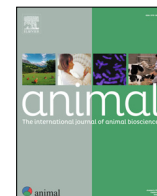




Animal

The international journal of animal biosciences



Early human contact and housing for pigs – part 2: resilience to routine husbandry practices



M.E. Lucas^{a,*}, L.M. Hemsworth^a, K.L. Butler^a, R.S. Morrison^b, A.J. Tilbrook^{c,d}, J.N. Marchant^{e,f}, J.-L. Rault^g, R.Y. Galea^a, P.H. Hemsworth^a

^a The Animal Welfare Science Centre, Faculty of Science, The University of Melbourne, Parkville, Victoria 3010, Australia

^b Rivalea Australia Pty Ltd, Corowa, Victoria 2464, Australia

^c Centre for Animal Science, Queensland Alliance for Agriculture and Food Innovation, The University of Queensland, St Lucia 4072, Australia

^d School of Veterinary Science, The University of Queensland, Gatton Campus, Gatton, Queensland 4343, Australia

^e Organic Plus Trust, Alexandria, VA 22302, USA

^f A World of Good Initiative Inc., Dover, DE 19901, USA

^g Institute of Animal Welfare Science, University of Veterinary Medicine, Vienna A-1210, Austria

ARTICLE INFO

Article history:

Received 6 July 2023

Revised 10 April 2024

Accepted 11 April 2024

Available online 18 April 2024

Keywords:

Animal welfare

Early experience

Handling

Human-animal interactions

Stress

ABSTRACT

The ability of pigs to cope with routine farming practices can affect their welfare. This paper is part of a series on early experiences and stress, and reports on the effects of early human contact and housing on the responses of pigs to routine husbandry practices. Using a 2×2 factorial design, 48 litters of pigs were raised in either a conventional farrowing crate (FC) or a loose farrowing pen (LP; PigSAFE pen) which was larger, more physically complex and allowed the sow to move freely. Piglets were provided with either routine contact from stockpeople (C), or routine contact plus regular opportunities for positive human contact (+HC) involving 5 min of scratching, patting and stroking imposed to the litter 5 days/week from 0 to 4 weeks of age. At 4 weeks of age, piglets were weaned and re-housed with controlled mixing of litters within treatment. At 4 days of age, after only 3 bouts of the handling treatment, +HC pigs showed less escape behaviour than C pigs after capture by a stockperson for vaccinations and tail docking, and shorter durations of vocalisations throughout the procedures. The +HC pigs also showed less escape behaviour when captured by a stockperson at 3 weeks of age. The FC pigs showed less escape behaviour than LP pigs after capture by a stockperson at 4 days of age but not at 3 weeks of age. Serum cortisol concentrations were lower in FC pigs than LP pigs 2 h after weaning but not at 49 h after weaning, whereas serum cortisol concentrations were lower in +HC pigs than C pigs at 49 h after weaning but not at 2 h after weaning. In the period from 0 to 1 h after weaning, C pigs from LP performed the most escape attempts, although escape attempts were rare overall. When being moved out of the home pen by a stockperson at 21 weeks of age, FC pigs showed less baulking than LP pigs, but there were no detected effects of human contact treatment. In conclusion, both housing system and human contact during lactation affected the stress responses of pigs to routine husbandry practices. The +HC and FC pigs appeared to cope better than C and LP pigs, based on lower responses indicative of stress including escape behaviour, vocalisations and cortisol concentrations. These findings are consistent with corresponding reductions in fear that were reported in Part 1 of this series of papers.

© 2024 The Authors. Published by Elsevier B.V. on behalf of The Animal Consortium. This is an open access article under the CC BY license (<http://creativecommons.org/licenses/by/4.0/>).

Implications

This research showed that early experiences can affect the ability of pigs to cope with stress associated with routine farming practices. Providing opportunities for positive experiences with people early in life through regular patting, stroking and scratching, improved the capacity of piglets to cope with husbandry practices

such as vaccinations and weaning. Compared to the loose farrowing and lactation housing system studied in the present research, rearing in farrowing crates appeared to reduce piglets' stress responses to routine husbandry challenges early in life, and improve ease of handling by stockpeople much later in life.

Introduction

In intensive production systems, pigs are regularly challenged with stressors including sudden weaning, regular close human

* Corresponding author.

E-mail address: megan.lucas@unimelb.edu.au (M.E. Lucas).

contact including handling, painful husbandry procedures and exposure to unfamiliar social and physical environments. The stress resilience of pigs to these routine challenges, defined as their ability to cope with and recover from stressors (Iacoviella and Charney, 2019), holds considerable ramifications for animal welfare as a lack of resilience can jeopardise the well-being of pigs. Stress resilience is characterised by low physiological and behavioural responses to stressors and subsequent rapid recovery (Chen, 2019). Factors that are likely to affect stress resilience in pigs include early experiences with the physical environment, the dam and with humans.

We and others have shown that fostering a positive human–animal relationship, through positive interactions such as patting, stroking and talking softly to pigs, can improve the capacity of pigs to handle routine stressors involving humans. For example, regular patting and scratching of sows decreased their avoidance of stockpeople during pregnancy testing and vaccination (Hayes et al., 2021a), and when imposed on piglets, reduced piglets' vocalisations (Hayes et al., 2021b) and escape behaviour (Muns et al., 2015; Hayes et al., 2021b) during husbandry procedures conducted in the lactation period. Furthermore, previous experience with a handler who used careful movements and spoke softly led to piglets showing more resting and less escape and agonistic behaviour after weaning, compared to piglets that had previous experience with a handler who moved unpredictably and shouted during routine checks and feeding (Sommavilla et al., 2011). These studies show that previous positive interactions with humans can reduce the aversiveness of many routine farming practices.

There is also evidence that the early housing system can affect the capacity of pigs to cope with routine stressors. It has been suggested that through increased maternal care, opportunity for sow–interaction, space and/or complexity of the environment, rearing in loose lactation systems as opposed to farrowing crates may better prepare pigs to cope with stress (Oostindjer et al., 2011). Chaloupková and colleagues (2007) found that cortisol responses to transport at 6 months of age were lower in pigs reared in straw-enriched loose lactation pens than in pigs reared in farrowing crates, and Oostindjer and colleagues (2011) found that after weaning, pigs reared in loose lactation pens showed more food exploration and less manipulative behaviour and belly nosing than pigs from farrowing crates. These findings suggest improved resilience in pen-reared pigs. In contrast, our previous research found that pigs raised in pens compared to farrowing crates were more likely to be nosing pen mates, nosing the floor, active and vocalising after weaning, which are behaviours that may indicate greater stress during the weaning process (Hayes et al., 2021b). Although pigs from the loose system were reared with more space, physical complexity and opportunity for sow–piglet interaction, they had less visual stimulation and opportunity for contact with people and other pigs in the particular housing system we studied. The latter factors may have affected their responses to weaning and other stressors.

In Part 1 of this series of papers on the effects of early human contact and lactation housing on stress resilience, we reported on the influence of these early experiences on the development of fear responses in pigs (Lucas et al., 2024a, submitted). Compared to pigs raised with routine human contact and pigs raised in loose pens respectively, pigs raised with opportunities for positive human interactions early in life and pigs raised in farrowing crates showed less fear, based on increased exploration and reduced avoidance, of humans, novelty, and social isolation. Reduced fear levels are likely to improve the stress resilience of pigs to routine challenges given that many routine stressors involve exposure to stockpeople, novel situations and isolation from conspecifics. In the present paper, Part 2 of this series, we report on the effects of early positive human contact and the lactation housing system on the resilience

of pigs to routine husbandry stressors. In measuring resilience, we consider the magnitude of pigs' physiological and behavioural responses to routine farm practices. This includes biological responses indicative of stress such as glucocorticoid and immune activity after stress exposure, as well as behavioural responses such as escape behaviour, vocalisation, abnormal behaviour and normal behaviour during and after routine stress exposure.

