HUMAN ENRICHMENT PROGRAM FOR BREEDING SOWS: PROOF OF CONCEPT – 1C-120

Final report prepared for the Co-operative Research Centre for High Integrity Australian Pork

By

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Executive Summary

Indoor production systems are considered by some to provide barren environments for animals and barren environments have been implicated in the development of stress and stereotypies in farm animals. Provision of effective environmental enrichment is one approach to reduce animal stress and improve animal welfare in intensive, indoor and non-bedded pig production systems. In general, enrichment therefore should present objects or situations that have functional relevance to the animal and act with a foreseeable rewarding outcome. The European Union Commission directive 2001/93/EC states that "pigs must have permanent access to a sufficient quantity of material to enable proper investigation and manipulation activities, such as straw, hay, wood, sawdust, mushroom compost or a mixture of such that does not compromise the health of animals". However, while some of these enrichments may not practical in some indoor pig production systems, the extent to which some of the currently-used so-called enrichments provide functional enrichment has been questioned by many authors. Clearly further research on opportunities to provide commercially practical enrichment for pigs is required. The implications of this topic are reflected in concerns about intensification of pig production, particularly the extensive use of fully or partially slatted, non-bedded and non-enriched environments for young and breeding pigs and the extent to which this provides functional enrichment.

While not studied extensively, there is evidence in the literature that regular positive human contact may be an effective form of environment enrichment. The present project is a proof of concept examining the effects of regular positive human contact on stress resilience.

A factorial treatment design with the following two main effects was applied to sows during gestation: (1) Human contact during gestation - Human enrichment (HE) and Control; and (2) Parity age - Parity 1-2 and Parity 3 and older. The HE treatment involved a stockperson entering and slowly walking through the group pens for 2 min daily, stopping at 30 s intervals for 10 seconds, squatting and talking and, if sows approached, patting them. The rationale for these treatments is that minimal human contact or additional and positive human contact with sows associated with routine husbandry will produce differences in environmental stimulation, while some of the behavioural and physiological measures studied are likely to differ due to age and/or experience.

The treatments were imposed from post-insemination mixing to entry to farrowing crates (16 wk post-insemination). Three hundred and sixty mixed-parity Landrace × Large White sows were studied in two-time replicates (i.e., 180 sows per replicate) in a 1-year experiment. Measurements studied to examine the effects on stress resilience and emotionality included behavioural, physiological (stress) and fitness (health and production) variables as well as brain-derived neurotrophic factor (BDNF), a neurotrophin that has been shown to increase in the brain and blood following provision of environment enrichment.

The results of this preliminary experiment provide no evidence that the human enrichment treatment affected stress resilience based on baseline (*in situ*) serum cortisol concentrations at 5 and 10 wk of gestation, baseline (*in situ*) salivary cortisol concentrations at 29 d of housing during lactation in farrowing crates, salivary cortisol responses (acute) to the human approach test (HAT) at wk 9 of gestation and salivary cortisol responses (acute) to

introduction to farrowing crates following gestation. The results of this experiment also provide no evidence that the human enrichment treatment affected stress resilience based on emotionality as measured by *in situ* aggression around feeding in wk 1, 4 and 8 of gestation. There were also no treatment effects on the behavioural response to the novel arena in the HAT (fear of novelty) at wk 9 of gestation, the behavioural response to humans in the HAT (fear of humans) at wk 9 of gestation, the behavioural response of sows to entry to farrowing crates or the behavioural responsiveness of sows to an audio recording of an unfamiliar piglet squealing (maternal responsiveness test) during wk 2 of lactation. There was also no treatment effect on aggression or the reproductive performance of sows, however the human enrichment treatment commenced post-mixing and this treatment is therefore unlikely to affect aggression and stress over the first few days post-mixing when stress has been shown to affect farrowing rate.

While the results of this experiment provide no evidence that the human enrichment treatment affected stress resilience based on stress physiology and fitness (reproductive performance), the results provide limited evidence that the opportunity for regular positive contact with humans affects emotionality of sows in terms of their fear responses to the husbandry practices of pregnancy testing and vaccination. Sows in the human enrichment treatment showed less withdrawal to the technician approaching to conduct pregnancy testing in the group pen and older sows in the human enrichment treatment generally withdrew less from the technician after the actual pregnancy test with the ultrasound probe in Replicate 2 of the experiment. Furthermore, less sows in the human enrichment treatment withdrew from an approaching technician during vaccination in the group pen. Thus, sows in the human enrichment treatment showed less avoidance of the technician during routine pregnancy testing and vaccinations indicating reduced fear of these procedures that is reduced emotionality.

Another important finding is that the human enrichment sows in Replicate 1 had higher serum brain-derived neurotrophic factor (BDNF) concentrations at 5 weeks of gestation. Several studies have shown that environmental enrichment increases BDNF, resulting in higher stress resilience. Higher concentrations of BDNF in the brain have been associated with greater stress resilience.

Therefore, the findings from this proof of concept project indicate that a better understanding of the effects of regular positive contact with humans may provide an enrichment strategy that is functionally relevant to pigs and acts with a foreseeable rewarding outcome. In other words, an enrichment strategy that leads to stress resilience and thus improved biological functioning in pigs. It is recommended that further, more extensive research is conducted on the effects of regular positive human contact on stress resilience in pigs. Indeed, the profound effects of early experience, including those experiential effects of humans, highlight the need for long-term research examining life time effects of positive human contact on the stress resilience and thus welfare and productivity of pigs.

An effective human enrichment strategy, that is one that provides sows with stress resilience, would offer the pork industry an enrichment program that (1) is practical, (2) provides sows with conditions that are functionally relevant and act with a foreseeable

rewarding outcome (elicit a positive emotional or affective experience), and (3) mitigates deleterious stress effects that the sows may encounter in commercial environments.

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1. Introduction

Animal welfare is a state and it is generally agreed that animal welfare relates to experienced sensations, that is, how the animal feels (Mellor et al. 2009; Mellor, 2012; Hemsworth et al., 2015). These experiences are all subjective, varying in their affective or emotional contents and, based on human experience, are likely to include negative emotional experiences such as thirst, hunger, nausea, pain and fear, and positive emotional experiences such as satiety, contentment, companionship, curiosity and playfulness.

The three main conceptual frameworks used by scientists to assess animal welfare and their associated measurements have been recently reviewed by Hemsworth (2018a,b). The conceptual framework of 'biological functioning' has been commonly used by scientists to assess risk to animal welfare. A key precept in this approach is that animals use a range of behavioural and physiological responses to assist them to cope with challenges in their lives and, while biological regulation in response to these challenges occurs continuously, successful adaptation is not always possible. Marked challenges may overwhelm an animal's capacity to adapt, leading to biological costs to the animal, such as damage, disease or even death. Thus, the rationale for this approach is that difficult or inadequate adaptation will generate welfare problems for the animal and that the risks to welfare can be assessed at the following two levels: first, the magnitude of the behavioural and physiological responses to the challenge and, second, the biological costs of these responses. Methodologically this approach has involved measuring a range of biological responses such behavioural (e.g. variables such as stereotypies, fear, pain and illness behaviours) and physiological stress (e.g. variables reflecting activation of the sympatho-adrenal medullary system and the hypothalamo–pituitary adrenal axis), and immune function measurements (e.g., heterophil to lymphocyte ratios, antibody production) as well as the fitness consequences of these behavioural, physiological stress and immunological responses (e.g. impairment of growth, reproduction and health) to assess risks to animal welfare.

Another common conceptual framework to assess animal welfare is the affective state framework, which emphasises that the welfare of an animal derives from its capacity for affective experiences. Thus, the welfare state is likely to be negative when the predominant experiences are unpleasant, and vice versa. It is well recognised that affective experiences are generated both by sensory inputs that reflect the animal's internal functional state and by other sensory inputs that reflect the animal's perception of its external circumstances. Thus, preference research, in which the strength of the preference for a chosen environmental option or the motivation to perform a type of behaviour is measured, has been used by scientists to make inferences about animal welfare. The rationale for these inferences is that an animal's preferences are influenced by either the animal's emotions, which have evolved to motivate behaviour to avoid harm and facilitate survival, growth and reproduction, and these emotions reflect important biological requirements of the animal. Other approaches utilised in assessing affective experiences, particularly negative ones, include measures of behaviour, such as fear, pain and illness behaviours, cognitive bias, such as deviation in judgement, and physiology, such as activation of the sympatho-adrenal medullary system and the hypothalamo–pituitary adrenal axis. Apart from preference research to identify preferred environmental options or preferred behaviours, measuring positive affective experiences is presently challenging, although there are several

behavioural indicators that are cautiously used, such as play, exploration and allogrooming in some species.

The third conceptual framework is 'natural living' which although not often well enunciated in the literature, is predicated on the view that the welfare of animals is improved when they can express their normal behaviour. For some people this also implies that the animal should be raised in a 'natural' environment and allowed to behave in 'natural' ways. However, the concept of natural is usually too poorly defined to provide a sound basis for animal welfare assessment, and thus, when applied uncritically it may lead to poorer welfare instead of an improvement. As many authors have recognised, encouraging captive animals to perform all the behavioural patterns evident in the wild is neither sensible nor humane. In the wild, many behavioural responses may be responses to adversity. Furthermore, "many of the 'wild' behaviours are perfectly natural, but their absence in captivity should not necessarily raise welfare concerns because they are elicited by external stimuli or physiological states that have already been fulfilled in animals whose safety, physical, social, health and nutritional needs are met" (Mason and Burns, 2011). There obviously is a need to define natural behaviours that are desirable or undesirable in terms of animal welfare and to clarify the rationale for their inclusion or exclusion. Indeed, there is an increasing focus on highly-motivated behaviours.

