jones & Waddington, 1992) suggested that positive interactions with people have a stimulus-specific effect, reducing animals' fear of humans specifically rather than reducing general fearfulness, while in contrast, other research has shown that stroking piglets while holding them leads to reduced fear of novelty, as evident by more play behaviour and less vocalising in a novel arena (de Oliveira et al., 2015; Zupan et al., 2016). One interpretation of reduced fear of novelty and reduced cortisol after isolation in the present experiment, is that the positive handling treatment improved general stress resilience. As stress resilience is typically fostered by overcoming experiences that are "challenging but not overwhelming" (Lyons et al., 2009; Lyons et al., 2010; Lyons & Schatzberg, 2019; Parker & Maestripieri, 2011), it is possible that close human contact during imposition of the handling treatment provided pigs with a minor challenge to overcome, that led to improved coping with other types of challenges. Furthermore, since humans were involved in conducting the novel object test and the isolation test, reduced fear of humans may have reduced the stressfulness of the test for the +HC pigs. Salivary concentrations of IgA have previously been shown to increase in pigs after isolation (Escribano et al., 2015), however early positive handling did not affect concentrations in serum 45 min after testing. There was also no evidence of effects of the handling treatment on the behaviour of pigs during the isolation test. A longer test duration may have resulted in stronger differences between the behaviour of +HC and C pigs.

At 3 wk of age, LP pigs showed less approach and interaction with a novel traffic cone, indicating greater fear of novelty. And at 7 wk of age during the isolation test, there was some evidence that LP pigs vocalised less and sooner than FC pigs. The cortisol response 45 min after isolation was also higher in LP pigs. Similarly, Brajon and colleagues (2017) found that pigs from loose farrowing and lactation pens enriched with straw showed greater stress in response to isolation at 17 d of age, based on more escape behaviour and a longer duration of vocalisation compared to piglets from farrowing crates. In the present experiment, one explanation for the increased stress response of LP pigs to novelty, weaning and isolation which we have previously speculated (Hayes et al., 2021b), is that pigs from the loose system were raised in a more isolated environment with fewer opportunities to learn to cope with stress. In the loose system, the high walls of the pens restricted piglets' contact with people outside of the pen, whereas in the farrowing crate system, piglets could more easily observe people in the room. There was also more opportunity for interactions at an earlier age between adjacent FC litters over the short dividing walls, compared to between adjacent LP litters through the windows in dividing walls, as LP piglets could only reach the pen windows at 2-3 wk of age. Furthermore, there was less routine contact with stockpeople in the loose system than in the farrowing crate system due to the layout of both rooms. For example, many of the loose pens were positioned along aisles which were dead ends while the farrowing crates were positioned along aisles that stockpeople used to access neighbouring sheds. Stockpeople also spent more time in the farrowing crate room conducting routine inspections and feeding as there were twice as many litters housed there than in the loose pen room. Therefore, due to both the design of the loose pens and the layout of the rooms, LP pigs may have been more vulnerable to stress due to fewer opportunities during rearing to cope with stressors such as regular and close exposure to stockpeople, their equipment and other pigs.

Fear of humans was higher in LP pigs than FC pigs in the present experiment as shown by more avoidance and less approach and interaction with an unfamiliar human at 3, 4 and 6 wk of age. In contrast, Kinane and colleagues (2021) found no effect of housing pigs in farrowing crates compared to loose lactation pens on fear of humans, however, the loose system differed markedly from that in the present experiment in terms of space and opportunities for sow-piglet interaction and human-pig interaction. As proposed earlier, less overall contact with stockpeople may have contributed to greater fear of humans in pigs from the loose system in the present experiment. Furthermore, it is also possible that the maternal behaviour of the sows played a role since sows in loose lactation systems show improved maternal behaviour and increased interactions with their piglets compared to sows from farrowing crates (Chidgey et al., 2016; Cronin et al., 1996; Singh et al., 2017; Thodberg et al., 2002). In response to unfamiliar piglet squeals in the maternal responsiveness test in wk 2 of lactation, there were more sows from loose pens than farrowing crates that changed their posture from resting to standing and showed behaviour directed towards their piglets. Therefore, it may be that sows in the loose system showed heightened maternal responses when stockpeople were close, which increased their piglets' fear of stockpeople. The design of the present experiment does not allow the contributions of effects of maternal behaviour of the sow, contact with humans, contact with neighbouring pigs and visual stimulation in general, as well as other differences between the housing systems to be determined. Further research is required to disentangle the specific characteristics of the housing systems that may affect fear and stress responses of piglets. There were no effects of the farrowing and lactation housing system on the fear responses of pigs to an unfamiliar person in the human approach test at 9 wk of age or in the flight distance test at 14 wk of age. It may be that the effect of the early housing system on fear of humans was ameliorated by pigs' subsequent interactions with humans, as previously discussed with respect to the effects of the early handling treatment**.**

- ➔ *Therefore, while the present experiment showed that the loose housing treatment increased pigs' fear of humans during lactation at 3 wk of age and after weaning at 4 and 6 wk of age, positive handling during lactation had the most profound and sustained reductions in fear of humans.*

4.5.3 Behavioural and Physiological Responses to Routine Stressors

Positive human contact piglets showed less escape behaviour during capture by a stockperson for husbandry practices at 4 d and 3 wk of age which supports our previous work on human contact and housing effects on stress in piglets (Hayes et al., 2021b). Despite no detectable effects of handling on the escape behaviour of piglets during oral vaccination and tail docking, or, on serum cortisol concentrations 1 h after processing, +HC pigs vocalised for shorter durations than C pigs throughout all procedures at processing, and furthermore, the average length of individual vocalisations was shorter in +HC pigs. There were no detected effects of handling on the total number of vocalisations or the maximum frequency of vocalisations during processing, and no detected effects of handling on piglet vocalisations during vaccination at 3 wk of age, but this may have been because vaccination was very short, usually involving only 3-5 seconds of handling for each pig. There were also no effects of early handling on the behavioural response of pigs to tattoo branding at 18 wk of age, or on the time taken to move pigs out of the pen, the number of interventions used by the stockperson or the occurrence of pigs baulking when being moved out of the home pens at 21 wk of age. Thus, while positive interactions with humans early in life may have reduced pigs' fear of people until at least 14 wk of age, there was no indication that the +HC treatment negatively affected ease of handling of pigs late in the experiment.

There were several effects of early handling and housing on the behavioural and physiological responses of pigs to weaning. In the period from 0-1 h after weaning and mixing, C pigs from LP were most frequently observed attempting to escape from the pen, although, overall escape attempts were rare. While there was no evidence of an effect of handling on serum cortisol concentrations 2 h after weaning, +HC pigs had considerably lower serum cortisol concentrations than C pigs at 49 h after weaning (30 vs 40 ng/ml, *p* = 0.02). Conversely, FC pigs had lower cortisol concentrations than LP pigs 2 h after weaning (60 vs 80 ng/ml, *p* = 0.03), but concentrations were similar at 49 h after weaning (30 vs 30 ng/ml). Cortisol may in part mediate increases in the ratio of neutrophil to lymphocyte cells (Sapolsky, 2000) and haptoglobin (Murata, 2007), both of which are part of the immune response to stress and have been shown to increase in pigs after weaning (De et al., 2017; Pomorska-Mol et al., 2012; Puppe et al., 1997; Sauerwein et al., 2005; Turpin et al., 2017; Turpin et al., 2016), but the handling and housing treatments in this experiment had no effects on these measurements.

One interpretation of the effects of the handling and housing treatments on escape attempt behaviour and cortisol concentrations, is that +HC pigs and FC pigs coped better with weaning and mixing compared to C and LP pigs respectively. Weaning involves several stressors for pigs including exposure to a novel physical and social environment and close and intense contact with stockpeople, and the greater fear responses of C and LP pigs to novelty and humans may have contributed to the greater stress responses of these pigs to weaning. Less contact between adjacent litters in the loose system may have also contributed to the increased stress response of LP pigs to weaning and mixing, since experience with non-littermates pre-weaning is known to mitigate stress at weaning (Kutzer et al., 2009). Furthermore, moving from an enriched to a barren environment has been shown to increase stress in pigs (Day et al., 2002; Luo et al., 2020; Munsterhjelm et al., 2009), and it is possible that the LP pigs experienced such a switch in housing conditions which increased their stress at weaning. Although, other than increased space there was little evidence of the LP environment being highly enriched. There are several limitations that need to be acknowledged in the interpretation of increased escape behaviour and cortisol concentrations in C and LP pigs after weaning. Firstly, it may be argued that since activation of the HPA axis is an effective mechanism which allows animals to adapt to challenges, increased cortisol may actually be an advantage and lead to animals being better able to cope with stressors. Secondly, in terms of the sampling method used for recording piglet behaviours post-weaning, we recognise that instantaneous sampling cannot always capture the true occurrence of events with short durations such as escape attempts. Thirdly, the effects of the handling and housing treatments on escape behaviour and cortisol concentrations were not well supported by effects of the treatments on other physiological or behavioural measurements after weaning.