Material and methods

Experimental design

From a design viewpoint, the experiment consisted of three consecutive phases (preweaning phase, weaner transition phase and weaner-finisher phases), with different experimental design considerations as described below. This led to different statistical analyses for the three phases (see Statistical Analysis section). The methodology of the first of the three phases was very similar, but not identical, to a separate previous study (Hayes et al., 2021b). The present research was conducted at the same research and innovation unit of a large commercial piggery in Corowa, NSW, Australia, as Hayes et al. (2021b).

Preweaning phase: experimental set-up

Preweaning, the experiment consisted of 24 litters from a farrowing crate housing system (FC), where the sow was confined throughout the farrowing and lactation period, and 24 litters from a larger and more structurally complex loose pen (LP), where the sow was free to move throughout the farrowing and lactation period. All litters were born from second parity Landrace × Large White sows that previously farrowed in a similar housing system, either farrowing crates or loose housing, at their first parity. Twelve FC litters and 12 LP litters were assigned to a routine human contact (C) treatment, and the remaining 12 FC litters and 12 LP litters were assigned to a positive human contact (+HC) treatment. This resulted in a 2 housing system × 2 human contact treatment factorial design during the lactation period. The housing systems and human contact treatments are described in subsequent sections, with further detail reported in Hayes et al. (2021b). Additionally, further detail related to experimental design, housing and management is reported in Part 1 of this series of papers (Lucas et al., 2024a, submitted).

Preweaning phase: housing treatment

Detailed diagrams of the housing treatments are available in Part 1 of this series (Lucas et al., 2024a, submitted). The farrowing crate and loose pen housing treatments had similar overhead lighting and ambient temperatures, and no bedding or enrichment was provided. Each farrowing crate contained a 2.3 × 1.7 m area for the piglets with slatted steel flooring, and a 1.1 × 0.41 m solid creep mat heated by an overhead lamp. The surrounding walls of each farrowing crate allowed sows and piglets to have visual contact with people in the aisles. Each loose pen (PigSAFE design; Baxter et al., 2015) contained a 3.6 × 2.4 m area for piglets and sows. The pens had a combination of solid and slatted plastic flooring and contained a covered piglet-only triangular creep area heated by a lamp. Although rarely used, there was a stalled area within each pen where the sow could be confined briefly to allow safe entry for stockpeople. The walls in the central and back areas of the pen contained sloped sides to reduce the risk of overlay by the sow. There were barred windows between pens which allowed limited interaction between adjacent pigs, and piglets' visual contact with people in the room was also minimal. Due to recent design modifications, at the back of each pen, there was a 2.4 × 0.4 m area of space which was not accessible to sows or piglets.

Prewaning phase: human contact treatment

The routine contact treatment involved human contact with stockpeople through the imposition of routine husbandry and management that was typical of a commercial environment. This included visual contact with stockpeople during health and welfare checks and sow feeding twice per day, and when piglet creep food was provided once per day after 14 days of age. In the positive human contact treatment, piglets received regular opportunities to interact with an experimenter in addition to routine contact with stockpeople. 5 days per week from 1 day of age until weaning, the +HC treatment involved an experimenter gently patting, stroking and scratching piglets. Two experimenters, one male and one female, were responsible for delivering the treatment, but only one of these experimenters imposed it each day. The treatment was delivered to any piglets that approached the experimenter or were sleeping in the creep area, and the experimenter attempted to interact with as many different piglets in the litter as possible. The experimenter remained silent during imposition of the treatment. The +HC treatment was delivered to the litter for a duration of 5 min in the morning, between 0700 and 1000 h. In farrowing crates, the experimenter crouched behind the sow's crate and next to the creep area to deliver the +HC treatment from inside the pen, and in loose pens, the experimenter crouched outside the pen and interacted with piglets over the pen wall by removing the creep roof.

Weaner transition phase

Weaning occurred at 4 weeks of age (mean age = 27 days; SD = 1.5; no difference between treatments). The process for weaning was somewhat complex, involving a two-stage mixing and moving procedure. The rationale behind this was to minimise disruption to other pigs during the collection of blood samples after weaning, and to observe the behavioural response of pigs in smaller groups during the human test at 4 weeks of age (reported in [Lucas et al., 2024a](#)). In summary, the procedure involved mixing half of the pigs at the beginning of the 'weaner transition phase' which lasted from 0 to 48 h postweaning, and mixing the remaining half 48 h postweaning at the beginning of the 'weaner-finisher phase'.

Prior to weaning, 2 litters of the same lactation housing system and human contact treatment were selected to be paired and mixed. This selection was based on there being enough pigs from each sex to eventually make up a pen of eight males and a pen of eight females in the weaner-finisher phase. The process for weaning and mixing was as follows. Firstly, sows were removed from the farrowing rooms. Then, four same-sex pigs from each pair of litters, referred to as cohort 1, were mixed and housed in 1.8 × 0.8 m pens containing slatted plastic flooring in an adjacent shed. Cohort 1 stayed in these pens for the 2 days of the weaner transition phase. Thus, during this phase, there were 24 pairs of pens. Each pair consisted of a pen of four male pigs and a pen of four female pigs, with all eight pigs in a pair originating from 2 litters of the same preweaning housing system and human contact treatment.

The remaining pigs from each pair, cohort 2, staying in farrowing house during this period. At 48 h after weaning, the cohort 1 and 2 pigs from each pair were mixed in the weaner facility which marked the start of the weaner-finisher phase. Essentially, this resulted in the cohort 1 pigs being exposed to moving pens and mixing with unfamiliar pigs twice (at 0 h and at 48 h postweaning), while the cohort 2 pigs were only moved and mixed once (at 48 h postweaning). 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.

Weaner-finisher phase

Two days after weaning (start of the weaner-finisher phase), the pigs from each cohort 1 pen were moved to the weaner facility and mixed with an additional four pigs of the same sex and pair from cohort 2 that had remained in the farrowing house. Thus, in the weaner facility, there were 24 pairs of pens each made up of a pen of eight male pigs (four from each cohort) and a pen of eight female pigs (four from each cohort), with all 16 pigs in a pair of pens being from 2 litters of the same farrowing and lactation housing system and human contact treatment. After the eight male and eight female pigs from each pair had been moved to the weaner facility, the remaining pigs from cohort 2 were removed from the experiment.

At 10 weeks of age, pigs remained in the same groups but were moved to the grower/finisher facility where they stayed until the conclusion of the experiment. The weaner and grower/finisher pens were spatially arranged in a six-block split-plot design with housing system associated with mainplots, human contact treatments associated with subplots and each subplot being two adjacent pens (one pen containing eight males, one pen containing eight females). The two pens in a subplot contained only pigs from 2 litters that were being paired. The pens in the weaner facility were 3.0 × 1.5 m, and the pens in the grower/finisher facility were 3.7 × 2.6 m. All pens contained ¾ slatted steel flooring and ¼ solid concrete flooring.

Measurements

The physiological and behavioural responses of pigs to routine husbandry practices were measured during piglet processing at 4 days of age, vaccination at 3 weeks of age, weaning at 4 weeks of age (during both the weaner transition and weaner-finisher phases of the experiment), and moving out of the home pens at 21 weeks of age.