The biological functioning and affective state frameworks were initially seen as competing, but a recent more unified approach is that biological functioning is taken to include affective experiences and affective experiences are recognised as products of biological functioning, and knowledge of the dynamic interactions between the two is considered to be fundamental to managing and improving animal welfare. For example, there is evidence that deprivation of laying hens for their preferred environment type is associated with reduced biological functioning in the hens based on a range of behavioural, physiological and fitness variables.

There is a burgeoning body of evidence that the interactions between stockpeople and their livestock can have a substantial effect on the behaviour, welfare and productivity of farm animals (Hemsworth and Coleman, 2011; Hemsworth et al., 2018). This research has focused on the adverse effects of a negative human-animal relationship: negative or aversive handling by affecting fear responses to humans has been shown to be stressful with adverse effects on animal welfare and productivity.

The nature of human contact may have profound effects on the behaviour, welfare and productivity of farm animals; this is best illustrated by the extensive research on pigs (Hemsworth and Coleman, 2011). For example, handling experiments in which pigs had the opportunity to avoid humans have shown that negative handling in comparison to handling of a positive nature, imposed briefly but regularly, increased their fear of humans and reduced their growth, feed conversion efficiency and reproduction. In these experiments, negative handling included slaps, hits and shouting whenever the pig approached the experimenter, while positive handling included squatting, talking and stroking the pig whenever it approached. A chronic stress response has been implicated in these effects on growth and reproduction, since for example in many experiments, handling treatments that resulted in high fear levels also produced a sustained elevation in the basal cortisol concentrations (Hemsworth and Coleman, 2011). Furthermore, most routine husbandry procedures involve

humans, and the presence of humans *per se* may contribute to fear, and thus stress in pigs, arising from the husbandry procedures (Marchant-Forde et al. 2009).

Stress can be defined as the biological response elicited when an individual perceives a threat to its homeostasis, i.e. a challenge (Moberg, 2000). An animal's stress response can be divided into three stages; the recognition of the stressor, the biological defense against the stressor, and the consequences of the stress response. It is the last stage of the stress response which determines whether stress is severe and is having a deleterious effect on an animal's welfare, or whether the animal is merely experiencing a brief acute stress response which will have no significant impact on its welfare (Moberg, 2000).

Stress has been shown to depress growth and reproduction and increase the prevalence of injury and ill-health (Hemsworth and Coleman, 2011; Sapolsky, 1992; Kaltas and Chrousos, 2007). It can also lead to behavioural problems, such as fearfulness, aggression and stereotypies. Stress can obviously compromise the welfare of animals (Broom and Johnson, 1993; Moberg, 2000), and in livestock production, such effects can have serious economic implications. Stress resilience, which generally refers to the resilience of animals to stressors (see reviews by Lyons et al., 2009; Parker and Maestripieri, 2011), thus has substantial implications on how well animals adapt to their environment and in turn animal emotionality and welfare and in agriculture animal productivity.

Stress resilience is generally studied by examining diminished emotionality, increased exploration, improved learning, and diminished hypothalamo–pituitary adrenal axis (HPA axis) activation (see reviews by Lyons et al., 2009; Parker and Maestripieri, 2011). Emotionality is commonly used to refer to the "degree we or other animals experience and express emotions". Traditionally emotionality has been often used as a synonym of fearfulness (Archer, 1973, Ramos and Mormede, 1998), but there is substantial overlap in the literature of the notions of emotionality, stress and anxiety (Archer, 1973; Ramos and Mormede, 1998). Fear and anxiety are two closely related emotions, with fear generally defined as a reaction to an explicit threatening stimulus, whereas anxiety is usually considered a more general state of distress, more long-lasting, prompted by less explicit or more generalized cues (Forkman et al., 2007). Animal studies on emotionality often measure fear- and anxiety-like behaviours, such as defecation, freezing and displacement activity of rats, and exploration in novel environments (Ramos and Mormède, 1998; Raineki et al., 2016).

In farming animals, we exercise varying degrees of control over the quality and duration of their lives and, because most of the animals that society uses can suffer, we have an ethical 'duty of care' towards them. In addition to this moral obligation, community concerns about restrictions in the lives of farms animals, such as restrictions in social contact, ability to exercise, and choice of stimuli (such as other conspecifics and additional features of the physical environment) to interact with, influence both public (Government) and private (NGOs and retailers) policy on animal welfare, which in turn together can influence production practices and consumer purchasing behaviour. Indeed, reviews by various Australian livestock industries have identified that a key to their success is the industry's ability to align their production practices with consumer and community expectations (e.g. recent Australian red meat industry strategic plan (Red Meat Advisory Council, 2015)).

Research to date has focused on strategies to reduce animal stress, either by modifying the animal's environment through the identification and elimination of the main stressors, by environmental enrichment or by genetic selection aimed at improved stress resilience (Galindo et al., 2011; Hocking et al., 2011). While there is considerable evidence of the effects of a negative human-animal relationship, little is known of the effects of a positive human-animal relationship on stress resilience in farm or other animals in captivity. Some authors have also proposed that positive human contact may provide a source of enrichment that confers stress resilience for domesticated and zoo animals in challenging situations (Wells, 2004; Claxton, 2011; Hemsworth and Coleman, 2011). Indeed, in addition to reducing the acute stress associated with husbandry practices imposed by humans, positive handling may also endow an ongoing positive emotional state with broader stress resilience. While not studied extensively, there is evidence that regular positive human contact (human enrichment programs) may ameliorate some of the stress associated with captivity. Brief daily positive human contact has been shown to reduce the magnitude of the physiological stress response to confinement housing (tether housing) of sows (Pedersen et al., 1998) and positive human contact stimulates brain oxytocin release in pigs, a hormone most often associated with positive social behaviours (Rault, 2016).

Animals in most production systems have to be moved and humans inadvertently interact with animals when inspecting them and their environment. Human-animal interactions also occur when animals are restrained in order to be subjected to routine husbandry practices. Some of these husbandry practices are undertaken to harvest a product (such as wool or milk), to prevent an animal behavioural or health problem or for human convenience, and some of these are contentious and involve surgical intervention, such as tail docking and castration. Other practices are less invasive, such as shearing, vaccination and early weaning, but nevertheless have the potential to elicit fear and stress. The general public is increasingly questioning the welfare impact of and the need for some routine husbandry practices, particularly surgical interventions that are likely to cause pain (Coleman, 2008). It may be possible to eliminate some of these practices such as tail docking and branding. However, it is likely that many will remain part of routine animal care and production for some time, but with the inclusion of pain relief drugs specifically targeting the pain component, not handling stress *per se*. It has been shown with piglet castration and tail docking that the fear and stress arising from handling *per se* may be as stressful as the pain component (Marchant-Forde et al., 2009). The association of fear and pain from husbandry practices performed by humans will also increase the fear of humans which animals exhibit in other situations, such as during routine inspections and handling. These detrimental effects on the human-animal relationship relate both to the aversiveness of the procedure, and the association by the animal of humans with that aversion.

Rewarding experiences, such as preferred feed or even positive handling, around the time of a stressful procedure, may ameliorate the aversiveness of the procedure and reduce the chances that animals associate the punishment of the procedure with humans. For example, studies with pigs have shown that they will associate the rewarding elements of feeding with humans if handlers are present at feeding (Hemsworth et al., 1996a). Daily injections were not highly aversive to pigs (Hemsworth et al., 1996b) and the authors suggested that there may have been some rewarding elements for the pigs in these handling bouts, such as the presence of the handler and the opportunity to closely approach and interact with the handler

before and after injection. There is also limited evidence that previous positive handling can improve ease of handling and reduce heart rates during loading of calves for transport (Lensink et al., 2001), reduce heart rate and salivary cortisol concentrations in lambs following tail docking (Tosi and Hemsworth, 2002) and reduce heart rates, kicking and restless behaviour in dairy cows during rectal palpation (Waiblinger et al., 2004). Recently our research team examined the effects of positive human contact on day-old piglets at 4 suckling bouts on their behavioural response to tail docking (Muns et al., 2015). Escape behaviour, based on the duration of struggling of the 'positively-conditioned' piglets was of shorter duration in response to tail docking at 2-days of age and capture at 15-days of age than piglets that were not handled at suckling. There was no treatment effect on the cortisol response to tail docking and vaccination, but the piglet's HPA axis may not be completely developed by the second day of life (Weaver et al., 2000). These results indicate that previous positive human contact may ameliorate the stress response associated with husbandry practices performed by humans.

These studies provide limited evidence that brief positive human contact can alter the animal's behavioural responses to subsequent stressful events involving humans. While social interactions can be a potent stressor, social interactions can buffer the response to an external stressor (Beery and Kaufer, 2015) and similarly a positive relationship with humans may ameliorate stressful events involving humans. Furthermore, as discussed above, a positive relationship with humans may eliminate an aversive component of many husbandry procedures, including those involving surgical interventions, since handling *per se* may be as stressful, based on behavioural and physiological responses, as the pain component (Marchant-Forde et al., 2009).

This project is a proof of concept to examine whether regular positive human contact is enriching for pigs and facilitates stress resilience. Thus, this project examined the effects of regular positive human contact on stress resilience in breeding sows. The hypothesis tested was that regular positive human contact leads to improved stress resilience with consequent effects on fitness.

2. Methodology

2.1 Animals, housing and experimental design

A factorial treatment design with the following two main effects was applied to sows during gestation:

1. **Human contact** – two treatments were examined:
    i. Human enrichment (HE) during gestation: this treatment was imposed for 2 min daily and involved 1 of 6 stockpeople entering and slowly walking through each group pen, and, at 30-s intervals for 10 seconds, stopping, squatting and talking and, if sows approached, patting them. These stockpeople were briefed prior to the commencement of the experiment.
    ii. Control during gestation: this treatment involved housing and routine husbandry as in the HE treatment but without 2 min of daily positive human contact in the pen.