At all observation timepoints after weaning, there was evidence for +HC and LP pigs being more active than C and FC pigs respectively, with +HC pigs and LP pigs more frequently observed walking and/or standing, and/or less frequently observed sitting and lying. Furthermore, +HC pigs from FC were most frequently observed interacting with the pen by sniffing, nosing or chewing physical components of the pen or floor. Only +HC pigs from LP were observed repetitively nosing other pigs in the period from 0-1 h after weaning, and these pigs were also most frequently observed investigating other pigs through gentle nasal contact, which may have preceded nosing behaviour. Repetitive nosing of pen mates, particularly directed towards the belly, has been reported to occur more frequently after weaning in early weaned pigs (O'Connell et al., 2005) and pigs housed in barren environments compared to pigs housed with increased space and enrichment materials (Oostindjer et al., 2011). At 0-1 h after weaning, +HC pigs showed more aggressive behaviour than C pigs, which may have been performed in conjunction with nosing behaviour. Despite these higher levels of aggression in the first hour post weaning, as mentioned previously +HC pigs had similar cortisol concentrations to C pigs 2 h after weaning, and if anything, they also had lower injury scores 1 wk after weaning. It is difficult to interpret the effects of the handling and housing treatments on some behaviours after weaning, particularly activity level and aggression, in terms of piglet welfare. A better understanding of the specific motivation(s) pertaining to nosing behaviour, as well as it's possible association with aggressive behaviour, is necessary to determine why the positive handling treatment increased the occurrence of these behaviours immediately after weaning.

When pigs were moved out of the home pens at 21 wk of age, there was some evidence that LP pigs baulked more than FC pigs suggesting that they were more difficult to handle. Fear of humans can affect ease of handling by stockpeople (Hemsworth & Coleman, 2011). However, since the fear responses to humans of pigs from the two housing treatments were similar at 9 and 14 wk of age, more baulks in this instance may have reflected greater fear of novel situations. The greater incidence of baulking in LP pigs did not increase the time taken for the stockperson to move the pigs. Nonetheless the lower incidence of baulking in FC pigs is a significant finding which shows that differences in the early housing environment can affect the behaviour of pigs, even after 4 months of housing in identical environments. Regarding the responses of pigs to other husbandry practices, there was little evidence of effects of the early housing system on the behavioural response of pigs to tattoo branding at 18 wk of age. Aligning with our previous work (Hayes et al., 2021b), there was an indication that LP piglets showed a higher intensity of escape behaviour than FC piglets during capture for piglet processing at 4 d of age (p = 0.04), but there were no housing system effects on escape behaviour during oral vaccination or tail docking, serum cortisol concentrations 1 h after processing, or on behavioural responses to capture for vaccination at 3 wk of age.

➔ *Not unexpectedly, positive handling during lactation reduced several biological responses indicative of stress, including escape behaviour, vocalisations and cortisol concentrations to routine husbandry practices such as processing, vaccination and weaning. Relative to* *housing in farrowing crates, the loose housing treatment increased escape behaviour and cortisol concentrations to routine stressors such as processing and weaning, and furthermore may have also increased the difficulty of the stockperson to move pigs out of the home pen at 21 wk of age.*

4.5.4 Time Budgets of Behaviour and Basal Physiological Measurements

Interactions with the dam can have a significant influence on the development of stress coping mechanisms of offspring (Liu et al., 1997) and have been shown to occur more frequently in loose lactation systems than in farrowing crates (Chidgey et al., 2016; Singh et al., 2017). In the present experiment, there were more LP piglets than FC piglets 'investigating' the sow which we defined as piglets initiating gentle nasal contact with any part of the sow's body, excluding the mammary area. This may have been a consequence of more opportunity based on more sow-initiated contact in the loose system. Interactions such as nasal contact between sows and piglets are important for the development of social bonds which may affect the maternal behaviour of the sow (Blackshaw & Hagelsø, 1990; Petersen et al., 1990). While we did not record sow maternal behaviour per se, we found that maternal responsiveness to unfamiliar piglet vocalisations was higher in sows from loose pens than sows from farrowing crates, based on more posture changes and piglet directed behaviour by LP sows in the maternal responsiveness test. While nasal contact with the sow was more frequent in loose pens, there were more piglets climbing on the sow in the farrowing crate system particularly at 2 and 3 wk of age. Additionally at these ages, when piglets were sitting or lying there was an indication that those from farrowing crates were more likely to be resting with the sow rather than resting away from the sow. Less time resting with the sow may reflect a more natural weaning process in the loose system where sows can move away their piglets, which may reduce stress at weaning by better preparing piglets for removal from the dam. However, this was not the case in the present experiment since LP pigs showed more stress at weaning based on greater cortisol concentrations and more escape attempts. The positive handling treatment had no effects on piglets' interactions with the sow.

In farrowing crates compared to loose pens, there was a higher frequency of nosing behaviour directed towards littermates, which involved repetitive vertical movements of the snout on any part of another pig's body. Singh and colleagues (2017) reported similar findings: housing sows and litters in loose pens from day 3 of lactation reduced piglet manipulation of pen mates through nibbling, sucking, chewing or rubbing with the nose any part of another pig's body. In contrast, Oostindjer and colleagues (2011) found reduced manipulative behaviour and increased play behaviour in piglets raised in lactation pens with space and straw however sow confinement per se had no effect. In the present experiment there were more piglets performing play behaviour at 2 wk of age in the loose system, aligning with research by Martin and colleagues (2015) which showed that play behaviour occurred more frequently and earlier in life in PigSAFE pens than in farrowing crates. In the present experiment, there was also a tendency for more play behaviour in pigs from the loose system at 3 wk of age ($p = 0.08$), as well as at 8 wk of age during the weaner period ($p = 0.07$). Greater play behaviour in the PigSAFE system may be facilitated by greater space, more comfortable flooring, non-restricted interactions with the sow and/or greater complexity of the physical environment. Play behaviour may both contribute to and indicate stress resilience, since it is generally associated with a positive affective state and has been suggested to improve stress coping abilities by enabling animals to recover from unexpected events (Spinka et al., 2001). There was also some evidence of more aggressive behaviour by LP than FC piglets at 2 wk of age ($p = 0.03$) which could have been a consequence of increased play. Martin and colleagues found a higher prevalence of active fighting in piglets reared in the same loose housing system as in the present experiment, while in the studies by Singh and colleagues and Oostindjer and colleagues, loose housing of the sow had no effect on piglet aggression during the lactation period. (Martin et al., 2015; Oostindjer et al., 2011; Singh et al., 2017).

At 17 wk of age, there was an indication that serum IgA concentrations were lower in LP pigs than in FC pigs ($p = 0.02$). IgA concentrations increase in response to acute stressors but decrease in the case of sustained levels of stress when immune activity is inhibited (Sapolsky, 2000; Sapolsky et al., 2000), which suggests a suppressed immune response at 17 wk of age in the LP pigs. However, there was no other evidence from the experiment to support this. For example, elevated glucocorticoids are thought to at least in part be responsible for suppressing immune activity, and there were no differences in cortisol concentrations of pigs from both housing systems at 17 wk of age. Furthermore, growth, which is known to reduce in the case of sustained stress (Moberg, 2000), was not affected by housing treatment at 18 wk of age. We did not record behavioural time budgets of pigs at 17 wk of age, but this may have provided further insight into whether the lower IgA concentrations of LP pigs were stress induced.