Piglet processing at 4 days of age

Processing occurred at 4 days 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 by one hind leg 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 using one hand under the abdomen, injected an iron supplement intramuscularly and passed the piglet to the second stockperson who held the piglet slightly inverted (head up) to administer an oral coccidiosis vaccination and before returning it 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 (head down) while the second stockperson docked approximately two-thirds of the tail with gas-heated cautery clippers before returning the piglet to the home pen. On average, processing took 4 min for each litter with no difference between treatments.

The behavioural responses of piglets immediately after being captured, and during oral vaccination and tail docking were assessed by one observer using video footage (GoPro Hero8 camera). 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. Similar to [Hayes et al. \(2021b\)](#), piglet vocalisations were recorded throughout processing using a microphone (Samson Meteor USB microphone), held approximately 1 m from piglets, connected to a laptop running Raven Pro sound analysis software ([K. Lisa Yang Center for Conservation Bioacoustics, 2014](#)). The duration and number of

vocalisations for each piglet, as well as the peak frequency the length of each individual call was determined. Blood samples for subsequent analysis of serum cortisol were collected 1 h after processing from two males and two females from each litter (see subsequent section “Sample Collection Details and Assay Characteristics” for further detail). The samples were collected from the first two piglets of each sex that were caught, prior to all piglets from the litter being weighed and receiving an ear tag for identification (piglet weights are reported in Part 3, [Lucas et al., 2024b](#), submitted).

Vaccination at 3 weeks of age

At 3 weeks of age, all piglets were administered an intramuscular vaccination against porcine circovirus-associated disease and additionally, male piglets were administered an immunisation against boar taint (Improvac). Vaccination was carried out by four stockpeople in loose pens and five stockpeople in farrowing crates, with three of these stockpeople imposing vaccination in both housing systems. 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 piglet to one of 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 (due to the differences in vaccination protocols between sexes) while a fifth stockperson administered vaccination.

Using the previously mentioned scale, the behavioural responses of piglets immediately after capture were assessed from video footage (GoPro Hero8 camera) by the same observer that assessed the responses of piglets to processing at 4 days of age. Additionally, as with processing, piglet vocalisations were recorded throughout vaccination and later analysed in Raven Pro ([K. Lisa Yang Center for Conservation Bioacoustics, 2014](#)).

Weaning and mixing at 4 weeks of age

[Fig. 1](#) shows a summary of the procedure followed and an overview of measurements collected at weaning and mixing at 4 weeks of age, and further detail on the process of weaning and mixing is described in previous sections (Experimental Design subsections, “Weaner Transition Phase” and “Weaner-Finisher Phase”). Behavioural observations were conducted by one observer using video footage (GoPro Hero8 camera) of pigs from cohort 1 at 0–1 h after weaning and mixing (start of the weaner transition phase), and of all pigs when cohorts 1 and 2 were mixed at 48–49 h after weaning (start of the weaner-finisher phase). Data were analysed in half-hour blocks to create four sets of behaviour observations postweaning: 0–30 and 30–60 min postweaning and 0–30 and 30–60 min postmixing of cohorts 1 and 2. Instantaneous scan sampling at 60 s intervals was used to record behaviours listed in the ethogram in [Table 1](#). The video cameras covered between 90 to 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 four pigs in 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).

Moving out of pens at 21 weeks of age

At 21 weeks of age, pigs were moved out of the home pen to be loaded for slaughter. An unfamiliar experienced 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 (GoPro Hero8 camera), the time taken to move all pigs out of the pen and the total number of times the stockperson used additional interventions to move

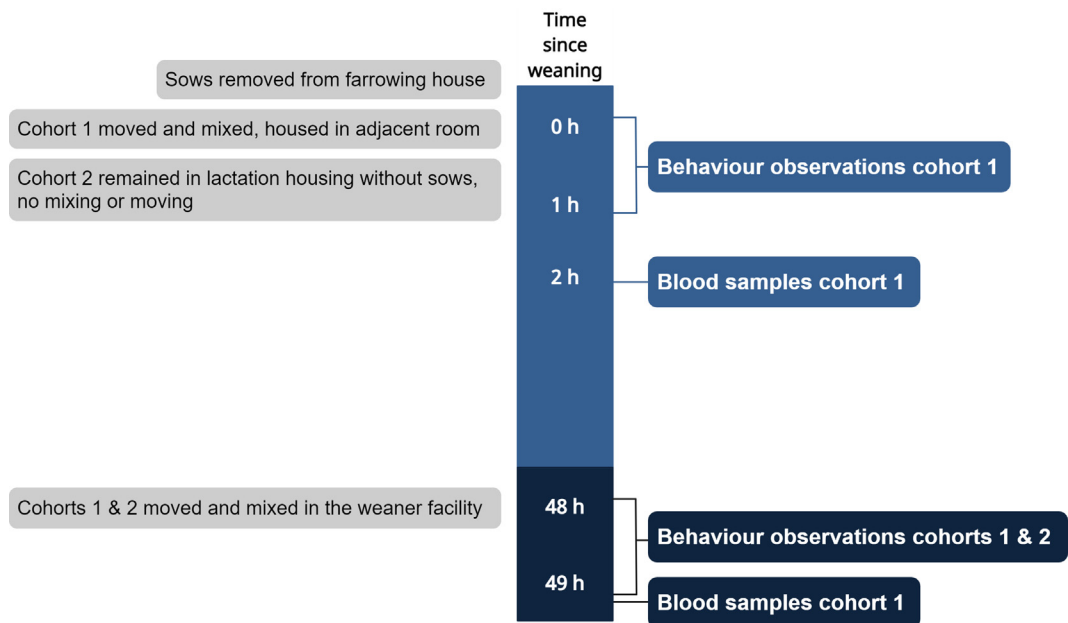


Fig. 1. Timeline and overview of measurements collected from pigs at weaning and mixing at 4 weeks of age. Light blue represents the weaner-transition phase of the experiment, and dark blue represents the weaner-finisher phase of the experiment.

Table 1
Ethogram of pig behaviours recorded after weaning and mixing.

Behaviour	Description
Play	Pig performs one of more of the following behaviours using bouncy, jerky movements (descriptions adapted from Martin and colleagues (2015)): <i>Scampering</i> – Sequence of at least two forward hops in rapid succession <i>Gamboling</i> – Running forward energetically <i>Pivoting</i> – Turning on the spot on a horizontal plane <i>Tossing head</i> – Circular, vertical or horizontal movement of the head <i>Flopping</i> – Rapid drop of the body from the upright position to recumbency <i>Hopping</i> – Two or all four feet off the floor in an energetic upwards movement <i>Rolling</i> – 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: <i>Knocking</i> – Vigorously thrusting the head against another pig <i>Biting</i> – Rapid opening and closing of the mouth on the body of another pig <i>Pushing</i> – 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 using the snout, directed to any part of another pig's body
Tail biting	Mouthing or chewing the tail of another pig
Ear biting	Mouthing or chewing the ear of another pig
Interacting with pen	Sniffing, nosing or chewing physical components of the pen including the floor
Escape attempt	Lifting at least two legs from the floor simultaneously, either in an attempt to jump or climb out of the pen

pigs forward was recorded. Additionally, the occurrence of pigs baulking (moving backwards rather than forwards out of the pen) was recorded.

Validation and quality assurance

Sample collection details and assay characteristics

Two teams of experienced technicians collected blood samples from pigs in neighbouring pens simultaneously. In each team, one technician held the pig inverted on the lap, and the other technician collected the sample via jugular venepuncture within 2 min of the pig being secured. Samples were collected in the aisle in front of the home pen of the sample pig. Samples obtained 2 h postweaning for haematology were collected into EDTA coated tubes (BD Vacutainer, New South Wales, Australia) and transported to a commercial laboratory where neutrophil and lymphocyte counts were conducted 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 1 300 × g. After centrifugation serum was transferred to polypropylene tubes and stored in a –20 °C freezer before being moved to a –80 °C 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 CV was 4.8%, and the inter-assay CV was 7.4%.