2. **Parity** - two parity groups were examined:
    i. Parity 1-2
    ii. Parity 3 and older

The rationale for studying these two main effects is that minimal human contact or additional and positive human contact with sows associated with routine husbandry will produce differences in environmental stimulation, while some of the behavioural and physiological measures studied are likely to differ due to age and/or experience (Waiblinger et al., 2006; Hemsworth and Coleman, 2011).

Three hundred and sixty mixed-parity Landrace × Large White sows were studied in two-time replicates (180 sows per replicate) in a 1-year experiment. The sows were in good health and were weaned and housed in mating stalls and artificially inseminated (2 inseminations, 24 h apart) once they were detected in oestrus. The sows were introduced to the treatments within 4 d post-insemination and remained in treatment from mixing to entry to farrowing crates (16 wk post-insemination). Introduction to the allocated treatment was considered d 1 of treatment. Each time replicate (2 replicates, conducted in late spring and early autumn, each consisting of 12 pens housing a total of 180 sows) was introduced into the experiment over 2 weeks as follows; within each time replicate on each of two successive Wednesdays, 45 parity 1-2 sows and 45 parity 3 and older sows (90 sows total), that had been inseminated within the previous 4 d, were assigned to the handling treatments (HE or Control) and then pens.

During gestation sows were housed in groups of 15 (24 pens of 15 sows in total) on partially-slatted floors with automatic overhead feed droppers. Feed was delivered onto the solid section of the floor via drop feeders (5/pen) that were evenly dispersed across the width of each pen. Feeding occurred twice/day (approximately 0700 and 0730 h) to provide a total of 2.5 kg/sow/d of a commercial diet (13.1 MJ/kg DM, and 13.5% crude protein). The sows were housed in 2 adjacent rows (6 pens/row) separated by the central corridor with a space allowance of 2.0 m^2/sow. 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 the corridor and the adjacent pens. Two closed-circuit TV (CCTV) cameras were positioned at the front and the back of each pen in order to capture the pen in its entirety. Sows were introduced to the farrowing crates at d 110 of gestation.

2.2 Measurements

Behaviour measurements

Behavioural response to the HE treatment

Using CCTV camera footage, the behaviour of the sows was observed during treatment imposition on d 2, 5, 9, 17, 28, 43 and 73 of treatment to assess interactions between the stockperson imposing the HE treatment and individual HE sows (continuous observations classifying behaviour as sow initiated and stockperson initiated tactile interactions) and proximity to stockperson (instantaneous point sampling at 10-s time points) in the home pens.

Behavioural response to humans (human approach test, HAT)

The behavioural response to a stationary human in a novel arena was assessed in six animals randomly selected from each pen during wk 9 of gestation (human approach test - HAT, Hemsworth et al., 1989, 1994). A test arena was constructed with solid black boarding inside an empty gestation pen (14.3 m^2) within the shed where the animals were housed. Marked lines on the floor split the arena into 4 quadrants in order to measure each sow's exploration of the arena. The mid-point of the wall adjacent to the entry of the arena was also marked, to create a 0.5m radius around the position in which the experimenter stood. Pens immediately adjacent to the test arena contained non-experimental pigs. Testing was undertaken from 08:30 to 15:00 h, allowing a minimum of 1 h to elapse after feeding.

Prior to testing, the six test sows from the first group were removed from their home pen and placed into a holding area located 8.8 m away from the test arena. These six sows were given 4 min to settle in the holding area, after which time 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, quadrants of the arena the sow entered were recorded as a measure of exploration of this novel area. At the end of the familiarisation period, an experimenter quietly entered the arena and slowly walked to the marked mid-point of the adjacent wall. The experimenter stood stationary in this position for the 3-min duration of the test, and verbally relayed the sow's behaviour to a recorder standing outside the arena who was not visible to the test sow. The behavioural variables recorded to assess fear of humans in the HAT included time to enter within 0.5m of experimenter, time spent within 0.5m of experimenter, time to interact and number of interactions initiated with the experimenter. Upon completion of the test, the test sow was moved back to the holding pen and the next sow from that group was tested. Once all sows from the group had been tested, they were moved back to the home pen and the six test sows in the next pen along the aisle were moved to the holding area. All sows were moved by experienced handlers with the assistance of a solid pig board.

Behavioural responses to routine husbandry practices

The behavioural responses of sows towards pregnancy testing with an ultrasound probe (wk 6), vaccination (wk 12) and introduction to farrowing accommodation (wk 16) were assessed. Using CCTV camera footage, the interactions between all sows and the technician, and the approach-withdrawal responses of all sows to the technician, were observed continuously around the imposition of the pregnancy test and vaccination in the home pens. When the approaching technician was within 1 m of a sow, the sow was recorded as either withdrawing (moved away from the technician immediately) or not withdrawing (approached or remained stationary) from the approaching technician conducting the pregnancy test or vaccination.

Aggression around feeding

Using CCTV camera footage, aggression around feeding was sampled for one day in each of wk 1, 4 and 8 of gestation. 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. The identity of each sow was not recorded because aggression at the level of the group was the focus. 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.

The number of aggressive interactions in each pen was expressed based on the average number of sows clearly visible in the field of view of the camera. Scan sampling at 30-sec intervals was used to count the number of sows in the field of view at each sampling point, providing an estimate of the average number of sows visible during each 15 min observation period. Aggression in each pen around feeding was then calculated by multiplying the number of aggressive interactions by the inverse of the average number of sows in the field of view.

Behavioural responses to introduction to farrowing crates

Direct observations were conducted to assess the behavioural responses of sows during introduction into farrowing crates at d 110 of gestation. Upon entry to the farrowing house, the behaviour of each sow was observed when the animal reached within 1 m of her farrowing crate. All sows were recorded as either voluntarily entering the farrowing crate (walked into the crate without freezing or stopping in the aisle) or resisting entry (attempted to turn around in the aisle or froze for more than 2 s in the aisle, requiring stockperson assistance). At 1.5, 3.5 and 4.5 h after all sows had been introduced to the farrowing accommodation, the behaviour of six randomly selected sows/pen was directly observed. For periods of 30 s at each of the three time points, one-zero sampling was used to record whether or not the behavioural variables of lying, feeding, drinking, rooting, manipulating the crate, sham chewing and vocalising occurred.

Behavioural response to piglet vocalisations (maternal responsiveness test, MRT)

Behavioural responsiveness of all sows towards an audio recording of an unfamiliar piglet squealing was measured in farrowing crates during wk 2 of lactation. Following the protocol for the MRT as described by Singh et al. (2016), 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 2 m) while walking slowly through the farrowing shed, pausing for 3 s in front of each sow's crate. The experimenter completed six laps (each lap took approximately 10 min to complete) of the farrowing house in succession, so that each sow experienced the recording playing in front of their crate for 3 s at six different timepoints. Two Go-Pro cameras were mounted on either side of the experimenter's head, which allowed each sow's behavioural response during each 3-s bout of the audio broadcasting in front of the crate, to later be assessed by one observer. Maternal responsiveness in the test was measured through changes in posture, disruptions to feeding and the occurrence of sows vocalising, manipulating crate fittings and displaying behaviour directed towards piglets.

Physiological and fitness measurements

Baseline cortisol during gestation (serum)

Blood samples were collected from three sows randomly selected from each pen during wk 5 and 10 of gestation, for subsequent analysis of serum cortisol.

Samples of 6 ml of blood were collected into serum collection tubes (BD Vacutainer, New South Wales, Australia) via jugular venepuncture from sows restrained with a snout snare. Sampling commenced at approximately 11:00-12:00 h and all blood samples were collected within 2 min of approaching the selected sow. After collection, tubes were gently inverted 5 times and allowed to clot for 1 h at room temperature. Samples were then centrifuged at 1000 x g for 15 min, before being divided into two collection tubes, and stored at -20°C until later analysis of serum cortisol and brain-derived neurotrophic factor (see below). Serum cortisol was quantified using a commercially available RIA kit from MP Biomedicals (Item #07-221105). The intra- and inter-assay CV's were 4.2 and 3.1%, respectively.

Baseline cortisol during lactation (saliva)

Saliva samples were collected from six sows randomly selected from each pen in wk 4 of lactation (at approximately 10:00-11:00 h) to assess basal stress in farrowing crates. Previous research has demonstrated that cortisol concentrations in gilts housed in farrowing crates increase at 4 wk postpartum (Cronin et al., 1991).

Saliva was collected using saliva collection tubes (Sarstedt; South Australia, Australia) and synthetic swabs (Salimetrics; Carlsbad, California, USA). The swab was secured to the end of a flexible cable tie, and the sow selected for sampling was allowed to chew on the swab for 1-2 min until it was suitably moistened. All saliva samples were collected within 2 min of approaching the selected sow. The swab was then placed into the collection tube, centrifuged at 2500 x g for 2 min and stored at -20°C until later analysis. Salivary cortisol was quantified using a commercially available RIA kit from MP Biomedicals (Item #07-221105). The intra- and inter-assay CV's were 2.9 and 3.0%, respectively.

Acute cortisol (saliva)

Saliva samples were collected from six sows randomly selected from each pen within 2 min after pregnancy testing (wk 6, at approximately 10:00-11:00 h), within 2 min after the HAT (wk 9), and within 2 min of attempting to take a sample at 2.5 h after introduction to the farrowing crate (wk 16, at approximately 10:00-11:00 h) to measure acute stress responses.

Saliva was collected and analysed as above. The intra-assay CV's for post-pregnancy testing, post-HAT and post-farrowing crate entry samples were 3.1, 3.3 and 2.9%, respectively.

Oxytocin concentration before and after human interaction (saliva)

Two saliva samples, obtained 5 min apart, were collected from six sows randomly selected from each pen to measure salivary oxytocin concentrations before (0 min) and 5 min after a 30-s opportunity to interact with a stationary experimenter (immediately following the first sample) in wk 11 of gestation.