Tear staining refers to the accumulation of a red/brown pigmented secretion released by the Harderian gland in the inner corner of the eye of many vertebrates, and in pigs increased levels of tear staining have been associated with isolation, lack of environment enrichment, increased tail damage and increased latency to approach a novel object (DeBoer et al., 2015; Larsen et al., 2019; Telkänranta et al., 2016). The mechanisms and function of stress-induced tear staining are not well understood, although it may be part of the immune response to stress (Payne, 1994). The severity of tear staining was higher in LP piglets than FC piglets at 4 wk of age during the lactation period (*p* < 0.0001 on all 3 severity measurements), and also the estimates of severity were higher at 1 and 7 wk of age with 2 of 6 measurements achieving statistical significance at the 10% level. Tear staining measurements at 4 wk of age were obtained 1-3 d after piglets had received vaccinations and participated in the novel object and human approach tests. Tear staining can increase after one day of pigs being introduced to new housing which is likely a stressor (DeBoer et al., 2015), and it is therefore possible that the increased staining in LP pigs at 4 wk of age reflected a greater response to the stressors of vaccination and behaviour testing that had occurred in the days prior. However, there may have been confounding factors such as air quality, humidity and ammonia levels that affected tear staining and differed between the housing systems. Additionally, tear staining has been positively correlated with growth in pigs as thyroid hormones involved in growth may also be involved in secretion release from the Harderian gland (Larsen et al., 2019). Weight gain during the lactation period was higher in the loose system, and it is therefore unclear if the higher levels of staining early in life in LP pigs were stress induced or simply a result of differences in growth or environmental conditions.

At 3 wk of age, +HC pigs appeared to engage in more nosing directed to other pigs, which was the same effect observed after weaning. Increased nosing may have occurred as a result of +HC pigs detecting odours of other litters and the experimenter after imposition of the positive handling treatment. However, of the 59 behavioural time budget measurements, this was the only one that achieved significance at the 5 % level with respect to the early handling treatment, which is less than expected by chance. There was some evidence of early handling reducing serum cortisol concentrations at 12 wk of age (*p* = 0.04), however there was no indication of greater stress in C pigs from behavioural observations at the same age. Although, there was a tendency for positively handled pigs to have lower tear staining scores and smaller perimeters of staining at 12 wk of age. Previous research has demonstrated a relationship between increased severity of tear staining and higher salivary cortisol concentrations in sows, although the correlation was weak (Schmitt et al., 2019). There were no other detected effects of the early positive handling treatment on basal measurements of cortisol and tear staining, and no effects on serum IgA concentrations at any ages.

Brain-derived neurotrophic factor (BDNF) is a neurotrophin which plays an important role in learning, memory and neuronal development and survival (Cunha et al., 2010; Huang & Reichardt, 2001; Miranda et al., 2019), and has been associated with stress resilience in pigs. Higher concentrations of serum BDNF have been reported in pigs provided with a foraging block as enrichment (Taliaz et al., 2011), and lower concentrations of hippocampal BDNF have been reported in pigs exposed to high-stress transport conditions compared to low-stress conditions (Arroyo et al., 2019). In the present experiment, serum BDNF concentrations were higher in +HC than C piglets at 4 wk of age during the treatment period ($p = 0.02$). While there were no detectable effects of handling on concentrations at 8 and 12 wk of age, +HC pigs appeared to also have higher BDNF concentrations than C pigs at 17 wk of age ($p = 0.04$), which may indicate greater stress resilience in +HC pigs. At 4 wk of age during the lactation period, LP piglets had higher BDNF concentrations than FC piglets. This, interpreted in conjunction with the greater levels of play behaviour and sow-piglet interactions and lower incidence of repetitive nosing, suggests the welfare of LP piglets in their undisturbed home pens may have been superior to that of the FC piglets. However, the LP piglets were evidently less able to cope with additional stressors, based on their stress responses to husbandry practices, weaning, isolation, novelty and humans. Concentrations of BDNF decreased in all groups over time, which is expected due to its central role in brain plasticity, which is known to be greatest early in life and reduce with age (Kolb & Gibb, 2011).

- ➔ *Therefore, while there was little evidence of positive handling early in life affecting the time budgets of behaviour of pigs, the +HC pigs had greater concentrations of serum BDNF during the lactation period and may have also had higher concentrations much later in life at 17 wk of age, suggesting improved stress resilience. Piglets in the loose housing treatment also had greater BDNF concentrations during the lactation period, and additionally, showed lower frequencies of repetitive nosing of pen mates and higher frequencies of play behaviour and interacting with the sow. The effects of the handling and housing treatments on basal physiology and tear staining clearly warrant further investigation.*

4.5.5 Growth, Injuries and Survival

Improved maternal responsiveness and increased interactions between sows and piglets in the loose system did not translate into improved piglet survival. There were more piglet mortalities in the loose system from 1 d of age until weaning, likely due to an increased prevalence of crushing although specific causes of mortality were not recorded. Notably, litter sizes were lower in the loose system even at only 1 d after farrowing, which highlights the mortality risk soon after parturition to piglets in loose farrowing and lactation systems. We also previously reported that while piglets born alive were similar in these two housing systems, piglets weaned were less in the loose system (Hayes et al., 2021b). While the literature is conflicting, there is considerable variability in pre-weaning piglet mortality in loose farrowing and lactation systems (Baxter et al., 2012; Moustsen et al., 2013), although the mortality of liveborn piglets in loose farrowing and lactation systems is usually higher than that in farrowing crates (Cronin et al., 2014; Glencorse et al., 2019). Management and the provision of environment enrichment or bedding are important factors that may be key to optimizing piglet survival in loose systems.

The positive handling treatment had no effects on piglet survival or the maternal responsiveness of sows. There were also no effects of early handling on growth at 4 d, 22 d or 18 wk of age, despite previous research showing weight gain to be higher at 12 wk of age in piglets habituated to human handling (de Oliveira et al., 2019) and human presence (de Oliveira et al., 2015) early in life. Piglet weights and growth rates at the end of the lactation period were higher in the loose system than in farrowing crates, which was most likely due to smaller litter sizes in loose pens. When there were equal stocking densities after weaning, growth rates and weights at 18 wk of age were similar between the housing systems.

Positive human contact piglets had fewer injuries on the head and the rest of the body at 4 wk of age during the lactation period. We previously found a similar effect at 2 d post-weaning (Hayes et al., 2021b). Injuries may be sustained from pen mates or collision with pen fittings during bouts of aggressive or play behaviour, however there was no evidence of effects of the handling treatment on these behaviours during the lactation period. It may be that +HC piglets sustained fewer injuries due to less avoidance of people in the farrowing facility, and therefore fewer collisions with pen fittings, the sow and/or pen mates. Although, fear of humans was also lower in FC pigs than LP pigs at 3, 4 and 6 wk of age, but housing system did not affect injury scores at any age. There was limited evidence of a sustained effect of early handing on injuries, with +HC pigs having fewer injuries on the head at 17 wk of age ($p = 0.04$), although, injuries on the rest of the body at this age were similar between the two handling treatments.

- ➔ *These limited data show increased piglet losses from d 1 of life in the loose housing treatment, despite improved sow maternal behaviour and more piglet-initiated interactions with sows in this system. These findings on piglet mortality, as well as our present and previous findings on reduced injuries in +HC pigs require further investigation because of their implications on pig welfare. Particularly, more information is needed on the specific causes of mortalities and injuries.*

4.6 Conclusion

This experiment showed that positive handling early in life, and, rearing in farrowing crates compared to loose farrowing and lactation pens, improved stress resilience in pigs on the basis of behavioural and physiological responses to stressors such as routine husbandry and management practices, isolation, novelty and humans. While the effects of the early +HC and FC housing treatments may have diminished over time with subsequent handling and housing, there was still some evidence of sustained effects of these early life experiences on stress resilience. These results highlight the importance of early human and housing experiences in shaping the development of stress coping mechanisms in pigs.

4.7 References

Arroyo, L., Valent, D., Carreras, R., Peña, R., Sabrià, J., Velarde, A., & Bassols, A. (2019). Housing and road transport modify the brain neurotransmitter systems of pigs: Do pigs raised in different conditions cope differently with unknown environments? *PLOS ONE, 14*(1), 1-20.