Observer reliability

Observers conducting video observations were blind to treatment, with the exception of behavioural responses to husbandry practices imposed in the home pens during lactation where it was impossible to be blind to housing treatment. For each husbandry practice, the observer repeated video observations for at least three groups of pigs (i.e., 3 litters or pens). Inter-observer reliability was assessed for all video observations using intraclass correlation

coefficient estimates based on a single measure, absolute agreement, two-way mixed effects models analysed in SPSS ([IBM Corp, 2020](#)). 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.

Statistical analysis

For each routine husbandry practice that was examined, a set of summary measurements to assess aspects of resilience was calculated for each litter during lactation (preweaning phase), or for a pair of adjacent pens (one all males; one all females) from weaning onwards (weaner transition and weaner-finisher phases). When measurements were assessed on individual pigs, an average value of the pigs for the litter (preweaning phase) or pair of adjacent pens (weaner transition and weaner-finisher phases) was calculated.

Table 2
ANOVA structures for statistical analysis of measurements collected during different phases of the experiment examining early human contact and housing for pigs.

Source of variation	df
Measurements collected during the preweaning phase ¹	
Housing system	1
Human contact treatment	1
Housing system by human contact treatment interaction	1
Residual	44
Measurements collected during the weaner transition phase (behaviour and physiology 0–2 h postweaning) ²	
Housing system	1
Human contact treatment	1
Housing system by human contact treatment interaction	1
Residual	20
Measurements collected during the weaner-finisher phase ³	
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

¹ Unit of analysis is litter/lactation pen.

² Unit of analysis is pair of temporary pens containing only cohort 1 pigs.

³ Unit of analysis is pair of pens; one pen containing females; one pen containing males.

lated. When measurements were assessed on a pen basis post-weaning, an average value of the two pens in a pair was calculated.

Each measurement was analysed using ANOVA with one of the structures presented in Table 2. The split-plot ANOVA in the weaner and grower/finisher facilities was used to account for the spatial distribution of the pens in the sheds. In addition, since stockperson behaviour may affect pig behaviour, Restricted Maximum Likelihood (REML) mixed models were used to analyse treatment effects on the number of baulks and the proportion of pigs that baulked when being moved out of the home pen at 21 weeks of age, after adjusting for the number of interventions used by the stockperson (INT). These REML models had random effects for blocks and rows within blocks, and fixed effects for INT, main effects of housing system and human contact and the interaction between housing and contact. When components of variance were estimated to be negative this was allowed to stand, so as to provide an analysis that was analogous to standard practice in analyses of variance. To be consistent with an experimental design approach, main effect means and standard errors were estimated using a model that excluded the interaction of housing and human contact, but utilised the estimates of variance components and residual variance obtained from an analysis that included the interaction.

Prior to analyses of variance, all behaviour measurements at 0–1 h and 48–49 h postweaning were angularly transformed ($y = \arcsin(\sqrt{P/100})$, where P is percentage of pigs in view that partook in the behaviour). All physiological measurements, and the number of vocalisations during processing and during vaccination, were logarithmically transformed. Transformations were chosen to ensure the distribution of the residuals was not markedly skewed and/or that the amount of residual variation did not increase as the mean increased.

Non-parametric permutation tests, based on the usual ANOVA F values, were used to calculate P -values for the proportion of pigs attempting escape, ear biting, tail biting, playing and nosing pen mates for all time periods after weaning and mixing (VSN International, 2022). In all these cases, there were many pens with no pigs partaking in the behaviour, and thus, the usual parametric P -values could not be considered as reliable.

Due to equipment failures (camera and microphone malfunctions), there were no vocalisation measurements for 2 litters at processing and one litter at vaccination, and no behaviour measurements for three pens in response to moving out of the home pens at 21 weeks of age.

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

Results

There were no statistically significant ($P > 0.05$) interactions between human contact treatment and housing system for measurements collected at piglet processing, vaccination, 2 h after weaning (on cohort 1, weaner transition phase), 48–49 h after weaning (after cohorts 1 and 2 mixed, weaner-finisher phase) and moving out of the home pens. Thus, the main effects of treatments on the measurements at these times are reported separately in these sections. Contrastingly, there was evidence of interactions between human contact treatment and housing system ($P < 0.05$) for several behavioural measurements collected on cohort 1 at 0–1 h after weaning, and thus, the results for these measurements are presented as separate means for the 4 treatment combinations.

Piglet processing at 4 days of age and vaccination at 3 weeks of age

In response to processing at 4 days of age and vaccination at 3 weeks of age, the intensity of escape behaviour after capture by the stockperson was lower in +HC than C piglets ($P < 0.05$; Table 3). The +HC piglets also vocalised for shorter durations and had shorter call lengths than C piglets during processing. There was no evidence ($P > 0.05$) of a human contact treatment effect on piglet escape behaviour during oral vaccination or tail docking at 4 days of age, stress physiology 1 h after processing at 4 days of age, or on vocalisations during vaccination at 3 weeks of age.

During processing at 4 days of age, the intensity of escape behaviour during capture by the stockperson was lower in FC piglets

Table 3

Effects of early housing and human contact on the behavioural and physiological responses of piglets to processing at 4 days of age and vaccination at 3 weeks of age. The number of calls during processing and vaccination and serum cortisol concentrations 1 h after processing were logarithmically transformed prior to analysis; back-transformed means are presented in parentheses.

Item	Housing System			Human Contact			P-value		
	FC	LP	SED	C	+HC	SED	Housing System	Human Contact	Housing × Contact
Processing 4 days of age									
Escape behaviour ¹									
At capture	1.7	2.1	0.17	2.1	1.6	0.17	0.041	0.010	0.52
At oral vaccination	2.2	2.0	0.17	2.2	2.0	0.17	0.18	0.13	0.27
At tail docking	1.7	1.9	0.16	1.9	1.7	0.16	0.22	0.35	0.78
Vocalisations									
Duration of calls (s)	6.3	5.1	0.83	6.7	4.8	0.83	0.16	0.028	0.47
Average call length (s)	0.5	0.5	0.03	0.5	0.4	0.03	0.91	0.00096	0.69
Number of calls	1.1 (12)	0.9 (8.7)	0.08	1.1 (11)	1.0 (9.3)	0.08	0.057	0.41	0.79
Peak frequency of calls (Hz)	2 980	2 810	80	2 910	2 890	85	0.052	0.85	0.57
Physiology									
Cortisol 1 h after processing (ng/ml)	2.5 (340)	2.5 (280)	0.07	2.5 (320)	2.5 (290)	0.07	0.21	0.50	0.084
Vaccination 3 weeks of age									
Escape behaviour ¹									
At capture	1.0	1.0	0.10	1.1	0.90	0.101	0.84	0.040	0.060
Vocalisations									
Duration of calls (s)	2.9	2.7	0.31	2.7	2.9	0.31	0.44	0.43	0.91
Average call length (s)	0.6	0.6	0.03	0.6	0.6	0.03	0.57	0.74	0.18
Number of calls	0.6 (4.4)	0.6 (4.2)	0.04	0.6 (4.2)	0.6 (4.4)	0.04	0.70	0.60	0.28
Peak frequency of calls (Hz)	2 910	2 940	97	2 870	2 980	97	0.76	0.27	0.80

Abbreviations: FC = farrowing crate; LP = loose pen; C = routine human contact; +HC = positive human contact.