The procedure for saliva collected was as described above. There are currently no commercially available kits designed to quantify oxytocin from pig saliva. However, as oxytocin is a highly conserved protein between species, a commercially available ELISA kit from Cayman Chemical (Item #500440) validated for measuring oxytocin from human samples was used. This kit was unable to accurately quantify oxytocin from the saliva samples collected for this study, and validation of the protocol for sample preparation and analysis is required. Therefore, these samples are yet to analysed until the assay is validated to measure oxytocin from pig saliva. The oxytocin data from this experiment will be available once this is completed.

Brain-derived neurotrophic factor (serum)

Blood samples were collected from three sows randomly selected from each pen during wk 5 and 10 of gestation, for subsequent analysis of Brain-derived neurotrophic factor (BDNF). Environmental enrichment has been shown to increase BDNF in rats, resulting in higher stress resilience, and reflecting better brain (hippocampal) development that facilitates learning and memory (Mosaferi et al., 2015).

Serum was collected and prepared as described above in the procedure for baseline cortisol during gestation. BDNF was quantified using a commercially available ELISA kit from Biosensis (Item #BEK-2211). The normal detectable range of the assay was 7.8 to 500 pg/ml and the mean intra- and inter-assay CV's were 2.9 and 7.8%, respectively.

General health and reproductive performance

Health treatments, culling including reasons, and reproductive performance (farrowing rate (proportion sows inseminated that farrowed), piglets born alive, stillborn piglets, mummified piglets and total piglets weaned) were collected for all sows studied.

2.3 Statistical analysis

The unit of analysis was the group of animals from a single treatment pen within a time replicate. Each measurement was analysed using a mixed model analysis of variance that included treatment, parity and replicate as fixed effects. All data were checked for homogeneity of variance (Levine's test for equality of error variances) and normality (Shapiro-Wilk test), and transformations were applied when necessary (square-root or logarithmic). All statistical analyses were performed using the statistical package IBM SPSS Statistics for Windows, Version 24.0 (IBM Corp., Armonk, NY, USA).

The four behavioural variables in the HAT, number of areas entered in the 2-min familiarisation, time to enter within 0.5m of experimenter, time spent within 0.5m of experimenter, time to interact and number of interactions initiated with the experimenter, were subjected to a principle component analysis (PCA) to identify sets of 'factors' that represent the underlying relationships among the variables in this test.

3. Outcomes

3.1 Results

Behaviour

Behavioural response to the HE treatment

The proportion of sows patted, the numbers of stockperson and sow interactions and the proportion of sows within 1 m of the stockperson during the imposition of the HE treatment on d 2, 5, 9, 17, 28, 43 and 73 of gestation in each of the two replicates are presented in Table 1. There were significant effects of replicate on proportion of sows patted (P=0.027), stockperson interactions (P=0.001) and proportion of sows within 1 m of the stockperson (P=0.015), with generally more sows patted and more stockperson interactions but less sows within 1 m of stockperson in Replicate 2. There were also effects of both day and replicate on number of sow interactions with the stockperson (P<0.001 and P=0.005, respectively), with sows generally showing increased interactions with the stockperson both in Replicate 2 and later in gestation. There were no day x replicate interactions on these variables (Table 1).

Table 1. Means (± standard errors) for the main effects of day and replicate (1 and 2) on the stockperson and sow behavior during the imposition of the human enrichment treatment on d 2 (wk 1), d 5 (wk 2), d 9 (wk 2), d 17 (wk 3), d 28 (wk 5), d 43 (wk 7) and d 73 (wk 11) of gestation (n=24 groups).

a. P values for main effects

Measurements	Day	Rep	Day x Rep
Sows patted (proportion of sows in pen)	0.367	0.027	0.388
Number of stockperson-initiated interactions (per/sow)	0.208	**0.001**	0.355
Number of sow-initiated interactions (per/sow)	<0.001	**0.005**	0.533
Sows within 1m of stockperson (proportion of sows)	0.051	**0.015**	0.876

b. Means (± standard error)

Measurements	Rep	D 2	D 5	D 9	D 17	D 28	D 43	D 73
Sows patted	1	0.60±0.06	0.43±0.09	0.60±0.05	0.53±0.09	0.58±0.08	0.49±0.09	0.39±0.06
	2	0.45±0.06	0.64±0.09	0.59±0.05	0.63±0.09	0.70±0.08	0.59±0.09	0.51±0.06
Stockperson initiated interactions	1	0.96±0.14	0.99±0.26	0.98±0.17	0.86±0.18	1.00±0.13	0.76±0.28	0.44±0.20
	2	0.86±0.14	1.14±0.26	1.45±0.17	1.46±0.18	1.54±0.13	1.50±0.28	1.13±0.20
Sow initiated interactions	1	0.11±0.11	0.42±0.12	0.61±0.18	0.68±0.09	0.50±0.09	0.29±0.19	0.07±0.07
	2	0.18±0.11	0.59±0.12	1.17±0.18	0.79±0.09	0.82±0.09	0.60±0.19	0.36±0.07
Sows within 1m of stockperson	1	0.19±0.02	0.17±0.03	0.20±0.01	0.24±0.02	0.24±0.01	0.25±0.03	0.21±0.03
	2	0.16±0.02	0.15±0.03	0.17±0.01	0.18±0.02	0.18±0.01	0.20±0.03	0.21±0.03

Behavioural response to humans (human approach test, HAT)

Prior to conducting the PCA on the behavioural variables in the HAT, the suitability of the data for the analysis was assessed using criteria outlined by Pallant (2013). An inspection of the correlation matrix revealed that the coefficients were all above the required 0.3. Furthermore, the Kaiser-Meyer-Olkin (KMO) value exceeded the recommended value of 0.6, and Bartlett's Test of Sphericity reached statistical significance. Thus, the factorability of the correlation matrix was supported. A PCA of the behavioural variables from HAT extracted two components. The first component labelled Fear of human score, with an eigenvalue greater than 1, included all the four variables, time to enter within 0.5m of experimenter, time spent within 0.5m of experimenter, time to interact and number of interactions initiated with the experimenter, with loadings of 0.77-0.92, and accounted for 60.1% of the variance. The second component labelled Novel score, with an eigenvalue greater than 1, included only the variable number of squares entered in the 2-min familiarisation period of the HAT, with a loading of 0.99, and accounted for 20.0% of variance.

There was no treatment effect on the Novel and Fear of human scores in the HAT (Table 2). However, there was a significant (P=0.025) replicate effect on the Fear of human score, with Replicate 2 sows being less fearful of the experimenter. There were no treatment x replicate interactions on these two scores (Table 2).

Table 2. Means (± standard errors) for the main effects of treatment (HE and Control), parity (P1&2 and P3+) and replicate (1 and 2) on the behavioural responses of sows towards novelty and humans in the HAT (wk 9) (n=24 groups).

a. P values for main effects and interactions

Measurements	Treat	Parity	Rep	Treat x Parity	Treat x Rep	Parity x Rep	Treat x Parity x Rep
Novelty score	0.637	0.915	0.650	0.658	0.295	0.913	0.859
Fear of humans score	0.477	0.326	**<0.001**	0.511	0.739	0.160	0.494

b. Means (± standard error)

Measurements	Rep	Parity	Control		HE	
Novelty score[1]	1	P1&2	-0.02	±0.24	-0.22	±0.24
		P3+	-0.16	±0.24	0.05	±0.24
	2	P1&2	0.19	±0.25	-0.11	±0.24
		P3+	0.15	±0.24	-0.07	±0.24
Fear of humans score[1]	1	P1&2	-0.42	±0.20	-0.67	±0.20
		P3+	-0.55	±0.20	-0.42	±0.20
	2	P1&2	0.77	±0.21	0.63	±0.20
		P3+	0.43	±0.20	0.28	±0.20

[1]The scores are component scores from the Principal Components Analysis conducted on pig behaviour variables in the HAT.

Behavioural responses to routine husbandry practices

There were significant treatment, parity and replicate effects on the proportion of sows withdrawing to both the approach of the technician (P=0.001, P=0.043 and P=0.046, respectively) and to the technician during the pregnancy test with the ultrasound probe (P=0.001, P=0.031 and P=0.036, respectively) (Table 3). Sows in the HE treatment showed less withdrawal to the approaching technician. There were treatment x parity and treatment x parity x replicate effects on the proportion of sows withdrawing from the technician after the actual pregnancy test with the ultrasound probe (P=0.003 and P=0.015 respectively, Table 3), with a higher proportion of younger C sows withdrawing, particularly in Replicate 2. There was also a significant treatment effect on the number of sow interactions with the technician during the pregnancy testing of the group (P<0.001, Table 3), with more HE sows interacting.

Table 3. Means (± standard errors) for the main effects of treatment (HE and Control), parity (P1&2 and P3+) and replicate (1 and 2) on the behavioural response of sows towards the technician during pregnancy testing (wk 6) (n=24 groups).

a. P values for main effects and interactions

Measurements	Treat	Parity	Rep	Treat x Parity	Treat x Rep	Parity x Rep	Treat x Parity x Rep
Withdrawal from approaching technician	**0.001**	**0.043**	**0.046**	0.137	0.939	0.352	0.894
Withdrawal from technician post-test	**<0.001**	**0.031**	**0.036**	**0.003**	0.915	0.307	**0.015**
Interactions with technician during test	**<0.001**	0.238	0.240	0.551	0.882	0.968	0.659

b. Means (± standard error)

Measurements	Rep	Parity	Control		HE	
Withdrawal from approaching technician (prop.)	1	P1&2	0.53	±0.88	0.18	±0.88
		P3+	0.35	±0.88	0.21	±0.88
	2	P1&2	0.45	±0.88	0.11	±0.88
		P3+	0.16	±0.88	0.00	±0.88
Withdrawal from technician post-test (prop.)	1	P1&2	0.61	±0.06	0.23	±0.06
		P3+	0.52	±0.06	0.20	±0.06
	2	P1&2	0.68	±0.06	0.05	±0.06
		P3+	0.27	±0.06	0.17	±0.06
Interactions with technician during test[1]	1	P1&2	5.67	±3.71	13.67	±3.71
		P3+	4.33	±3.71	13.67	±3.71
	2	P1&2	8.67	±3.71	23.00	±3.71
		P3+	5.33	±3.71	16.50	±3.71

[1]Logarithmic transformation and back-transformed means presented

There was a significant treatment effect (P=0.003) on the withdrawal response of sows to the approaching technician during vaccination, with fewer HE sows withdrawing (Table 4).