Baxter, E. M., Andersen, I. L., & Edwards, S. A. (2018). Sow welfare in the farrowing crate and alternatives. In M. Špinka (Ed.), *Advances in pig welfare* (pp. 27-72). Duxford, United Kingdom: Woodhead Publishing.

Baxter, E. M., Lawrence, A. B., & Edwards, S. A. (2012). Alternative farrowing accommodation: Welfare and economic aspects of existing farrowing and lactation systems for pigs. *Animal, 6*(1), 96-117.

Blackshaw, J. K., & Hagelsø, A. M. (1990). Getting-up and lying-down behaviours of loose-housed sows and social contacts between sows and piglets during day 1 and day 8 after parturition. *Applied Animal Behaviour Science, 25*(1), 61-70.

Brajon, S., Ringgenberg, N., Torrey, S., Bergeron, R., & Devillers, N. (2017). Impact of prenatal stress and environmental enrichment prior to weaning on activity and social behaviour of piglets (sus scrofa). *Applied Animal Behaviour Science, 197*, 15-23.

Carlstead, K., & Shepherdson, D. J. (2000). Alleviating stress in zoo animals with environmental enrichment. In G. P. Moberg & J. A. Mench (Eds.), *The biology of animal stress: Basic principles and implications for animal welfare* (pp. 337-354). Wallingford, United Kingdom: CABI Publishing.

Center for Conservation Bioacoustics. (2014). Raven pro: Interactive sound analysis software (version 1.5). Ithaca, NY: The Cornell Lab of Ornithology.

Chaloupkova, H., Illmann, G., Neuhauserova, K., Tomanek, M., & Valis, L. (2007). Preweaning housing effects on behavior and physiological measures in pigs during the suckling and fattening periods. *Journal of Animal Science*, 1741-1749.

Chen, A. (2019). Preface. In A. Chen (Ed.), *Stress resilience: Molecular and behavioral aspects* (pp. xvii-xx). London, England: Academic Press.

Chidgey, K. L., Morel, P. C. H., Stafford, K. J., & Barugh, I. W. (2016). Observations of sows and piglets housed in farrowing pens with temporary crating or farrowing crates on a commercial farm. *Applied Animal Behaviour Science, 176*, 12-18.

Clarkson, J. M., Baxter, E. M., & Martin, J. E. (2021). Who plays with whom: Farrowing environment influences isolation of foster piglets in play. *Frontiers in Animal Science, 2*, 724080.

Crofton, E. J., Zhang, Y., & Green, T. A. (2015). Inoculation stress hypothesis of environmental enrichment. *Neuroscience & Biobehavioral Reviews, 49*, 19-31.

Cronin, G. M., Rault, J. L., & Glatz, P. C. (2014). Lessons learned from past experience with intensive livestock management systems. *Revue Scientifique et Technique, 33*(1), 139-151.

Cronin, G. M., Simpson, G. J., & Hemsworth, P. H. (1996). The effects of the gestation and farrowing environments on sow and piglet behaviour and piglet survival and growth in early lactation. *Applied Animal Behaviour Science, 46*(3), 175-192.

Cunha, C., Brambilla, R., & Thomas, K. (2010). A simple role for bdnf in learning and memory? *Frontiers in Molecular Neuroscience, 3*.

Day, J. E. L., Burfoot, A., Docking, C. M., Whittaker, X., Spoolder, H. A. M., & Edwards, S. A. (2002). The effects of prior experience of straw and the level of straw provision on the behaviour of growing pigs. *Applied Animal Behaviour Science, 76*(3), 189-202.

de Oliveira, D., Keeling, L. J., & Paranhos da Costa, M. J. R. (2019). Individual variation over time in piglet´s reactions to early handling and its association to weight gain. *Applied Animal Behaviour Science, 215*, 7-12.

de Oliveira, D., Paranhos da Costa, M. J. R., Zupan, M., Rehn, T., & Keeling, L. J. (2015). Early human handling in non-weaned piglets: Effects on behaviour and body weight. *Applied Animal Behaviour Science, 164*, 56-63.

De, U. K., Nandi, S., Mukherjee, R., Gaur, G. K., & Verma, M. R. (2017). Identification of some plasma biomarkers associated with early weaning stress in crossbred piglets. *Comparative Clinical Pathology, 26*(2), 343-349.

DeBoer, S. P., Garner, J. P., McCain, R. R., Lay Jr, 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*(1), 15-27.

Escribano, D., Gutiérrez, A. M., Tecles, F., & Cerón, J. J. (2015). Changes in saliva biomarkers of stress and immunity in domestic pigs exposed to a psychosocial stressor. *Research in Veterinary Science, 102*, 38-44.

Glencorse, D., Plush, K., Hazel, S., D'Souza, D., & Hebart, M. (2019). Impact of non-confinement accommodation on farrowing performance: A systematic review and meta-analysis of farrowing crates versus pens. *Animals, 9*(11).

Gunnar, M., & Quevedo, K. (2007). The neurobiology of stress and development. *Annual Review of Psychology, 58*, 145-173.

Hayes, M. E., Hemsworth, L. M., Morrison, R. S., Butler, K. L., Rice, M., Rault, J.-L., & Hemsworth, P. H. (2021a). Effects of positive human contact during gestation on the behaviour, physiology and reproductive performance of sows. *Animals, 11*(1), 214.

Hayes, M. E., Hemsworth, L. M., Morrison, R. S., Tilbrook, A. J., & Hemsworth, P. H. (2021b). Positive human contact and housing systems impact the responses of piglets to various stressors. *Animals, 11*(6), 1619.

Hemsworth, P. H. (2018). Key determinants of pig welfare: Implications of animal management and housing design on livestock welfare. *Animal Production Science, 58*(8), 1375-1386.

Hemsworth, P. H., & Barnett, J. L. (1992). The effects of early contact with humans on the subsequent level of fear of humans in pigs. *Applied Animal Behaviour Science, 35*, 83-90.

Hemsworth, P. H., Barnett, J. L., Hansen, C., & Gonyou, H. W. (1986). The influence of early contact with humans on subsequent behavioural response of pigs to humans. *Applied Animal Behaviour Science, 15*, 55-63.

Hemsworth, P. H., & Coleman, G. J. (2011). *Human-livestock interactions: The stockperson and the productivity and welfare of intensively farmed animals* (2nd ed.). Wallingford, United Kingdom: CABI Publishing.

Huang, E. J., & Reichardt, L. F. (2001). Neurotrophins: Roles in neuronal development and function. *Annual Review of Neuroscience, 24*(1), 677-736.

Jones, R. B., & Waddington, D. (1992). Modification of fear in domestic chicks, gallus gallus domesticus, via regular handling and early environmental enrichment. *Animal Behaviour, 43*(pt.6), 1021-1033.

Kinane, O., Butler, F., & O'Driscoll, K. (2021). Freedom to grow: Improving sow welfare also benefits piglets. *Animals, 11*(4).

Kolb, B., & Gibb, R. (2011). Brain plasticity and behaviour in the developing brain. *Journal of the Canadian Academy of Child and Adolescent Psychiatry 20*(4), 265-276.

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. *Applied Animal Behaviour Science, 121*(1), 16-24.

Larsen, M. L. V., Gustafsson, A., Marchant-Forde, J. N., & Valros, A. (2019). Tear staining in finisher pigs and its relation to age, growth, sex and potential pen level stressors. *Animal, 13*(8), 1704-1711.

Leidig, M. S., Hertrampf, B., Failing, K., Schumann, A., & Reiner, G. (2009). Pain and discomfort in male piglets during surgical castration with and without local anaesthesia as determined by vocalisation and defence behaviour. *Applied Animal Behaviour Science, 116*(2), 174-178.

Liu, D., Diorio, J., Tannenbaum, B., Caldji, C., Francis, D., Freedman, A., Sharma, S., Pearson, D., Plotsky, P. M., & Meaney, M. J. (1997). Maternal care, hippocampal glucocorticoid receptors, and hypothalamic- pituitary-adrenal responses to stress. *Science, 277*(5332), 1659-1662.

Luna, D., González, C., Byrd, C. J., Palomo, R., Huenul, E., & Figueroa, J. (2021). Do domestic pigs acquire a positive perception of humans through observational social learning? *Animals, 11*(1), 127.

Luo, L., Reimert, I., Middelkoop, A., Kemp, B., & Bolhuis, J. E. (2020). Effects of early and current environmental enrichment on behavior and growth in pigs. *Frontiers in Veterinary Science, 7*(268).