¹ Escape behaviour was scored on an ordinal scale from 0 to 4 as described by Leidig et al. (2009).

compared to LP piglets ($P = 0.04$; Table 3). There was no other evidence ($P > 0.05$) of a housing system effect on the behavioural responses of piglets to husbandry procedures at 4 days or 3 weeks of age, or on stress physiology after piglet processing.

Weaning and mixing at 4 weeks of age

Nearly, all of the observations of pigs attempting to escape from the pen postweaning were in the C/LP treatment (Human contact treatment $\times$ housing system interaction $P < 0.05$, for both 0–30 and 30–60 min postweaning in the cohort 1 pigs, Table 4). No escape attempts from the pen were observed in any treatment postmixing when pigs were moved to larger pens during the start

of the weaner-finisher phase of the experiment (Table 5). There were more FC and +HC pigs interacting with the pen after weaning, with this behaviour being observed most frequently in FC than LP pigs from 0 to 30 min postweaning ($P = 0.014$), +HC/FC pigs from 30 to 60 min postweaning (Human contact treatment $\times$ housing system interaction $P = 0.010$), and +HC than C pigs from 0 to 30 and 30–60 min postmixing of cohorts 1 and 2 days after weaning ($P = 0.001$ for 0–30 min, $P = 0.01$ for 30–60 min, Tables 4 and 5).

There was evidence for +HC and FC pigs engaging in more aggression immediately after weaning, but less aggression 2 days postweaning when cohorts 1 and 2 were mixed (Tables 4, 5). Compared to C and LP pigs respectively, +HC and FC pigs were more frequently observed engaging in aggressive behaviour from 0 to

Table 4

Effects of early housing and human contact on the behaviour of cohort 1 pigs from 0 to 1 h after weaning during the weaner transition phase of the experiment. All data were angularly transformed prior to analysis; back-transformed means are presented in parentheses. Note that this table contains the means of each of the four treatment combinations, rather than the main effect means.

Item	C/FC	+HC/FC	C/LP	+HC/LP	SED	P-value		
						Housing System	Human Contact	Housing × Contact
Behaviour 0–30 min postweaning (% of pigs in view)								
Playing	2.3 (0.16)	0 (0)	0 (0)	0 (0)	0.71	0.035 ¹	0.035 ¹	0.035 ¹
Aggression	7.8 (1.8)	11.0 (3.6)	4.0 (0.48)	8.1 (2.0)	2.05	0.030	0.021	0.76
Nosing pen mate	0 (0)	0 (0)	0 (0)	2.3 (0.17)	1.04	0.21 ¹	0.21 ¹	0.21 ¹
Investigating pen mate	8.1 (2.0)	6.6 (1.3)	6.8 (1.4)	13.9 (5.7)	2.66	0.13	0.15	0.033
Tail biting	1.5 (0.065)	0 (0)	0 (0)	0.8 (0.021)	0.88	0.86 ¹	0.86 ¹	0.22 ¹
Ear biting	3.6 (0.39)	1.1 (0.039)	3.2 (0.32)	3.6 (0.40)	1.84	0.43 ¹	0.45 ¹	0.28 ¹
Attempting escape	0 (0)	0 (0)	2.5 (0.19)	0 (0)	0.81	0.039 ¹	0.039 ¹	0.039 ¹
Interacting with pen	48 (55)	53 (64)	43 (47)	43 (47)	3.9	0.014	0.35	0.33
Behaviour 30–60 min postweaning (% of pigs in view)								
Playing	0.8 (0.021)	3.4 (0.34)	0 (0)	2.2 (0.14)	1.39	0.30 ¹	0.035 ¹	0.80 ¹
Aggression	4.1 (0.52)	4.9 (0.72)	4.3 (0.57)	7.4 (1.6)	2.50	0.46	0.30	0.53
Nosing pen mate	0 (0)	0 (0)	0 (0)	4.5 (0.63)	1.14	0.011 ¹	0.011 ¹	0.011 ¹
Investigating pen mate	3.9 (0.46)	7.7 (1.8)	4.8 (0.69)	12.1 (4.4)	2.55	0.15	0.0058	0.33
Tail biting	0 (0)	0 (0)	0.8 (0.019)	0 (0)	0.55	1.0 ¹	1.0 ¹	1.0 ¹
Ear biting	0.9 (0.024)	1.7 (0.086)	2.3 (0.17)	0.8 (0.019)	1.34	0.95 ¹	0.79 ¹	0.32 ¹
Attempting escape	0 (0)	0.9 (0.026)	3.0 (0.28)	0 (0)	1.18	0.24 ¹	0.23 ¹	0.028 ¹
Interacting with pen	28 (22)	43 (46)	36 (35)	31 (26)	5.0	0.56	0.20	0.010

Abbreviations: FC = farrowing crate; LP = loose pen; C = routine human contact; +HC = positive human contact.

¹ P-values calculated using permutation tests.

Table 5

Effects of early housing and human contact on the behaviour of pigs from 48–49 h after weaning, which also coincided with the period from 0 to 1 h after cohorts 1 and 2 were mixed (i.e., the second mixing for cohort 1 and the first for cohort 2; start of the weaner-finisher phase of the experiment). All data were angularly transformed prior to analysis; back-transformed means are presented in parentheses.

Item	Housing System			Human Contact			P-value		
	FC	LP	SED	C	+HC	SED	Housing System	Human Contact	Housing × Contact
Behaviour 0–30 min postmixing of cohorts 1 and 2 (% of pigs in view)									
Playing	4.2 (0.52)	4.6 (0.63)	1.54	3.5 (0.37)	5.2 (0.83)	1.46	0.82 ¹	0.27 ¹	0.13 ¹
Aggression	12.6 (4.8)	11.6 (4.0)	2.88	14.5 (6.2)	9.8 (2.9)	1.77	0.73	0.025	0.85
Nosing pen mate	0 (0)	0 (0)	–	0 (0)	0 (0)	–	1.0 ¹	1.0 ¹	1.0 ¹
Investigating pen mate	5.8 (1.0)	6.4 (1.2)	1.60	6.1 (1.1)	6.1 (1.1)	0.87	0.75	0.98	0.74
Tail biting	0.4 (0.0051)	1.3 (0.046)	0.83	0.3 (0.0021)	1.5 (0.065)	0.83	0.25 ¹	0.22 ¹	0.69 ¹
Ear biting	2.0 (0.12)	1.4 (0.061)	1.00	1.9 (0.11)	1.5 (0.070)	1.00	0.60 ¹	0.78 ¹	0.43 ¹
Attempting escape	0 (0)	0 (0)	–	0 (0)	0 (0)	–	1.0 ¹	1.0 ¹	1.0 ¹
Interacting with pen	42 (45)	43 (47)	1.1	39 (40)	46 (52)	1.4	0.22	0.00055	0.62
Behaviour 30–60 min postmixing of cohorts 1 and 2 (% of pigs in view)									
Playing	2.0 (0.12)	1.3 (0.048)	1.35	1.3 (0.046)	1.9 (0.12)	1.20	0.54 ¹	0.59 ¹	0.46 ¹
Aggression	8.2 (2.0)	12.3 (4.6)	1.51	9.6 (2.8)	10.9 (3.6)	0.99	0.040	0.20	0.18
Nosing pen mate	0.4 (0.0054)	0 (0)	0.42	0 (0)	0.4 (0.0054)	0.42	1.0 ¹	1.0 ¹	1.0 ¹
Investigating pen mate	5.5 (0.93)	6.0 (1.1)	1.62	5.4 (0.88)	6.1 (1.12)	1.56	0.80	0.67	0.65
Tail biting	0.6 (0.0099)	1.8 (0.094)	0.82	1.4 (0.057)	1.0 (0.027)	0.82	0.13 ¹	0.52 ¹	0.11 ¹
Ear biting	2.9 (0.25)	0.5 (0.0067)	0.92	1.8 (0.10)	1.5 (0.069)	0.92	0.19 ¹	0.60 ¹	0.060 ¹
Attempting escape	0 (0)	0 (0)	–	0 (0)	0 (0)	–	1.0 ¹	1.0 ¹	1.0 ¹
Interacting with pen	41 (43)	42 (44)	1.8	39 (40)	44 (48)	1.6	0.77	0.010	0.49

Abbreviations: FC = farrowing crate; LP = loose pen; C = routine human contact; +HC = positive human contact.