Table 4. Means (± standard errors) for the main effects of treatment (HE and Control), parity (P1&2 and P3+) and replicate (1 and 2) on the behavioural response of sows towards the technician during vaccination (wk 12) (n=24 groups).

a. P values for main effects and interactions

Measurements	Treat	Parity	Rep	Treat x Parity	Treat x Rep	Parity x Rep	Treat x Parity x Rep
Withdrawal from approaching technician	**0.003**	0.727	0.570	0.362	0.341	0.676	0.114
Withdrawal from technician post-vaccination	0.374	0.813	0.679	0.714	0.700	0.949	0.691

b. Means (± standard error)

Measurements	Rep	Parity	Control		HE	
Withdrawal from approaching technician (prop.)	1	P1&2	0.53	±0.10	0.53	±0.10
		P3+	0.70	±0.10	0.35	±0.10
	2	P1&2	0.72	±0.10	0.36	±0.10
		P3+	0.72	±0.10	0.47	±0.10
Withdrawal from technician post-vaccination (prop.)[1]	1	P1&2	0.91	±0.09	0.88	±0.09
		P3+	0.89	±0.09	0.86	±0.09
	2	P1&2	0.98	±0.09	0.85	±0.09
		P3+	0.92	±0.09	0.92	±0.09

[1]Logarithmic transformation and back-transformed means presented

Aggression around feeding

There were no treatment effects on aggression in wk 1, 4 and 8 (Table 5). There was however a significant replicate effect on aggression in wk 8, with higher levels in Replicate 2.

Table 5. Means (± standard errors) for the main effects of treatment (HE and Control), parity (P1&2 and P3+) and replicate (1 and 2) on the number of aggressive interactions between sows during morning feeding on wk 1, 4 and 8 of gestation. Aggression is expressed as frequency per sow (n=24 groups).

a. P values for main effects and interactions

Measurements	Treat	Parity	Rep	Treat x Parity	Treat x Rep	Parity x Rep	Treat x Parity x Rep
Aggression Wk 1	0.492	0.922	0.955	0.985	0.634	0.137	**0.035**
Aggression Wk 4	0.984	0.616	0.806	0.936	0.618	**0.030**	0.350
Aggression Wk 8	0.902	0.347	**0.002**	0.762	0.033	0.338	0.843

b. Means (± standard error)

Measurements	Rep	Parity	Control		HE	
Aggression Wk 1	1	P1&2	6.19	±2.63	8.23	±2.63
		P3+	13.17	±2.63	6.71	±2.63
	2	P1&2	12.38	±2.63	7.65	±2.63
		P3+	4.96	±2.63	8.88	±2.63
Aggression Wk 4[1]	1	P1&2	7.80	±1.33	8.84	±1.33
		P3+	10.93	±1.33	10.57	±1.33
	2	P1&2	11.65	±1.33	10.52	±1.33
		P3+	8.65	±1.33	9.10	±1.33
Aggression Wk 8	1	P1&2	5.67	±1.02	7.34	±1.02
		P3+	5.76	±1.02	7.27	±1.02
	2	P1&2	10.53	±1.02	9.12	±1.02
		P3+	9.49	±1.02	7.35	±1.02

[1]Logarithmic transformation and back-transformed means presented

Behavioural response to introduction to farrowing crates

There was no effect of treatment on the proportion of sows resisting entry to the farrowing crate or the proportion of observation periods in which sows were observed lying, feeding, drinking, rooting, manipulating crate fittings or vocalising at 1.5, 3.5 and 4.5 h after introduction to the farrowing crate (Table 6). There were effects of parity and replicate on several of the behaviours. For example, parity affected manipulation of the crate at 1.5 h, with more older sows manipulating the crate and replicate affected sham chewing at 4.5 h (P=0.012) and vocalisations at 3.5 and 4.5 h after entry to crate (P<0.001). There was a significant treatment x parity x replicate interaction (P=0.008) on manipulating the crate at 4.5 h with more younger HE sows manipulating the crate than younger Control sows and more older Control sows manipulating the crate than older HE sows in Replicate 1, with the reverse in Replicate 2.

Table 6. Means (± standard errors) for the main effects of treatment (HE and Control), parity (P1&2 and P3+) and replicate (1 and 2) on the behaviour of sows after introduction to farrowing crates at d 110 of gestation (n=24 groups). Resistance to entry of the crate presented as proportion of sows and other behaviours presented as proportion of observation periods in which the behavior was observed.

a. P values for main effects and interactions

Measurements	Time after entry (h)	Treat	Parity	Rep	Treat x Parity	Treat x Rep	Parity x Rep	Treat x Parity x Rep
Resistance to enter crate	Upon entry	0.510	0.219	0.196	0.271	0.497	0.423	0.146
Lying	1.5	0.354	0.712	**0.001**	0.515	0.629	0.119	0.193
	3.5	0.724	0.085	0.950	0.787	0.634	0.787	0.496
	4.5	0.088	0.932	0.448	0.071	0.302	0.631	0.194
Feeding	1.5	0.905	0.478	**0.002**	0.858	0.905	0.478	0.677
	3.5	0.496	**0.039**	0.283	0.859	0.561	0.561	0.939
	4.5	0.689	0.689	0.058	0.238	0.689	0.689	0.238
Drinking	1.5	1.000	1.000	0.624	0.153	0.332	0.332	0.624
	3.5	0.238	0.238	0.058	0.698	0.058	0.698	0.698
	4.5	1.000	0.176	1.000	1.000	0.176	1.000	0.176
Rooting	1.5	0.600	0.301	0.128	**0.048**	0.600	0.301	0.301
	3.5	0.421	0.421	0.208	0.208	0.886	0.421	0.738
	4.5	0.176	1.000	1.000	1.000	1.000	0.176	0.176
Manipulating crate	1.5	0.514	**0.017**	0.514	0.514	1.000	0.514	1.000
	3.5	0.898	0.170	0.014	0.898	0.898	0.170	0.898
	4.5	0.519	0.183	0.432	0.432	0.183	0.519	**0.008**
Sham chewing	1.5	0.490	0.490	0.176	1.000	0.490	**0.050**	1.000
	3.5	0.201	1.000	0.201	0.514	0.514	0.063	0.201
	4.5	0.678	0.678	**0.012**	0.320	0.320	0.858	0.122
Vocalising	1.5	0.381	0.106	0.174	0.011	0.797	0.274	0.589
	3.5	1.000	0.100	**<0.001**	0.209	0.396	**0.003**	0.209
	4.5	0.195	0.914	**<0.001**	0.641	0.801	0.801	0.136

b. Means (± standard error)

Measurements	Time after entry (h)	Rep	Parity	Control		HE	
Resistance to enter crate	Upon entry	1	P1&2	0.44	±0.11	0.47	±0.11
			P3+	0.42	±0.11	0.40	±0.11
		2	P1&2	0.67	±0.11	0.57	±0.11
			P3+	0.31	±0.11	0.61	±0.11
Lying	1.5	1	P1&2	0.44	±0.13	0.78	±0.13
			P3+	0.83	±0.13	0.78	±0.13
		2	P1&2	0.41	±0.13	0.39	±0.13
			P3+	0.22	±0.13	0.33	±0.13
	3.5	1	P1&2	0.50	±0.19	0.67	±0.19
			P3+	0.83	±0.19	0.89	±0.19
		2	P1&2	0.70	±0.19	0.56	±0.19
			P3+	0.78	±0.19	0.89	±0.19
	4.5	1	P1&2	1.00	±0.14	0.78	±0.14
			P3+	1.00	±0.14	0.67	±0.14
		2	P1&2	0.64	±0.14	0.89	±0.14
			P3+	1.00	±0.14	0.61	±0.14
Feeding	1.5	1	P1&2	0.06	±0.13	0.00	±0.13
			P3+	0.00	±0.13	0.06	±0.13
		2	P1&2	0.29	±0.13	0.33	±0.13
			P3+	0.44	±0.13	0.44	±0.13
	3.5	1	P1&2	0.50	±0.15	0.33	±0.15
			P3+	0.17	±0.15	0.06	±0.15
		2	P1&2	0.24	±0.15	0.22	±0.15
			P3+	0.06	±0.15	0.06	±0.15
	4.5	1	P1&2	0.00	±0.05	0.00	±0.05
			P3+	0.00	±0.05	0.00	±0.05
		2	P1&2	0.11	±0.05	0.06	±0.05
			P3+	0.00	±0.05	0.11	±0.05
Drinking	1.5	1	P1&2	0.00	±0.08	0.17	±0.08
			P3+	0.06	±0.08	0.00	±0.08
		2	P1&2	0.06	±0.08	0.06	±0.08
			P3+	0.17	±0.08	0.06	±0.08
	3.5	1	P1&2	0.17	±0.05	0.06	±0.05
			P3+	0.11	±0.05	0.00	±0.05
		2	P1&2	0.00	±0.05	0.06	±0.05
			P3+	0.00	±0.05	0.00	±0.05
	4.5	1	P1&2	0.00	±0.03	0.00	±0.03
			P3+	0.06	±0.03	0.00	±0.03
		2	P1&2	0.00	±0.03	0.00	±0.03
			P3+	0.00	±0.03	0.06	±0.03
Rooting	1.5	1	P1&2	0.06	±0.07	0.00	±0.07
			P3+	0.00	±0.07	0.06	±0.07
		2	P1&2	0.11	±0.07	0.00	±0.07
			P3+	0.06	±0.07	0.23	±0.07