Lyons, D. M., Parker, K. J., Katz, M., & Schatzberg, A. F. (2009). Developmental cascades linking stress inoculation, arousal regulation, and resilience. *Frontiers in Behavioral Neuroscience, 3*(32).

Lyons, D. M., Parker, K. J., & Schatzberg, A. F. (2010). Animal models of early life stress: Implications for understanding resilience. *Developmental Psychobiology, 52*(5), 402-410.

Lyons, D. M., & Schatzberg, A. F. (2019). Resilience as a process instead of a trait. In A. Chen (Ed.), *Stress resilience: Molecular and behavioral aspects* (pp. 33-44). London, England: Academic Press.

Marchant-Forde, A., E.N. , & Marchant-Forde, J. N. (2014). *Social status and tear staining in nursery pigs.* Paper presented at the The Proceedings of the 48th Congress of the International Society for Applied Ethology, Vitoria-Gasteiz, Spain.

Martin, J. E., Ison, S. H., & Baxter, E. M. (2015). The influence of neonatal environment on piglet play behaviour and post-weaning social and cognitive development. *Applied Animal Behaviour Science, 163*, 69-79.

Mason, G., Wilson, D., Hampton, C., & Würbel, H. (2004). Non-invasively assessing disturbance and stress in laboratory rats by scoring chromodacryorrhoea. *Alternatives to Laboratory Animals, 32*, 153-159.

Meaney, M. J., Aitken, D. H., van Berkel, C., Bhatnagar, S., & Sapolsky, R. M. (1988). Effect of neonatal handling on age-related impairments associated with the hippocampus. *Science, 239*(4841), 766-768.

Meaney, M. J., Bhatnagar, S., Larocque, S., McCormick, C., Shanks, N., Sharma, S., Smythe, J., Viau, V., & Plotsky, P. M. (1993). Individual differences in the hypothalamic-pituitary-adrenal stress response and the hypothalamic crf system. *Annals of the New York Academy of Sciences, 697*, 70-85.

Miranda, M., Morici, J. F., Zanoni, M. B., & Bekinschtein, P. (2019). Brain-derived neurotrophic factor: A key molecule for memory in the healthy and the pathological brain. *Frontiers in Cellular Neuroscience, 13*.

Moberg, G. P. (2000). Biological response to stress: Implications for animal welfare. In G. P. Moberg & J. A. Mench (Eds.), *The biology of animal stress: Basic principles and implications for animal welfare* (pp. 1-21). Wallingford, United Kingdom: CABI Publishing.

Morgan, L., Meyer, J., Novak, S., Younis, A., Ahmad, W. A., & Raz, T. (2021). Shortening sow restraint period during lactation improves production and decreases hair cortisol concentrations in sows and their piglets. *Animal, 15*(2).

Moustsen, V. A., Hales, J., Lahrmann, H. P., Weber, P. M., & Hansen, C. F. (2013). Confinement of lactating sows in crates for 4 days after farrowing reduces piglet mortality. *Animal, 7*(4), 648-654.

Muns, R., Rault, J. L., & Hemsworth, P. (2015). Positive human contact on the first day of life alters the piglet's behavioural response to humans and husbandry practices. *Physiology & Behavior, 151*, 162-167.

Munsterhjelm, C., Peltoniemi, O. A. T., Heinonen, M., Hälli, O., Karhapää, M., & Valros, A. (2009). Experience of moderate bedding affects behaviour of growing pigs. *Applied Animal Behaviour Science, 118*(1), 42-53.Murata, H. (2007). Stress and acute phase protein response: An inconspicuous but essential linkage. *Veterinary Journal, 173*(3), 473-474.

O'Connell, N. E., Beattie, V. E., Sneddon, I. A., Breuer, K., Mercer, J. T., Rance, K. A., Sutcliffe, M. E. M., & Edwards, S. A. (2005). Influence of individual predisposition, maternal experience and lactation environment on the responses of pigs to weaning at two different ages. *Applied Animal Behaviour Science, 90*(3), 219-232.

Oostindjer, M., van den Brand, H., Kemp, B., & Bolhuis, J. E. (2011). Effects of environmental enrichment and loose housing of lactating sows on piglet behaviour before and after weaning. *Applied Animal Behaviour Science, 134*(1), 31-41.

Parker, K. J., & Maestripieri, D. (2011). Identifying key features of early stressful experiences that produce stress vulnerability and resilience in primates. *Neuroscience & Biobehavioral Reviews, 35*(7), 1466-1483.

Payne, A. P. (1994). The harderian gland: A tercentennial review. *Journal of Anatomy, 185*, 1-49.

Pederson, V., Barnett, J. L., Hemsworth, P. H., Newman, E. A., & Schirmer, B. (1998). The effects of handling on behavioural and physiological responses to housing in tether-stalls among pregnant pigs. *Animal Welfare, 7*(2), 137-150.

Petersen, V., Recén, B., & Vestergaard, K. (1990). Behaviour of sows and piglets during farrowing under free-range conditions. *Applied Animal Behaviour Science, 26*(1), 169-179.

Pomorska-Mol, M., Kwit, K., & Markowska-Daniel, I. (2012). Major acute phase proteins in pigs from birth until slaughter. *Bulletin of the Veterinary Institute in Pulaway, 56*(4), 553-557.

Puppe, B., Tuchscherer, M., & Tuchscherer, A. (1997). The effect of housing conditions and social environment immediately after weaning on the agonistic behaviour, neutrophil/lymphocyte ratio, and plasma glucose level in pigs. *Livestock Production Science, 48*(2), 157-164.

Rincón-Cortés, M., & Sullivan, R. (2014). Early life trauma and attachment: Immediate and enduring effects on neurobehavioral and stress axis development. *Frontiers in Endocrinology, 5*(33).

Sapolsky, R. M. (2000). Stress hormones: Good and bad. *Neurobiology of Disease, 7*(5), 540-542.

Sapolsky, R. M., Romero, L. M., & Munck, A. U. (2000). How do glucocorticoids influence stress responses? Integrating permissive, suppressive, stimulatory, and preparative actions. *Endocrine Reviews, 21*(1), 55-89.

Sauerwein, H., Schmitz, S., & Hiss, S. (2005). The acute phase protein haptoglobin and its relation to oxidative status in piglets undergoing weaning-induced stress. *Redox Report, 10*(6), 295-302.

Schmitt, O., Baxter, E. M., Boyle, L. A., & O'Driscoll, K. (2019). Nurse sow strategies in the domestic pig: I. Consequences for selected measures of sow welfare. *Animal, 13*(3), 580-589.

Schneider, C. A., Rasband, W. S., & Eliceiri, K. W. (2012). Nih image to imagej: 25 years of image analysis. *Nature Methods, 9*(7), 671-675.

Singh, C., Verdon, M., Cronin, G. M., & Hemsworth, P. H. (2017). The behaviour and welfare of sows and piglets in farrowing crates or lactation pens. *Animal, 11*(7), 1210-1221.

Spinka, M., Newberry, R. C., & Bekoff, M. (2001). Mammalian play: Training for the unexpected. *Quarterly Review of Biology, 76*(2), 141-168.

Taliaz, D., Loya, A., Gersner, R., Haramati, S., Chen, A., & Zangen, A. (2011). Resilience to chronic stress is mediated by hippocampal brain-derived neurotrophic factor. *The Journal of Neuroscience, 31*(12), 4475.

Tallet, C., Sy, K., Prunier, A., Nowak, R., Boissy, A., & Boivin, X. (2014). Behavioural and physiological reactions of piglets to gentle tactile interactions vary according to their previous experience with humans. *Livestock Science, 167*, 331-341.

Tanida, H., Miura, A., Tanaka, T., & Yoshimoto, T. (1995). Behavioural response to humans in individually handled weanling pigs. *Applied Animal Behaviour Science, 42*, 249-259.

Telkänranta, H., Marchant-Forde, J. N., & Valros, A. (2016). Tear staining in pigs: A potential tool for welfare assessment on commercial farms. *Animal, 10*(2), 318-325.

Thodberg, K., Jensen, K. H., & Herskin, M. S. (2002). Nursing behaviour, postpartum activity and reactivity in sows: Effects of farrowing environment, previous experience and temperament. *Applied Animal Behaviour Science, 77*(1), 53-76.