¹ P-values calculated using permutation tests.

Table 6
Effects of early housing and human contact on the physiology of pigs 2 h (weaner transition phase) and 49 h after weaning (weaner-finisher phase). The timepoint 49 h after weaning was also 1 h after the pigs that were blood sampled, cohort 1, had been mixed with cohort 2. All data were logarithmically transformed prior to analysis; back-transformed means are presented in parentheses.

Item	Housing System			Human Contact			P-value		
	FC	LP	SED	C	+HC	SED	Housing System	Human Contact	Housing × Contact
Physiology 2 h after weaning									
Cortisol (ng/ml)	1.8 (57)	1.9 (83)	0.07	1.8 (68)	1.8 (70)	0.07	0.032	0.88	0.41
N:L ratio	−0.06 (0.87)	−0.07 (0.85)	0.047	−0.05 (0.90)	−0.09 (0.82)	0.047	0.85	0.38	0.92
Physiology 49 h after weaning (1 h after cohorts 1 and 2 were mixed)									
Cortisol (ng/ml)	1.5 (32)	1.5 (29)	0.09	1.6 (37)	1.4 (25)	0.09	0.62	0.015	0.15
Haptoglobin (µg/ml)	3.0 (900)	2.8 (690)	0.08	2.9 (770)	2.9 (800)	0.05	0.23	0.81	0.81

Abbreviations: FC = farrowing crate; LP = loose pen; C = routine human contact; +HC = positive human contact; N:L ratio = Neutrophil lymphocyte ratio.

Table 7
Effects of early housing and human contact on behavioural responses of pigs to being moved out of the home pen at 21 weeks of age.

Item	Housing System			Human Contact			P-value		
	FC	LP	SED	C	+HC	SED	Housing System	Human Contact	Housing × Contact
Number of stockperson interventions (per pig) ¹	1.0	1.0	0.05	0.9	1.1	0.28	0.74	0.63	0.084
Time to move out of pen (s)	3.8	3.6	0.35	3.7	3.7	0.66	0.62	0.99	0.41
Number of baulks									
Unadjusted	0.2	0.3	0.05	0.3	0.3	0.07	0.052	0.86	0.75
Adjusted for number of stockperson interventions ¹	0.2	0.3	0.04	0.3	0.3	0.05	0.034	0.71	0.12
% Pigs that baulked									
Unadjusted	15	23	4.5	19	20	5.0	0.15	0.81	0.68
Adjusted for number of stockperson interventions ¹	16	23	4.7	20	20	5.0	0.18	0.97	0.40

Abbreviations: FC = farrowing crate; LP = loose pen; C = routine human contact; +HC = positive human contact.

¹ Number of times the stockperson hit the stockboard to the ground or used tactile contact to encourage pigs to move forward.

30 min after weaning (*P* between 0.02 and 0.03 for both main effects). There were no detected effects (*P* > 0.1) of human contact treatment or housing system on aggressive behaviour from 30 to 60 min after weaning. However, when cohorts 1 and 2 were mixed 2 days after weaning, C pigs showed more aggression than +HC pigs from 0 to 30 min after mixing (*P* = 0.03), and LP pigs showed more aggression than LP pigs from 30 to 60 min postmixing (*P* = 0.04). From 0 to 30 min after weaning, only C/FC pigs were observed playing (Human contact treatment × housing system interaction *P* = 0.04), and from 30 to 60 min after weaning, nearly, all play was observed in +HC pigs (*P* = 0.04, Table 4). Treatments did not affect the small amount of play observed after mixing during the start of the weaning-finisher phase of the experiment (*P* > 0.1).

The +HC /LP pigs were most frequently observed investigating pen mates through gentle nasal contact from 0 to 30 min after weaning (Human contact treatment × housing system interaction *P* = 0.03), and these pigs were also the only observed to repetitively nose pen mates from 30 to 60 min after weaning (Human contact treatment × housing system interaction *P* = 0.01, Table 4). There was no evidence (*P* > 0.1) of a housing system or human contact treatment effect on ear or tail biting at any time periods after weaning and mixing (Tables 4 and 5).

Serum cortisol concentrations were higher in LP pigs than FC pigs 2 h after weaning, and higher in C pigs than +HC pigs at 49 h after weaning/1h after cohorts 1 and 2 were mixed (*P* < 0.05, Table 6). There was no evidence (*P* > 0.1) of a housing system effect on cortisol concentrations at 49 h after weaning, or of a human contact treatment effect at 2 h after weaning. There was also no evidence (*P* > 0.1) of any treatment effects on neutrophil to lymphocyte cell counts 2 h after weaning or serum haptoglobin concentrations 49 h after weaning.

Moving out of pens at 21 weeks of age

When being moved out of the home pen at 21 weeks of age, there was an indication (*P* < 0.1) that LP pigs baulked more than FC pigs, and after accounting for the number of interventions used by stockpeople to move pigs out of the pen, this effect was significant (*P* < 0.05, Table 7). There was no evidence (*P* > 0.05) of a housing system effect 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, and no evidence (*P* > 0.05) of a human contact treatment effect on any behaviours measured when pigs were moved at 21 weeks of age.

Discussion

The present research found that 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 used in this study increased escape behaviour and cortisol concentrations to routine stressors such as processing and weaning, and increased the incidence of pigs baulking when being moved out of the home pen at 21 weeks of age.

Muns and colleagues (2015) showed that around 36 min of positive handling imposed on the litter on the first day of life reduced piglets' escape behaviour during husbandry procedures at 2 days of age, and the present research also demonstrates that brief positive handling may produce the same benefit. Fifteen minutes of positive human contact imposed over 3 days reduced escape behaviour, the durations of vocalising, and call lengths during

vaccinations and tail docking at 4 days of age. Although there were no detected effects of human contact treatment on the escape behaviour of piglets during oral vaccination and tail docking or on serum cortisol concentrations 1 h after piglet processing, the present research, as well as our previous findings (Hayes et al., 2021b) and those of Muns and colleagues (2015), highlight that previous brief positive human interaction is effective in reducing the stress responses of piglets to routine husbandry practices. During vaccination at 3 weeks of age, a similar effect of handling was found to that seen earlier in life, with +HC pigs showing less escape behaviour than C pigs after being caught by the stockperson. There were no detected effects of human contact treatment on piglet vocalisations during vaccination at 3 weeks of age, but this may have been because vaccination was very short, usually involving only 3–5 s of handling for each pig.

In agreement 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 after being caught for piglet processing at 4 days of age ($P = 0.04$), but there were no effects of lactation housing on escape behaviour during oral vaccination or tail docking, serum cortisol concentrations 1 h after processing, or behavioural responses to being caught for vaccination at 3 weeks of age. Therefore, in comparison to the housing treatment, the +HC treatment had a stronger effect on improving resilience to routine stressors during the lactation period, based on the behavioural responses of pigs to husbandry practices.