	3.5	1	P1&2	0.11	±0.08	0.00	±0.08
			P3+	0.06	±0.08	0.06	±0.08
		2	P1&2	0.24	±0.08	0.11	±0.08
			P3+	0.06	±0.08	0.11	±0.08
	4.5	1	P1&2	0.00	±0.03	0.06	±0.03
			P3+	0.00	±0.03	0.00	±0.03
		2	P1&2	0.00	±0.03	0.00	±0.03
			P3+	0.00	±0.03	0.06	±0.03
Manipulating crate	1.5	1	P1&2	0.00	±0.06	0.00	±0.06
			P3+	0.17	±0.06	0.11	±0.06
		2	P1&2	0.00	±0.06	0.00	±0.06
			P3+	0.11	±0.06	0.06	±0.06
	3.5	1	P1&2	0.00	±0.06	0.00	±0.06
			P3+	0.00	±0.06	0.00	±0.06
		2	P1&2	0.19	±0.06	0.17	±0.06
			P3+	0.06	±0.06	0.06	±0.06
	4.5	1	P1&2	0.00	±0.05	0.06	±0.05
			P3+	0.11	±0.05	0.00	±0.05
		2	P1&2	0.07	±0.05	0.00	±0.05
			P3+	0.00	±0.05	0.22	±0.05
Sham chewing	1.5	1	P1&2	0.17	±0.06	0.17	±0.06
			P3+	0.06	±0.06	0.06	±0.06
		2	P1&2	0.00	±0.06	0.06	±0.06
			P3+	0.06	±0.06	0.11	±0.06
	3.5	1	P1&2	0.00	±0.06	0.00	±0.06
			P3+	0.00	±0.06	0.17	±0.06
		2	P1&2	0.11	±0.06	0.17	±0.06
			P3+	0.06	±0.06	0.06	±0.06
	4.5	1	P1&2	0.00	±0.07	0.06	±0.07
			P3+	0.00	±0.07	0.00	±0.07
		2	P1&2	0.24	±0.07	0.06	±0.07
			P3+	0.11	±0.07	0.17	±0.07
Vocalising	1.5	1	P1&2	0.33	±0.14	0.22	±0.14
			P3+	0.06	±0.14	0.39	±0.14
		2	P1&2	0.66	±0.14	0.39	±0.14
			P3+	0.06	±0.14	0.44	±0.14
	3.5	1	P1&2	0.06	±0.09	0.00	±0.09
			P3+	0.17	±0.09	0.11	±0.09
		2	P1&2	0.72	±0.09	0.61	±0.09
			P3+	0.22	±0.09	0.44	±0.09
	4.5	1	P1&2	0.00	±0.11	0.17	±0.11
			P3+	0.06	±0.11	0.06	±0.11
		2	P1&2	0.48	±0.11	0.44	±0.11
			P3+	0.33	±0.11	0.61	±0.11

Behavioural response of lactating sows to the maternal responsiveness test (MRT)

There was a significant treatment effect (P=0.014) as well as a significant treatment x replicate interaction (P=0.026) on the proportion of sows vocalizing, with more Control sows vocalizing, particularly in Replicate 2 than Replicate 1 (Table 7).

Table 7. Means (± standard errors) for the main effects of treatment (HE and Control), parity (P1&2 and P3+) and replicate (1 and 2) on the behavioural responses of sows towards piglet vocalisations in the MRT (wk 2 lactation) (n=24 groups).

a. P values for main effects and interactions

Measurements	Treat	Parity	Rep	Treatx Parity	Treat x Rep	Parity x Rep	Treat x Parity x Rep
Changed posture (lying/sitting to upright)	0.282	0.957	0.336	0.053	0.276	0.366	0.048
Ceased feeding	0.260	0.939	0.177	0.214	0.443	0.911	0.223
Vocalising	**0.014**	0.429	0.122	0.926	**0.026**	0.064	0.086
Piglet directed behaviour	0.833	0.482	0.128	0.796	0.940	0.626	0.881
Manipulating crate	0.061	0.482	0.994	0.102	0.713	0.985	0.162

b. Means (± standard error)

Measurements	Rep	Parity	Control		HE	
Changed posture (lying/sitting to upright)[1]	1	P1&2	0.29	±0.11	0.31	±0.11
		P3+	0.22	±0.11	0.23	±0.11
	2	P1&2	0.50	±0.11	0.03	±0.11
		P3+	0.22	±0.11	0.31	±0.11
Ceased feeding[1]	1	P1&2	0.34	±0.11	0.30	±0.11
		P3+	0.34	±0.11	0.30	±0.11
	2	P1&2	0.19	±0.11	0.27	±0.11
		P3+	0.47	±0.11	0.13	±0.11
Vocalising[1]	1	P1&2	0.57	±0.16	0.72	±0.16
		P3+	0.89	±0.16	0.65	±0.16
	2	P1&2	1.55	±0.16	0.70	±0.16
		P3+	0.92	±0.16	0.57	±0.16
Piglet directed behaviour[1]	1	P1&2	0.40	±0.14	0.42	±0.14
		P3+	0.54	±0.14	0.49	±0.14
	2	P1&2	0.30	±0.14	0.29	±0.14
		P3+	0.33	±0.14	0.36	±0.14
Manipulating crate[1]	1	P1&2	0.13	±0.08	0.17	±0.08
		P3+	0.02	±0.08	0.20	±0.08
	2	P1&2	0.14	±0.08	0.09	±0.08
		P3+	0.01	±0.08	0.20	±0.08

[1]Square-root transformation and back-transformed means presented

Physiology

Cortisol

As shown in Table 8, there were no main effects of treatment or parity on serum cortisol concentrations at 5 and 10 wk of treatment, or salivary cortisol concentrations 2 min after the HAT at wk 9 of gestation, 2.5 h after entry to the farrowing crate and d 29 of lactation. However, there were effects of replicate on cortisol concentrations at 5 wk of treatment (P<0.001), 2 min after the HAT at wk 9 of gestation (P=0.001) and 2.5 h after entry to the farrowing crate (P<0.001). There was also a significant treatment x parity x replicate interaction on cortisol concentrations at d 29 of lactation (P=0.026). This 3-factor interaction is one of the three significant 3-factor interactions statistically significant at the 5% level (P<0.05) and only one at the 1% level (P<0.01), and thus this interaction may be significant (P<0.05) by chance.

Table 8. Means (± standard errors) for the main effects of treatment (HE and Control), parity (P1&2 and P3+) and replicate (1 and 2) on measurements of cortisol (n=24 groups).

a. P values for main effects and interactions

Measurements	Treat	Parity	Rep	Treat x Parity	Treat x Rep	Parity x Rep	Treat x Parity x Rep
Wk 5 gestation[1]	0.712	0.833	**<0.001**	0.079	0.764	0.482	0.094
Wk 10 gestation[1]	0.569	0.985	0.823	0.282	0.785	0.294	0.130
2-min post-HAT (wk 9 gestation)[2]	0.663	0.144	**0.001**	0.052	0.529	0.293	0.898
2.5 h post-farrowing crate entry (d 1 lactation)[2]	0.521	0.276	**<0.001**	0.520	0.808	0.231	0.131
D 29 lactation[2]	0.250	0.715	**0.020**	0.828	0.935	**0.050**	**0.026**

b. Means (± standard error)

Measurements	Rep	Parity	Control		HE	
Wk 5 gestation[1]	1	P1&2	10.81	±1.39	7.28	±1.39
		P3+	6.30	±1.39	9.96	±1.39
	2	P1&2	3.51	±1.39	4.09	±1.39
		P3+	3.91	±1.39	4.68	±1.39
Wk 10 gestation[1]	1	P1&2	11.21	±2.01	6.93	±2.01
		P3+	8.88	±2.01	12.30	±2.01
	2	P1&2	10.56	±2.01	10.03	±2.01
		P3+	9.68	±2.01	7.77	±2.01
2-min post-HAT (wk 9 gestation)[2]	1	P1&2	4.13	±0.77	5.24	±0.77
		P3+	5.10	±0.77	3.78	±0.77
	2	P1&2	2.30	±0.77	3.96	±0.77
		P3+	1.95	±0.77	1.47	±0.77
	1	P1&2	1.44	±0.13	1.60	±0.13

2.5hr post-farrowing crate entry (d 1 lactation)[2]	2	P3+	1.51	±0.13	1.51	±0.13
		P1&2	1.04	±0.13	0.88	±0.13
		P3+	1.05	±0.13	1.29	±0.13
D 29 lactation[2]	1	P1&2	1.59	±0.21	1.42	±0.21
		P3+	1.63	±0.21	2.13	±0.21
	2	P1&2	1.15	±0.21	1.73	±0.21
		P3+	1.28	±0.21	1.07	±0.21

[1] and [2] serum and salivary cortisol (ng/mL) concentrations, respectively.

Brain-derived neurotrophic factor (BDNF)

There were significant parity (P=0.001) and replicate (P=0.007) effects on the serum BDNF concentration at 5 wk and significant parity (P=0.001) effects on the serum BDNF concentration at 10 wk of gestation (Table 9). There was also a significant treatment x replicate effect (P=0.015) at 5 wk of gestation, with HE sows in Replicate 1 having higher serum BDNF concentrations at 5 wk of gestation. There were significant parity x replicate effects at 5 wk and 10 of gestation (P=0.001).