Turpin, D. L., Langendijk, P., Sharp, C., & Pluske, J. R. (2017). Improving welfare and production in the peri-weaning period: Effects of co-mingling and intermittent suckling on the stress response, performance, behaviour, and gastrointestinal tract carbohydrate absorption in young pigs. *Livestock Science, 203*, 82-91.

Turpin, D. L., Langendijk, P., Tai-Yuan, C., Lines, D., & Pluske, J. R. (2016). Intermittent suckling causes a transient increase in cortisol that does not appear to compromise selected measures of piglet welfare and stress. *Animals, 6*(3), 24.

Villain, A. S., Lanthony, M., Guérin, C., Noûs, C., & Tallet, C. (2020). Manipulable object and human contact: Preferences and modulation of emotional states in weaned piglets. *Frontiers in Veterinary Science, 7.*

VSN International. (2018). Genstat for windows 19th edition. Hemel Hempstead, United Kingdom: VSN International.

Widowski, T. M., Cottrell, T., Dewey, C. E., & Friendship, R. M. (2003). Observations of piglet-directed behavior patterns and skin lesions in eleven commercial swine herds. *Journal of Swine Health and Production, 11*(4), 181-185.

Zupan, M., Rehn, T., de Oliveira, D., & Keeling, L. J. (2016). Promoting positive states: The effect of early human handling on play and exploratory behaviour in pigs. *Animal, 10*(1), 135-141.

Chapter 5

General Discussion

5.1 Overview of Aims and Main Findings

Pigs in production systems are routinely exposed to several stressors and an impaired ability to cope with stress may compromise their welfare and productivity. The aim of this thesis was to examine the effects of early life experiences, including positive human contact and housing, and positive human contact later in life on stress resilience in pigs. The research presented in Chapter 2 studied the effects of 2 min of patting, stroking and scratching imposed daily on multiparous sows during gestation. The research presented in Chapters 3 and 4 studied the effects of 3 and 5 min respectively of daily patting, stroking and scratching imposed on piglets during the lactation period, in addition to the effects of the early farrowing and lactation housing environment. The research presented in Chapter 3 examined these human contact and housing effects in the short-term through studying pigs from birth until weaning, while the research presented in Chapter 4 examined in more detail both immediate and long-term effects of these early life experiences on stress resilience, studying pigs from birth until slaughter. Stress resilience was measured on the basis of behavioural and physiological responses to stressors such as routine husbandry and management practices, isolation, novelty and humans, basal behavioural and physiological measurements that reflect how pigs cope with their general environment, as well as measurements of biological fitness including reproductive performance, growth and survival.

In general, providing opportunities for positive interactions with humans reduced pigs' fear of humans and their biological stress responses to routine husbandry practices imposed by stockpeople. There was an indication that positive handling early in life had a prolonged effect on reducing pigs' fear of humans, since positively handled pigs showed less avoidance of humans well beyond the period when the handling treatment was imposed. Positive handling early in life also appeared to confer broader benefits on stress resilience in addition to reducing fear of humans.

The early housing system during the farrowing and lactation period had a considerable effect on the human-animal relationship, with pigs reared in farrowing crates showing less fear of humans compared to pigs reared in loose farrowing and lactation pens with more space and opportunity for interaction with the sow. Furthermore, pigs reared in farrowing crates were better able to cope with a range of stressors, both before and after weaning. Less contact with stockpeople, other pigs and less visual stimulation in general in the loose system, may have contributed to the greater fear and stress responses of pigs from loose farrowing and lactation pens.

5.2 Positive Human Contact and the Resilience of Pigs to Routine Husbandry Practices

Most of the challenges that pigs encounter as part of routine production involve close or intense interactions with humans and therefore pigs' responses to humans can affect their stress responses to these challenges. A finding across all experiments presented in this thesis was that brief but regular opportunities for positive human contact improved the ability of pigs to cope with routine stressors. Positive handling reduced pigs' avoidance of stockpeople, vocalisations, escape behaviour and cortisol concentrations to the husbandry practices of piglet processing, vaccination, weaning and pregnancy testing, showing that previous positive interactions with humans can ameliorate pigs' stress responses to routine challenges. Notably, only a small amount of positive human contact was necessary in order to see this effect. Muns and colleagues (2015) showed that around 36 minutes of positive handling imposed at a litter level on the first day of life reduced piglets' escape behaviour during tail docking at 2 days of age, and the present research suggests even a lesser amount of positive handling can produce the same benefit. In the experiments presented in Chapters 3 and 4, less than 15 minutes of previous positive handling reduced the duration of piglets' vocalisations and their intensity of escape behaviour during piglet processing at 3-4 days of age. It would be beneficial to further understand the specific duration and timing of positive human interaction that would produce the most benefit in terms of sustaining reduced fear and stress responses throughout the pig's life in production. While increasing concern for animal welfare has led to a number of husbandry practices to be modified or eliminated in the pig industry, some practices are necessary and consequently pigs will unavoidably have negative experiences with humans in production systems (Hemsworth & Coleman, 2011). Given that contact with and handling by stockpeople may be one of the most stressful elements of many routine husbandry practices (Marchant-Forde et al., 2009), finding opportunities for pigs to have positive experiences with humans may be a successful strategy to reduce the stressfulness of these routine challenges.

5.1 Broader Benefits of Positive Human Contact

In the experiments presented in Chapters 3 and 4, the positive human contact treatment was highly effective in reducing pigs' fear of humans but also appeared to have broader benefits on stress resilience. Late in the lactation period positive human contact pigs showed less fear of a novel object and had fewer injuries and higher serum BDNF concentrations compared to routine contact pigs. Furthermore, positive human contact pigs showed less escape behaviour and lower cortisol concentrations after weaning, and lower cortisol concentrations after isolation. One explanation for these broader benefits of the positive handling treatment is that they were mediated by reduced fear of humans. For example, since close human contact and handling were part of the procedure for the novel object test, the isolation test and weaning, reduced fear of humans may have reduced the stressfulness of these situations for positively handled pigs. Another explanation, which is not mutually exclusive to the first, is that general stress resilience was fostered by providing piglets with frequent opportunities to learn to cope with the small challenge of regular and close human contact through the imposition of positive handling. In other words, the positive human contact treatment may have provided piglets with a stressor of minor magnitude to overcome, that improved their ability to cope with a range of stressors. In rodents, early life stressors such as regular handling and maternal separation increase the sensitivity of the hippocampus to glucocorticoids, which enhances negative feedback on HPA axis activity and results in reduced behavioural and physiological responses to a range of stressors later in life (Meaney et al., 1988; Meaney et al., 1993). Furthermore, BDNF, which is associated with neuronal growth, learning and memory due its important role in brain plasticity, is thought to mediate stress resilience by regulating HPA axis activity early in life (Taliaz et al., 2011). The higher concentrations of BDNF in positively handled pigs may have reflected that the handling treatment provided pigs with opportunities for learning that enhanced their general competence to cope with stress. A better understanding of the mechanism by which positive human contact reduced piglets' injuries, fear of novelty, and their stress responses to weaning and isolation would be valuable.

5.2 Visual Contact with Humans

Findings from the three experiments presented in this thesis suggest that visual contact with humans is likely to play a significant role in reducing pigs' fear of humans and thus their stress responses to routine challenges involving humans. In the experiment presented in Chapter 2, positive handling was successful in reducing sows' avoidance of stockpeople imposing pregnancy testing and vaccination in the home pen, but did not affect sows' behaviour towards an unfamiliar human or other stressors imposed outside of the home pen, acute or basal physiological measures of stress, or reproductive performance. One of the challenges in conducting this experiment was eliminating human contact in the control treatment other than that associated with routine management. Although measures were taken to reduce it, imposition of the positive handling treatment in neighbouring pens resulted in sows in the control contact group unavoidably being exposed to up to 30 min daily of additional visual, auditory and olfactory contact with stockpeople, on top of human interaction associated with regular management. This additional human exposure may have diluted any effects of the positive handling treatment. In particular, visual contact with humans may have had the largest contribution since pigs appear to rely on visual cues more than other sensory cues when discriminating humans (Koba & Tanida, 2001). Other research has shown that pigs' fear of humans is reduced when they observe a human stroking other pigs (Luna et al., 2021), and furthermore, research on poultry has shown that visual human contact reduces birds' fear of handling (Zulkifli et al., 2002) and in some cases is even more effective than gentle tactile contact in reducing fear of humans (Jones, 1993). In a commercial system, visual contact may more feasible and practical to implement compared to gentle tactile contact.