There were several effects of early human contact and housing on the physiological and behavioural responses of pigs to weaning and mixing. Nearly all escape attempts from the pen were observed during the 0–1 h period after weaning by C pigs from LP, although even in this treatment, there was a low incidence of escape attempts. In the 0–30 min period after weaning, FC pigs were more frequently observed exploring the pen through physical interactions such as sniffing, nosing or chewing, whilst in the period 0–1 h after mixing of cohorts 1 and 2 (48–49 h after weaning), there was more interaction with the pen by +HC than C pigs. Although there was no evidence of an effect of handling on serum cortisol concentrations 2 h after weaning, +HC pigs had lower concentrations than C pigs at 49 h after weaning. Conversely, FC pigs had lower cortisol concentrations than LP pigs 2 h after weaning, but concentrations were similar at 49 h after weaning. Cortisol may in part mediate increases in the proportion of neutrophil to lymphocyte cells (Sapolsky, 2000) and haptoglobin release (Murata, 2007), both of which are part of the immune response to stress and have been reported to increase in pigs postweaning (Puppe et al., 1997; Sauerwein et al., 2005; Pomorska-Mol et al., 2012; Turpin et al., 2016; De et al., 2017; Turpin et al., 2017), but the early handling and housing treatments in this experiment had no effects on these other physiological measurements.

A reasonable interpretation of the effects of these treatments on escape attempt behaviour from the pen, exploration of the pen, and cortisol concentrations postweaning is that +HC pigs and FC pigs were more resilient and coped better with weaning and mixing compared to C and LP pigs. In Part 1 of this series of papers (Lucas et al., 2024a, submitted), we reported that C pigs and LP pigs showed more fear, based on less exploration and greater avoidance, of humans and novelty prior to weaning. Weaning involves several stressors for pigs including exposure to a novel physical, nutritional and social environment and close and intense contact with stockpeople, and thus, 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. Furthermore, during lactation, the LP pigs had less opportunity for interaction with adjacent litters due to the high dividing pen walls, compared to FC pigs that anecdotally were observed interacting with neighbouring pigs over the short dividing walls. This apparent reduced 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 preweaning is known to mitigate stress at weaning (Kutzer et al., 2009). Additionally, switching from an enriched to a barren environment has been shown to increase stress in pigs (Day et al., 2002a; Munsterhjelm et al., 2009; Luo et al., 2020), and it is possible that the increased stress at weaning experienced by LP pigs was due to the change in housing being greater for these pigs. As discussed in Part 1 of this series, further research is necessary to determine which specific characteristics of the two lactation housing systems studied are responsible for affecting the stress responses of piglets (Lucas et al., 2024a, submitted).

Care is required in our interpretation of the physiological and behavioural responses of the pigs to routine husbandry practices. Firstly, the limitations in using cortisol as an indicator of stress are well known (Ralph and Tilbrook, 2016). Secondly, we recognise that the procedure of instantaneous scan sampling may have resulted in many events of pig behaviour postweaning being missed. We believe that a more expansive sampling of the time course of the behavioural and physiological responses in relation to husbandry disturbances is required to fully appreciate the ability of pigs to cope with these stressors. This is particularly important when considering the resilience of pigs to weaning, since in the present research, many behaviours postweaning were not affected by the treatments at all, or were not affected at all time-points examined. Furthermore, the effects of the treatments on some behaviours postweaning were challenging to interpret.

After weaning, +HC pigs from LP were most frequently observed interacting with pen mates, evident by these pigs showing more investigation of pen mates using gentle nasal contact (0–30 min postweaning), and more repetitive nosing directed to any part of a pen mate's body (30–60 min postweaning). The meaning of these findings together is difficult to interpret as gentle nasal contact may be a form of positive social interaction, while in contrast, 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). Effects of the treatments on play behaviour after weaning were somewhat inconsistent, with C/FC pigs engaging in the most play behaviour from 0 to 30 min after weaning, but +HC pigs engaging in more play behaviour than C pigs from 30 to 60 min after weaning. Overall, the animal welfare implications, if any, of the effects of treatments on interaction with pen mates and playing postweaning are not clear cut.

The +HC pigs were more frequently observed performing aggressive behaviour from 0 to 30 min after weaning, but not from 30 to 60 min after weaning. Despite these initial higher levels of aggression, as mentioned previously, +HC pigs had similar cortisol concentrations to C pigs 2 h after weaning, and as reported in Part 3 of this series of papers, if anything, they also had lower injury scores 1 week after weaning (Lucas et al., 2024b, submitted). Furthermore, 2 days after weaning, the +HC pigs actually showed less aggression and had lower cortisol concentrations after cohorts 1 and 2 were mixed. This may indicate the positive handling treatment resulted in pigs being able to form a social hierarchy more quickly after weaning. The mechanism behind this effect is unclear, but it may be that the +HC pigs were more adaptable to the weaning process overall. There was an indication that the housing treatment resulted in a similar effect: the FC pigs engaged in more aggressive behaviour than LP pigs at 0–30 min after weaning, but not at 30–60 min after weaning, and although aggression was similar at 0–30 min after cohorts 1 and 2 were mixed, there was some evidence ($P = 0.04$) of LP pigs being more aggressive 30–60 min

after mixing. As raised earlier, a more detailed examination of responses to weaning, including a longer duration of behaviour observation is necessary to better understand the effects of the early experience treatments on social behaviour postweaning. Additionally, no manipulative material was provided to pigs in either housing system and this should be considered when comparing findings from the present research to those from other studies.

When pigs were moved out of the home pens at 21 weeks of age, there was some evidence that LP pigs baulked more than FC pigs. This suggests the LP pigs were more difficult to handle. Fear of humans can affect ease of handling by stockpeople (Hemsworth and Coleman, 2011), but the fear responses to humans of pigs from the two housing treatments were similar at 9 and 14 weeks of age (Lucas et al., 2024a, submitted), and thus, 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, this is evidence that differences in the early housing environment can affect the behaviour of pigs, even after 4 months of housing in identical environments.

Day and colleagues (2002b) found that positive handling increased the time taken for grower pigs to exit their home pen, suggesting that pigs with less fear of humans were more difficult to move. In contrast, Hemsworth and colleagues (1994) found that grower pigs with greater fear of humans, based on less interaction with a person, were more difficult to move, as evident by more baulking and increased time to move. In the present experiment, +HC pigs were less fearful of humans at 14 weeks of age (Lucas et al., 2024a, submitted), but there were no effects of early human contact 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 ushered out of the home pens at 21 weeks of age. Thus, while positive experiences with humans early in life may have reduced pigs' fear of people until at least 14 weeks of age, there was no indication that the +HC treatment negatively affected ease of handling of pigs late in the experiment.

In conclusion, both the early housing environment and early experiences with humans affected the capacity of pigs to cope with stress associated with routine husbandry challenges. Overall, stress resilience was better in pigs reared with opportunities for positive human interaction early in life and pigs reared in farrowing crates, based on lower biological responses indicative of stress such as escape behaviour, vocalisations, and cortisol concentrations. Reduced stress responses of treatments to routine husbandry practices reported in this paper are consistent with corresponding reductions in fear responses to humans and novelty that were reported in Part 1 of this series of papers (Lucas et al., 2024a, submitted).

Ethics approval

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).

Data and model availability statement

The data were not deposited in an official repository but are available from the corresponding author upon request.