Table 9. Means (± standard errors) for the main effects of treatment (HE and Control), parity (P1&2 and P3+) and replicate (1 and 2) on measurements of serum BDNF (pg/ml) in wk 5 and wk 10 of gestation (n=23 groups).

a. P values for main effects and interactions

Measurements	Treat	Parity	Rep	Treat x Parity	Treat x Rep	Parity x Rep	Treat x Parity x Rep
Wk 5 gestation	0.363	**0.001**	**0.007**	0.990	**0.015**	**0.024**	0.075
Wk 10 gestation	0.441	**0.001**	0.093	0.623	0.074	**0.010**	0.468

b. Means (± standard error)

Measurements	Rep	Parity	Control		HE	
Wk 5 gestation	1	P1&2	915	±1206	2394	±1128
		P3+	4767	±1842	9583	±1206
	2	P1&2	1105	±1303	1278	±1128
		P3+	4015	±1128	895	±1206
Wk 10 gestation	1	P1&2	810	±839	935	±1061
		P3+	6333	±1187	4648	±1370
	2	P1&2	730	±791	2481	±1061
		P3+	1163	±1061	3266	±839

Reproductive performance

There were no treatment effects on any of the reproductive variables (Table 10). There were significant effects of replicate on farrowing rate (P=0.006), litter size (born alive, P=0.003), and mummified piglets per litter (P=0.032), with a higher farrowing rate, larger litter sizes and more mummified piglets per litter in Replicate 1. There was also a

significant treatment x parity x replicate interaction on litter size (born alive, P=0.031), but again this interaction may be significant (P<0.05) by chance. There was also a significant effect of parity on stillborn piglets per litter (P=0.030), with more stillborn piglets in older sows.

Table 10. Means (± standard errors) for the main effects of treatment (HE and Control), parity (P1&2 and P3+) and replicate (1 and 2) on measurements of reproductive performance (n=24 groups). Farrowing rate presented as proportion of sows farrowed that were inseminated.

a. P values for main effects and interactions

Measurements	Treat	Parity	Rep	Treat x Parity	Treat x Rep	Parity x Rep	Treat x Parity x Rep
Farrowing rate	0.645	0.877	**0.006**	0.758	0.877	0.645	1.000
Average number of piglets born alive	0.719	0.581	**0.003**	0.545	0.872	0.978	**0.031**
Average number of stillborn piglets	0.557	**0.031**	0.216	0.455	0.422	0.916	0.613
Average number of mummified piglets	0.707	0.389	**0.032**	0.265	0.497	0.724	0.265
Average number of piglets weaned[1]	0.155	0.318	0.913	0.254	0.697	0.524	0.208

b. Means (± standard error)

Measurements	Rep	Parity	Control		HE	
Farrowing rate	1	P1&2	0.911	±0.100	0.911	±0.100
		P3+	0.911	±0.100	0.956	±0.100
	2	P1&2	0.711	±0.100	0.733	±0.100
		P3+	0.644	±0.100	0.731	±0.100
Average number of piglets born alive	1	P1&2	13.20	±0.60	12.55	±0.60
		P3+	12.21	±0.60	13.04	±0.60
	2	P1&2	10.61	±0.60	12.11	±0.60
		P3+	11.65	±0.60	10.61	±0.60
Average number of stillborn piglets	1	P1&2	1.18	±0.25	1.09	±0.25
		P3+	1.53	±0.25	1.53	±0.25
	2	P1&2	0.87	±0.25	0.90	±0.25
		P3+	1.08	±0.25	1.56	±0.25
Average number of mummified piglets	1	P1&2	0.12	±0.07	0.29	±0.07
		P3+	0.26	±0.07	0.20	±0.07
	2	P1&2	0.08	±0.07	0.06	±0.07
		P3+	0.14	±0.07	0.12	±0.07
Average number of piglets weaned[1]	1	P1&2	9.79	±1.06	10.09	±1.06
		P3+	10.24	±1.06	7.11	±1.06
	2	P1&2	9.76	±1.06	9.00	±1.06
		P3+	9.53	±1.06	8.66	±1.06

[1]including fostered piglets

3.2 Discussion

Lack of stimulation in the pig's environment may lead to boredom, stereotypies and stress, but the welfare implications of indoor and non-bedded systems common in current Australian pig production systems are poorly understood. The utilisation of enrichment objects has been studied, but the effects of enrichment on stress adaptability and indicators denoting poor welfare have not been extensively studied in pigs (Van de Weerd and Day 2009), particularly sows (Marchant-Forde, 2009; Hemsworth, 2018). While not studied extensively, there is evidence that regular positive human contact may ameliorate some of the stress associated with captivity. Brief daily positive human contact has been shown to reduce the magnitude of the physiological stress response to confinement housing (tether housing) of sows (e.g., Pedersen et al., 1998). There is evidence in other domesticated species that animals that experience positive emotional experiences in the presence of humans may have reduced stress responses in stressful situations. For example, social isolation stress is reduced in dogs and sheep that show a strong affinity to humans (see review by Hemsworth et al., 2018). Previous positive handling has also been shown to reduce the heart rate and salivary cortisol concentrations in lambs following tail docking (Tosi and Hemsworth, 2002), and reduce restlessness and heart rate in cows undergoing rectal palpation (Waiblinger et al., 2004). This preliminary experiment examined whether or not regular positive human contact is enriching for group-housed sows and thus, facilitates stress resilience including reduced emotionality.

There were two treatments imposed in this experiment. The Human enrichment treatment consisted of regular (2 min daily in the sows' pen) positive human contact, while the Control sows had the same housing and routine husbandry as in the Human enrichment treatment but without 2 min of daily positive human contact in their group pens. All sows were housed indoors and in non-bedded pens with floor feeding. Stress resilience in the sows was measured in their gestation pens (*in situ*) and also when they were challenged with common husbandry practices of pregnancy testing and vaccination, as well as introduction to farrowing crates (acute effects).

The results of this preliminary experiment provide no evidence that the human enrichment treatment affected stress resilience based on baseline (*in situ*) serum cortisol concentrations at 5 and 10 wk of gestation, baseline (*in situ*) salivary cortisol concentrations at 29 d of housing during lactation in farrowing crates, salivary cortisol responses (acute) to the human approach test (HAT) at wk 9 of gestation and salivary cortisol responses (acute) to introduction to farrowing crates following gestation.

The results of this experiment provide no evidence that the human enrichment treatment affected stress resilience based on emotionality as measured by *in situ* aggression around feeding in wk 1, 4 and 8 of gestation. There were also no treatment effects on the behavioural response to the novel arena in the HAT (fear of novelty) at wk 9 of gestation, the behavioural response to humans in the HAT (fear of humans) at wk 9, behavioural response of sows to entry to farrowing crates or the behavioural responsiveness of sows to an audio recording of an unfamiliar piglet squealing (maternal responsiveness test) during wk 2 of lactation. However, there was a treatment x replicate interaction on the behavioural responses of

lactating sows to the maternal responsiveness test, with more Control sows vocalizing, particularly in Replicate 1 than Replicate 2.

Agonistic behaviour of sows in conventional-sized groups functions to establish a stable hierarchy in the group. Furthermore, a number of factors will affect aggression such as floor space, competitive feeding, time of mixing relative to the stage of reproduction, and static versus dynamic groups. However, to the best of the authors' knowledge, there is no evidence in the scientific literature that environmental enrichment reduces aggression in sows. The human enrichment treatment commenced post-mixing and since aggression is most intense early after mixing, the protocol of this experiment meant that the effects of human enrichment on aggression when it is most intense (Verdon et al., 2013) is unlikely to have been tested.

Housing pre-parturient sows in farrowing crates without bedding/nesting material reduces their level of maternal behaviour, in particular pre-farrowing nest-building behaviour, compared with sows in more enriched environments (see Barnett et al., 2001). Housing sows in farrowing crates which limits sows of the opportunity to freely interact with their piglets, also appears to reduce their maternal responsiveness to piglets (Cronin et al., 1996; Thodberg et al., 2002; Singh et al., 2017). While there were no effects of treatment on the behavioural responses of sows to an audio recording of unfamiliar piglet screams in the present experiment, there was a treatment x replicate interaction, with more Control sows vocalizing, particularly in Replicate 1 than Replicate 2. It is unclear why the human enrichment sows were vocally less responsive in this test, but clearly this requires further study.

The human approach test (HAT), which measures the approach behaviour of pigs individually held in a test arena to a stationary experimenter, has been used extensively to study fear of humans in pigs (see Hemsworth and Coleman, 2011). The validity of this test to assess an animal's fear of humans has been shown by the findings of behavioural and physiological correlates in the HAT (Hemsworth et al., 1987; Hemsworth and Barnett, 1987), together with findings that imposition of handling treatments designed to differentially affect an animal's fear of humans generally produced the expected variations in the behavioural responses of the pigs to humans in this test (see review by Hemsworth and Coleman, 2011). One of the difficulties in conducting this experiment was eliminating human contact in the Control treatment other than that associated with routine husbandry. The pens containing the human enrichment sows were randomly allocated to one area of the gestation shed in which cameras were located. The imposition of the human enrichment treatment in the sows' pens therefore unavoidably involved Control sows receiving additional daily visual contact that clearly exceeded that normally associated with routine husbandry. However, the behavioural responses of sows to a human actively approaching and imposing threatening tasks for sows, as indicated by the sows' avoidance responses, suggests that an active and more threatening encounter with humans may provide a better indication of the animal's perception of routine husbandry practices involving humans, that is their fear responses to the husbandry practices.