Unexpectedly, in the research presented in Chapters 3 and 4, pigs from the loose farrowing and lactation housing treatment showed much greater fear of humans than pigs from the farrowing crate treatment, based on more avoidance and less exploration of an unfamiliar human in tests at 2, 3, 4 and 6 weeks of age. In both of these experiments, piglets in farrowing crates received much more visual human exposure for several reasons. Firstly, the loose pens had high walls to contain the sow which greatly restricted piglets' visual contact outside of the pen, to the extent that people were only visible to piglets when standing directly in front of the pen. In contrast, piglets in farrowing crates could more easily observe people in the room. The farrowing crates were positioned along aisles that stockpeople used to regularly access adjacent rooms, while many of the loose pens were positioned along aisles that were dead ends, which resulted in more human traffic in the farrowing crate room. Additionally, stockpeople spent more time in the farrowing crate room conducting routine management as there were double the number of pigs housed there compared to the loose pen room. Although there were several other differences between the housing systems, it is likely that less visual exposure with people was a contributing factor in the higher levels of fear towards humans shown by loose pen pigs. While further investigation is needed to test whether restrictions in visual contact contribute to the fear responses of pigs to humans, the results of the present research suggests that the human-animal relationship may be an important consideration that is overlooked in the design of housing systems for pigs.

5.3 The Early Housing Environment

The early housing environment affected the welfare of pigs in several ways. Compared to piglets reared in farrowing crates, piglets reared in loose farrowing and lactation pens with greater space, complexity in their physical environment and opportunity for interaction with the sow, showed more play behaviour and less repetitive nosing directed towards pen mates during the lactation period. Play behaviour may have been facilitated in the loose system through increased space, more comfortable flooring, non-restricted interactions with the sow and/or greater complexity of the physical environment. During the lactation period piglets from loose pens also had higher concentrations of serum BDNF. BDNF plays a role in brain plasticity, learning and memory (Cunha et al., 2010; Huang & Reichardt, 2001; Miranda et al., 2019), and in rodents has been associated with improved spatial learning and memory (Mizuno et al., 2003; Mizuno et al., 2000), increased maternal care (Branchi et al., 2006; Liu et al., 2000), and social enrichment (Branchi et al., 2006). Serum BDNF concentrations may have been higher in the loose system due to piglets having more opportunities for spatial learning (in their larger and more complex physical environment), maternal care (based on increased sow maternal responsiveness and more piglet-initiated sow interactions) and play. Spinka and colleagues (2001) proposed that the function of play behaviour is for animals to build flexibility in response to unexpected events. They suggest that greater play experience improves the ability of animals to cope with future challenges, but unexpectedly, the opposite was observed in the present research. Piglets reared in the loose system were clearly less able to cope with challenges before and after the lactation period, based on their behavioural and physiological responses to husbandry practices, weaning, isolation, novelty and humans. Compared to piglets reared in farrowing crates, piglets reared in loose pens showed greater fear of novelty, more escape behaviour during capture by a stockperson and more injuries early in the lactation period, higher cortisol concentrations after weaning, more vocalising and a higher cortisol response to isolation during the weaner period, and more baulking when being moved out of the home pen during the grower/finisher period. These results certainly raise questions on the stress resilience of pigs reared in loose housing systems, which, in general, are considered superior in terms of animal welfare (Baxter et al., 2018; Johnson & Marchant-Forde, 2009; Martin et al., 2015; Oostindjer et al., 2011; Singh et al., 2017). There may have been features, such as high solid walls and restricted human-contact, that were unique to the loose housing system studied in the present experiment.

While the loose housing treatment in our experiments offered piglets more space, physical complexity and opportunity for interaction with the sow, as mentioned previously it was more restrictive in terms of piglets' contact with people. Furthermore, in the loose system there was less opportunity for interaction with adjacent pigs. In fact, piglets from the loose system had less visual stimulation in general. Stress resilience is conferred by learning to overcome small challenges (Lyons et al., 2009; Lyons et al., 2010; Lyons & Schatzberg, 2019; Parker & Maestripieri, 2011), and piglets in the loose system were reared in a more isolated environment where there may have been less capacity for this to occur. Therefore, pigs from the loose housing treatment may have been more vulnerable to stress due to fewer opportunities during rearing to learn to overcome stressors such as frequent and/or close exposure to stockpeople and other pigs. In addition, the sow, in terms of her stress, fear and interaction with piglets, may have impacted her offspring's ability to cope with stress. In the experiment presented in Chapter 4, sows from loose pens showed greater maternal responsiveness towards piglets vocalisations, and piglets from loose pens initiated more interactions with sows, suggesting differences in maternal behaviour between the housing systems. Our research did not allow the separate contributions of effects on stress resilience of the role of the sow, contact with humans, contact with neighbouring pigs and overall visual stimulation, as well as other possible differences between the housing systems studied, to be determined. The same challenge arises when reviewing the literature on the effects of early housing on piglet welfare since significant variation exists in the design of farrowing and lactation housing systems, particularly with regard to space, physical complexity, provision of substrates, and the length and timing of confinement of the sow. Furthermore, differences between studies in management, stockperson behaviour, and gestational experience of the dam. Due to the implications of fear and stress on animal welfare, clearly further examination is warranted to unravel which specific features of the early housing environment have the greatest effect on piglet and sow fear and stress responses.

5.4 Sustained Effects of Early Life Experiences on Stress Resilience

Early life experiences can play an important role in shaping how animals behave and respond to stress as adults (Knudsen, 2004; Lyons & Schatzberg, 2019; Parker & Maestripieri, 2011; Short et al., 2019). Overall, there is a lack of research assessing impacts of early life experiences on pigs beyond weaning. A unique aspect of our experiment presented in Chapter 4 was that it followed pigs from birth until slaughter at 21 weeks of age, to explore both immediate and long-term consequences of early experiences on stress resilience. There were clear immediate effects of the human contact and housing treatments on the responses of pigs to stressors during the lactation period, that suggested improved coping in positive human contact and farrowing crate pigs, compared to routine contact and loose pen pigs respectively. There was also some evidence that these effects were sustained after the treatment period had finished (i.e., after weaning at 4 weeks of age).

Firstly, pigs reared with positive human contact in the first 4 weeks of life showed less avoidance of an unfamiliar human at 3 weeks of age during the lactation period, but also in tests at 6, 9 and 14 weeks of age. The effects of the early handling treatment on fear responses at 9 and 14 weeks of age were not as strong as when pigs were tested earlier in life, suggesting that subsequent interactions with humans may have diluted the effects of early positive handling, and/or reduced the fear responses of routine contact pigs. Nevertheless, early positive handling still reduced fear of humans for most of the study period, providing further support for a sensitive period in pigs (Hemsworth & Barnett, 1992). The positive human contact treatment also reduced the cortisol response of pigs to isolation at 7 weeks of age. Furthermore, positive human contact pigs had fewer injuries and higher BDNF concentrations at 4 weeks of age prior to weaning, and there was some indication that these same effects were present much later in life at 17 weeks of age. However, at 17 weeks of age, only injuries on the head were reduced in positive human contact pigs, while injuries on the rest of the body were similar between the handling treatment. Additionally, there were no effects of the early human contact treatment on BDNF concentrations at 8 and 12 weeks of age. While this is only limited evidence of positive handling imposed early in life resulting in sustained improvements to stress resilience, it warrants further research nonetheless.

Rearing in farrowing crates from 0-4 weeks of age reduced piglets' avoidance of novelty and humans at 2-3 weeks of age and escape behaviour during capture by a stockperson at 3-4 days of age, and there was evidence of some maintenance of better stress resilience in farrowing crate pigs after weaning when all pigs were in identical housing. Farrowing crate pigs showed less escape behaviour from the pen 0-1 h after weaning, lower cortisol concentrations 2 h after weaning and less vocalisation and lower cortisol concentrations in response to isolation at 7 weeks of age. Additionally, farrowing crate pigs showed less fear of humans in tests at 4 and 6 weeks of age after weaning, but not at 9 or 14 weeks of age. However, at 21 weeks of age when being moved out of the home pen, there was less baulking by farrowing crate pigs. These findings suggest that while the effects of the early housing treatment on pigs' fear of humans may have weakened over time, effects on fear responses to novel situations such as isolation and moving pens were more enduring. Furthermore, early handling appeared to have a more profound and sustained effect on fear responses to humans compared to the early housing environment.