Declaration of Generative AI and AI-assisted technologies in the writing process

During the preparation of this work the author(s) did not use any AI and AI-assisted technologies.

Author ORCIDs

Megan E. Lucas: <https://orcid.org/0000-0002-5730-7535>.

Lauren M. Hemsworth: <https://orcid.org/0000-0002-7752-8917>.

Kym L. Butler: <https://orcid.org/0000-0002-1958-3942>.

Rebecca S. Morrison: <https://orcid.org/0000-0002-8412-4945>.

Alan J. Tilbrook: <https://orcid.org/0000-0002-1116-1470>.

Jeremy N. Marchant: <https://orcid.org/0000-0002-5287-2914>.

Jean-Loup Rault: <https://orcid.org/0000-0001-6015-8318>.

Rutu Y. Galea: <https://orcid.org/0000-0002-8740-0589>.

Paul H. Hemsworth: <https://orcid.org/0000-0002-0211-0751>.

CRediT authorship contribution statement

M.E. Lucas: Writing – original draft, Visualization, Project administration, Methodology, Investigation, Formal analysis. **L.M. Hemsworth:** Writing – review & editing, Supervision, Project administration, Methodology, Funding acquisition, Conceptualization. **K.L. Butler:** Writing – review & editing, Methodology, Formal analysis. **R.S. Morrison:** Writing – review & editing, Supervision, Methodology. **A.J. Tilbrook:** Writing – review & editing, Supervision, Methodology, Funding acquisition. **J.N. Marchant:** Writing – review & editing, Methodology. **J.-L. Rault:** Writing – review & editing, Methodology. **R.Y. Galea:** Project administration, Investigation. **P.H. Hemsworth:** Writing – review & editing, Supervision, Project administration, Methodology, Funding acquisition, Conceptualization.

Declaration of interest

None.

Acknowledgements

This research was conducted as part of a PhD Thesis: Lucas, M., 2022. Human-animal interactions, early life experiences and stress resilience in pigs. Doctoral dissertation, The University of Melbourne, Melbourne, Victoria, Australia.

The authors would like to gratefully acknowledge Jessalyn Taylor and Will Coombs from the Animal Welfare Science Centre for assisting with data collection, Erin Ford, Steve Smith and the Rivalea Research and Innovation team for providing technical support, and John Pluske, Darryl D'Souza and Kate Plush for their contributions to the larger experiment within which this research was conducted.

Financial support statement

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

References

- Baxter, E.M., Adeleye, O.O., Jack, M.C., Farish, M., Ison, S.H., Edwards, S.A., 2015. Achieving optimum performance in a loose-housed farrowing system for sows: the effects of space and temperature. *Applied Animal Behaviour Science* 169, 9–16. <https://doi.org/10.1016/j.applanim.2015.05.004>.

- Chaloupková, H., Illmann, G., Neuhauserova, K., Tomanek, M., Valis, L., 2007. Prewaning housing effects on behavior and physiological measures in pigs during the suckling and fattening periods. *Journal of Animal Science* 85, 1741–1749. <https://doi.org/10.2527/jas.2006-504>.
- Chen, A., 2019. Preface. In *Stress Resilience: Molecular and Behavioral Aspects* (ed. Chen, A.), Academic Press, London, England, UK, pp. xvii–xx. <https://doi.org/10.1016/B978-0-12-813983-7.05001-1>.
- 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, 189–202. [https://doi.org/10.1016/S0168-1591\(02\)00017-5](https://doi.org/10.1016/S0168-1591(02)00017-5).
- 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, 177–192. [https://doi.org/10.1016/S0168-1591\(01\)00199-X](https://doi.org/10.1016/S0168-1591(01)00199-X).
- 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, 343–349. <https://doi.org/10.1007/s00580-016-2379-x>.
- 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, 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, 1619.
- Hemsworth, P.H., Coleman, G.J., 2011. *Human-Livestock Interactions: the stockperson and the productivity and welfare of intensively farmed animals*. CABI Publishing, Wallingford, UK.
- 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, 129–142. [https://doi.org/10.1016/0168-1591\(94\)90077-9](https://doi.org/10.1016/0168-1591(94)90077-9).
- Iacoviella, B.M., Charney, D.S., 2019. Cognitive and behavioral components of resilience to stress. In: Chen, A. (Ed.), *Stress Resilience: Molecular and Behavioral Aspects*. Academic Press, London, England, UK, p. 23.
- IBM Corp., 2020. IBM SPSS Statistics for Windows. Version 27.0. IBM Corp, Armonk, NY.
- K. Lisa Yang center for conservation bioacoustics at the cornell lab of ornithology, 2014. Raven Pro: Interactive Sound Analysis Software (Version 1.5). The Cornell Lab of Ornithology, Ithaca, NY, USA.
- 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, 16–24. <https://doi.org/10.1016/j.applanim.2009.08.004>.
- 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, 174–178. <https://doi.org/10.1016/j.applanim.2008.10.004>.
- Lucas, M.E., Hemsworth, L.M., Butler, K.L., Morrison, R.S., Tilbrook, A.J., Marchant, J.N., Rault, J.-L., Galea, R.Y., Hemsworth, P.H., 2024a. Early Human Contact and Housing for Pigs - Part 1: Responses to Humans, Novelty and Isolation. *Animal*, 101164.
- Lucas, M.E., Hemsworth, L.M., Butler, K.L., Morrison, R.S., Tilbrook, A.J., Marchant, J. N., Rault, J.-L., Galea, R.Y., Hemsworth, P.H., 2024b. Early Human Contact and Housing for Pigs - Part 3: Ability to Cope with the Environment. *Animal*, 101166.
- 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. <https://doi.org/10.3389/fvets.2020.00268>.
- 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. <https://doi.org/10.1016/j.applanim.2014.11.022>.
- 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. <https://doi.org/10.1016/j.physbeh.2015.06.030>.
- 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, 42–53. <https://doi.org/10.1016/j.applanim.2009.01.007>.
- Murata, H., 2007. Stress and acute phase protein response: an inconspicuous but essential linkage. *Veterinary Journal* 173, 473–474. <https://doi.org/10.1016/j.tvjl.2006.05.008>.
- 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, 219–232. <https://doi.org/10.1016/j.applanim.2004.08.012>.
- 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, 31–41. <https://doi.org/10.1016/j.applanim.2011.06.011>.
- 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 Pulawy* 56, 553–557. <https://doi.org/10.2478/v10213-012-0097-y>.
- 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, 157–164. [https://doi.org/10.1016/S0301-6226\(97\)00006-7](https://doi.org/10.1016/S0301-6226(97)00006-7).
- Ralph, C.R., Tilbrook, A.J., 2016. The usefulness of measuring glucocorticoids for assessing animal welfare. *Journal of Animal Science* 94, 457–470. <https://doi.org/10.2527/jas.2015-9645>.
- Sapolsky, R.M., 2000. Stress hormones: good and bad. *Neurobiology of Disease* 7, 540–542. <https://doi.org/10.1006/nbdi.2000.0350>.
- 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, 295–302. <https://doi.org/10.1179/135100005X83725>.
- 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, 1426–1431. <https://doi.org/10.1017/S1751731111000358>.
- 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, 24. <https://doi.org/10.3390/ani6030024>.
- 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. <https://doi.org/10.1016/j.livsci.2017.07.006>.
- VSN International, 2018. *Genstat for Windows*. VSN International, Hemel Hempstead, UK.
- VSN International, 2022. *Genstat Reference Manual (Release 22), Part 3 Procedures*. In *Genstat®* (ed. sine nomine). VSN International, Hemel Hempstead, UK, pp. 187–188.