While the results of this experiment provide no evidence that the human enrichment treatment affected stress resilience based on stress physiology, the results provide limited evidence that the opportunity for regular positive contact with humans affects emotionality

of sows in terms of their fear responses to the husbandry practices of pregnancy testing and vaccination. Sows in the human enrichment treatment showed less withdrawal to the technician approaching to conduct pregnancy testing in the group pen and older sows in the human enrichment treatment generally withdrew less from the technician after the actual pregnancy test with the ultrasound probe in Replicate 2 of the experiment. While group size was reduced with the removal of sows that failed to conceive or retain pregnancy, a group size of 15 with a space allowance of 2.0 m^2/sow is unlikely to restrict withdrawal responses to the technician, particularly in sows that are highly fearful. Furthermore, less sows in the human enrichment treatment withdrew from an approaching technician during vaccination in the group pen. While it is difficult to standardize the timing of blood sampling of individual sows following the imposition of these husbandry practices in groups of 15, measuring the acute physiological stress response to these stressors would be valuable in appreciating the effects of regular human enrichment on stress resilience to routine husbandry practices. Nevertheless, sows in the human enrichment treatment showed less avoidance of the technician during routine pregnancy testing and vaccinations indicating reduced fear of these procedures, that is reduced emotionality.

Another important finding was that there was a significant treatment x replicate effect on serum brain-derived neurotrophic factor (BDNF) concentrations at 5 weeks of gestation: the human enrichment sows in Replicate 1 had higher BDNF concentrations at 5 weeks of gestation. Environmental enrichment has been shown to increases BDNF, resulting in higher stress resilience. Environment enrichment in the form of physical exercise, early social environment and objects simulating hiding or exercise has been shown to increase brain and blood BDNF concentrations (Branchi et al., 2006; Zhu et al.,2006; Rasmussen et al.,2009). Recently, Rault et al. (2018) found that pigs provided with a foraging enrichment during lactation had higher serum BDNF concentration at 8 weeks than pigs housed in barren pens before weaning, and that pigs provided with enrichment after weaning tended to have higher serum BDNF concentration than pigs housed in barren pens after weaning. The environmental enrichment used in the study by Rault et al. (2018) was a foraging block and the nutritional content of the block may have confounded the enrichment properties of the foraging block. However, the results of the present study and those of Rault et al. (2018) indicate that environmental features that are of functional relevance to the animal and act with a foreseeable rewarding outcome may provide pigs with functional enrichment and increased stress resilience and emotionality. It is of interest that there is evidence of seasonal effects on BDNF. In a study of human patients from mental health care, primary care and in the general population humans, BDNF concentrations were found to increase in the spring-summer period and decrease in the autumn-winter period (Molendijk et al., 2012).

There were no treatment effects on any of the reproductive variables. However, there were significant effects of replicate on farrowing rate, litter size (born alive), and mummified piglets, with higher farrowing rates, litter sizes and mummified piglets in Replicate 1. Sows in Replicate 2 were inseminated in March and thus this reduction seen in reproductive performance in Replicate 2 may be due to seasonal infertility. There was also a significant effect of parity on stillborn piglets, with more stillborn piglets in older sows. There were no treatment effects on baseline serum cortisol concentrations at 5 and 10 weeks of gestation, and thus stress-related effects of treatment during this stage of gestation on reproduction are unlikely to affect reproductive performance. The human enrichment treatment

commenced post-mixing and, as indicated earlier, this treatment is therefore unlikely to affect aggression and stress over the first few days post-mixing when aggression and stress have been shown to affect farrowing rate (Hemsworth et al., 2013).

As indicated previously, one of the difficulties in conducting this experiment was eliminating human contact in the Control treatment other than that associated with routine husbandry. Thus, in light of the results, examination of the influence of regular positive human contact in comparison to routine human contact associated with normal husbandry practices on stress resilience is recommended. Furthermore, the fact that the opportunity for regular positive contact with humans affected emotionality of sows to the short-term fear responses associated with the routine husbandry practices of pregnancy testing and vaccination indicates the need for more extensive examination of the effects of human enrichment on stress resilience of the breeding sow. This need for further research is strengthened by the finding in this preliminary experiment that the human enrichment sows in Replicate 1 had higher serum brain-derived neurotrophic factor (BDNF) concentrations, a trophic agent associated with greater stress resilience.

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3. Application of Research

With concerns about intensification of pig production, the extensive use of fully or partially-slatted, non-bedded and non-enriched environments for young and breeding pigs, and the extent to which this provides functional enrichment, the present research is highly relevant.

Animal stress has substantial implications on animal productivity, health and welfare of farm animals and thus farm profitability. Furthermore, public concerns and policy debates about animal production often involve the conditions that guarantee animal welfare. Animal welfare is a high priority to the Australian pork industry and the community, as well as Australian governments. It is also one of international significance. Many argue that there are conflicts between animal welfare and efficient farming. However, there is increasing evidence that improved animal welfare can increase profits through reduced morbidity and mortality (through reduced stress and improved disease resistance), improved product quality, lower risk of zoonoses and foodborne diseases and the ability to command higher prices from consumers (Moberg, 2000: Spoolder et al., 2009: Dawkins, 2016).

While there were no treatment effects on baseline stress physiology in the gestation pens or late in lactation or acute stress physiology in response to testing in the human approach test and introduction to farrowing crates, the sows in the human enrichment treatment showed less avoidance of the technician during routine pregnancy testing and vaccinations indicating reduced fear of these procedures, that is reduced emotionality. Furthermore, the human enrichment sows in Replicate 1 had higher serum brain-derived neurotrophic factor (BDNF) concentrations at 5 weeks of gestation. Further research is required to understand factors affecting BDNF and the usefulness of BDNF as measure of effective enrichment reflecting neural changes.

Therefore, the findings from this proof of concept project indicate that a better understanding of the effects of regular positive contact with humans may provide an enrichment strategy that is functionally relevant to pigs and acts with a foreseeable rewarding outcome. In other words, an enrichment strategy that leads to stress resilience and thus improved biological functioning in pigs.

However, before a human enrichment strategy can be proposed to industry, it is recommended that further, more extensive research is conducted on the effects of regular positive human contact on stress resilience in pigs. Indeed, the profound effects of early experience, including those experiential effects of humans, highlight the need for long-term research examining life time effects of positive human contact on stress resilience and thus welfare and productivity in pigs.

4. Conclusion

These results indicate that the human enrichment treatment, involving regular positive contact with humans in addition to human contact associated with routine husbandry practices, did not affect stress resilience based on both baseline cortisol concentrations during gestation and the acute cortisol response in the human approach test in gestation and introduction to farrowing crates following gestation. However, the results provide limited evidence that the opportunity for regular positive contact with humans diminishes emotionality of sows in terms of fear responses to the routine husbandry practices of pregnancy testing and vaccination. Furthermore, some of the human enrichment sows had higher serum brain-derived neurotrophic factor (BDNF) concentrations, a trophic agent associated with greater stress resilience.

Therefore, the findings from this proof of concept project indicate that a better understanding of the effects of regular positive contact with humans may provide an enrichment strategy that is functionally relevant to pigs and acts with a foreseeable rewarding outcome. In other words, an enrichment strategy that leads to stress resilience and thus improved biological functioning in pigs.

5. Limitations/Risks

- A limitation in conducting this experiment was eliminating human contact in the Control treatment other than that associated with routine husbandry as would occur in typical commercial group housing settings. The pens containing the human enrichment sows were randomly allocated to one area of the gestation shed in which cameras were located. While group pens had solid sides to limit visual contact between adjacent pens, the gates on the front of the pens were not solid, allowing visual contact with people and pigs in the corridor and in the opposite pens. The imposition of the human enrichment therefore unavoidably involved Control sows receiving additional daily visual contact that clearly exceeded that normally associated with routine husbandry. Thus, the additional human contact that the Control sows received most likely meant that this experiment did not fully test the effects of additional positive human contact as compared to human contact associated routine husbandry in typical commercial group housing settings.

- The results indicate that the opportunity for regular positive contact with humans affects stress resilience in terms of sows' fear responses to the husbandry practices of pregnancy testing and vaccination. The preliminary nature of this experiment and the fact that it was conducted under commercial conditions precluded the opportunity to collect blood samples from individual sows in groups at a predetermined time after the actual imposition of the specific husbandry practice on the animal. Further research perhaps in smaller groups or even individually housed, in which individual sows are blood sampled at predetermined times after the actual imposition of the specific husbandry practice would provide valuable data on the effects of human enrichment on the physiological stress response of sows to husbandry practices.
- The human enrichment treatment commenced post-mixing and since aggression is most intense early after mixing, the protocol of this experiment meant that the effects of human enrichment on aggression when it is most intense is unlikely to have been tested. Furthermore, in addition to the effects of previous human enrichment on not only aggression at mixing post-insemination but also on other stressors, such as transport of growing-finishing pigs, weaning of sows, and oestrus detection and insemination, would be valuable in appreciating the effects of human enrichment on the physiological stress response of sows to a wide range of husbandry practices practiced in the pork industry.

6. Recommendations

Based on the findings from this preliminary experiment, together with limited data in the literature on pigs and other species, it is recommended that further, more extensive research is conducted on the effects of regular positive human contact on stress resilience in pigs. Indeed, the profound effects of early experience, including those experiential effects of humans, highlight the need for long-term research examining life time effects of positive human contact on pigs on stress resilience and welfare and productivity.

The importance of this topic is reflected in concerns about intensification of pig production, particularly the extensive use of fully or partially slatted, non-bedded and non-enriched environments for young and breeding pigs and the extent to which this provides functional enrichment. The European Union Commission directive 2001/93/EC states that 'pigs must have permanent access to a sufficient quantity of material to enable proper investigation and manipulation activities, such as straw, hay, wood, sawdust, mushroom compost or a mixture of such that does not compromise the health of animals'. However, the extent to which this provides effective enrichment has been questioned by many authors.

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