5.5 Conclusion

This research showed that interactions with humans and early human and housing experiences can impact both the immediate and longer-term resilience of pigs to stressors. Providing opportunities for positive human interaction, particularly early in life, reduced pigs' fear of humans and their biological stress responses to various challenges, providing further support that humans are a key determining factor in the welfare of pigs in a production setting. The pig industry should look for opportunities to increase positive experiences between pigs and humans. Increasing visual human contact may also be of benefit, although further investigation is required to examine the effects of visual contact on the human-animal relationship. Contrary to our initial expectations, rearing in farrowing crates improved stress resilience relative to rearing in loose farrowing and lactation pens. Given that an impaired ability to cope with stress can compromise animal welfare, and, given the continued interest in Australia but also internationally to reduce confinement for sows during the farrowing and lactation period, clearly more research is needed to determine specific characteristics of the early housing environment that contribute to the development of stress coping mechanisms in pigs.

5.6 References

Baxter, E. M., Andersen, I. L., & Edwards, S. A. (2018). Sow welfare in the farrowing crate and alternatives. In M. Špinka (Ed.), *Advances in pig welfare* (pp. 27-72). Duxford, United Kingdom: Woodhead Publishing.

Branchi, I., D'Andrea, I., Fiore, M., Di Fausto, V., Aloe, L., & Alleva, E. (2006). Early social enrichment shapes social behavior and nerve growth factor and brain-derived neurotrophic factor levels in the adult mouse brain. *Biological Psychiatry, 60*(7), 690-696.

Cunha, C., Brambilla, R., & Thomas, K. (2010). A simple role for bdnf in learning and memory? *Frontiers in Molecular Neuroscience, 3*.

Hemsworth, P. H., & Barnett, J. L. (1992). The effects of early contact with humans on the subsequent level of fear of humans in pigs. *Applied Animal Behaviour Science, 35*, 83-90.

Hemsworth, P. H., & Coleman, G. J. (2011). *Human-livestock interactions: The stockperson and the productivity and welfare of intensively farmed animals* (2nd ed.). Wallingford, United Kingdom: CABI Publishing.

Huang, E. J., & Reichardt, L. F. (2001). Neurotrophins: Roles in neuronal development and function. *Annual Review of Neuroscience, 24*(1), 677-736.

Johnson, A. K., & Marchant-Forde, J. N. (2009). Welfare of pigs in the farrowing environment. In J. N. Marchant-Forde (Ed.), *The welfare of pigs* (pp. 141-188). Dordrecht, Netherlands: Springer.

Jones, R. B. (1993). Reduction of the domestic chick's fear of human beings by regular handling and related treatments. *Animal Behaviour, 46*(5), 991-998.

Knudsen, E. I. (2004). Sensitive periods in the development of the brain and behavior. *Journal of Cognitive Neuroscience, 16*(8), 1412-1425.

Koba, Y., & Tanida, H. (2001). How do miniature pigs discriminate between people?. Discrimination between people wearing coveralls of the same colour. *Applied Animal Behaviour Science, 73*(1), 45-58.

Liu, D., Diorio, J., Day, J. C., Francis, D. D., & Meaney, M. J. (2000). Maternal care, hippocampal synaptogenesis and cognitive development in rats. *Nature Neuroscience, 3*(8), 799.

Luna, D., González, C., Byrd, C. J., Palomo, R., Huenul, E., & Figueroa, J. (2021). Do domestic pigs acquire a positive perception of humans through observational social learning? *Animals, 11*(1), 127.

Lyons, D. M., Parker, K. J., Katz, M., & Schatzberg, A. F. (2009). Developmental cascades linking stress inoculation, arousal regulation, and resilience. *Frontiers in Behavioral Neuroscience, 3*(32).

Lyons, D. M., Parker, K. J., & Schatzberg, A. F. (2010). Animal models of early life stress: Implications for understanding resilience. *Developmental Psychobiology, 52*(5), 402-410.

Lyons, D. M., & Schatzberg, A. F. (2019). Resilience as a process instead of a trait. In A. Chen (Ed.), *Stress resilience: Molecular and behavioral aspects* (pp. 33-44). London, England: Academic Press.

Marchant-Forde, J. N., Lay, D. C., McMunn, K. A., Cheng, H. W., Pajor, E. A., & Marchant-Forde, R. M. (2009). Postnatal piglet husbandry practices and well-being: The effects of alternative techniques delivered in combination. *Journal of Animal Science*(3), 1150-1161.

Martin, J. E., Ison, S. H., & Baxter, E. M. (2015). The influence of neonatal environment on piglet play behaviour and post-weaning social and cognitive development. *Applied Animal Behaviour Science, 163*, 69-79.

Meaney, M. J., Aitken, D. H., van Berkel, C., Bhatnagar, S., & Sapolsky, R. M. (1988). Effect of neonatal handling on age-related impairments associated with the hippocampus. *Science, 239*(4841), 766-768.

Meaney, M. J., Bhatnagar, S., Larocque, S., McCormick, C., Shanks, N., Sharma, S., Smythe, J., Viau, V., & Plotsky, P. M. (1993). Individual differences in the hypothalamic-pituitary-adrenal stress response and the hypothalamic crf system. *Annals of the New York Academy of Sciences, 697*, 70-85.

Miranda, M., Morici, J. F., Zanoni, M. B., & Bekinschtein, P. (2019). Brain-derived neurotrophic factor: A key molecule for memory in the healthy and the pathological brain. *Frontiers in Cellular Neuroscience, 13*.

Mizuno, M., Yamada, K., He, J., Nakajima, A., & Nabeshima, T. (2003). Involvement of bdnf receptor trkb in spatial memory formation. *Learning and Memory, 10*(2), 108-115.

Mizuno, M., Yamada, K., Olariu, A., Nawa, H., & Nabeshima, T. (2000). Involvement of brain-derived neurotrophic factor in spatial memory formation and maintenance in a radial arm maze test in rats. *The Journal of Neuroscience, 20*(18), 7116-7121.

Muns, R., Rault, J. L., & Hemsworth, P. (2015). Positive human contact on the first day of life alters the piglet's behavioural response to humans and husbandry practices. *Physiology & Behavior, 151*, 162-167.

Oostindjer, M., van den Brand, H., Kemp, B., & Bolhuis, J. E. (2011). Effects of environmental enrichment and loose housing of lactating sows on piglet behaviour before and after weaning. *Applied Animal Behaviour Science, 134*(1), 31-41.

Parker, K. J., & Maestripieri, D. (2011). Identifying key features of early stressful experiences that produce stress vulnerability and resilience in primates. *Neuroscience & Biobehavioral Reviews, 35*(7), 1466-1483.

Short, A. K., Bolton, J. L., & Baram, T. Z. (2019). Mechanisms by which early-life experiences promote enduring stress resilience or vulnerability. In A. Chen (Ed.), *Stress resilience: Molecular and behavioral aspects* (pp. 165). London, England: Academic Press.

Singh, C., Verdon, M., Cronin, G. M., & Hemsworth, P. H. (2017). The behaviour and welfare of sows and piglets in farrowing crates or lactation pens. *Animal, 11*(7), 1210-1221.

Spinka, M., Newberry, R. C., & Bekoff, M. (2001). Mammalian play: Training for the unexpected. *Quarterly Review of Biology, 76*(2), 141-168.

Taliaz, D., Loya, A., Gersner, R., Haramati, S., Chen, A., & Zangen, A. (2011). Resilience to chronic stress is mediated by hippocampal brain-derived neurotrophic factor. *The Journal of Neuroscience, 31*(12), 4475.

Zulkifli, I., Gilbert, J., Liew, P. K., & Ginsos, J. (2002). The effects of regular visual contact with human beings on fear, stress, antibody and growth responses in broiler chickens. *Applied Animal Behaviour Science, 79*(2), 103-112.