Optimising the management of group-housed gestating sows

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By

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Executive Summary

In order for aggression not to mitigate the positive welfare aspects of group housing, it should be carefully managed. Fighting is highest when sows are first mixed and subsides relatively quickly once a social hierarchy has been established. Altering the way in which we manage sows during this initial period, or the use of a dedicated mixing pen, may prove a useful compromise to producers who strive to limit aggression in sows but are constrained by available resources. There may also be an opportunity to exploit the current interest in alternate accommodations that induce ovulation in lactation to alter the timing of mixing in order to reduce aggression, stress and so improve reproduction. The experiments conducted in this project investigated potential manipulations, as well as the timing of grouping, in order to reduce sow aggression at mixing.

The most logical resource to allocate sows in the mixing pen was space. We hypothesised that high space allowances for the discrete period around hierarchy formation would act to reduce aggression, injuries and stress when sows were mixed into groups, and that when space was standardised after hierarchy formation, no increase in aggression would be observed. In this first experiment, sows were mixed into groups of six and allowed $2m^2$/sow (LOW n = 48 sows), $4m^2$/sow (MEDIUM, n = 42 sows) or $6m^2$/sow (HIGH, n = 42 sows) for four days after mixing at which point all pens were equalised to $2m^2$/sow. In the primary analyses where measures where considered at the pen level, there were no effects of space allowance on fighting behaviours. These measures were higher on d0 ($P < 0.05$), and no increase in aggression was observed on d4 when pen sizes were standardized. Secondary analyses conducted to examine individual sow behavior within each pen identified increased injuries in the lowest ranked sows in LOW pens ($P < 0.05$). The provision of high space allowances did not reduce aggression overall, but given that aggression subsided very quickly, and that there was no increase in aggression when pens were standardized, we recommend that increased space in a mixing pen can be provided on a short term basis to improve the welfare of low ranked sows.

The second experiment determined the effect of novel materials in a mixing pen on aggression and stress in gestating sows. One hundred and forty four sows were mixed in groups of twelve into either a standard pen (concrete, partially slatted; n = 72 sows) or a novel pen (concrete, partially slatted and containing two rubber mats, eight strands of sisal rope and two plastic discs, suspended from the roof; n = 72 sows). Play behaviours were observed in the novel pen, and were higher on d4, d7 and d20, compared to d0 and d1 ($P < 0.05$). No play was observed in standard housing. Aggression measures, salivary free cortisol concentrations and injury counts were unaffected by treatment. Although the enrichment materials did not appear to be an effective way of decreasing aggression at mixing, they maintained the interest of sows for the experimental period, and also appeared to improve the affect (or emotional state) of the sows as play behaviour was induced.

Synthetic olfactory agonists mimic odours secreted by mammary glands and their application can reduce anxiety levels. The third experiment determined the effect of a commercially available olfactory agonist on grouped sows. Sows were divided between two treatments: 24 control sows (CON), and 24 that received the synthetic olfactory agonist by being housed with diffusing blocks (SOA). On d0, the average number of aggressive events was reduced in the SOA (10.6 events) when compared with CON treatment (5.9 events; $P < 0.05$). These results were mirrored on almost all other measurement days ($P < 0.05$). When averaged across the experimental period, sows from the SOA treatment ate for longer (69.6 min) and more often (13.2 events) when compared with CON sows (54.7 min and 10.4 times respectively; $P < 0.05$), but there was no impact on weight gain. No treatment effects were observed on injury counts, salivary cortisol concentrations, or conception rate. These findings support the hypothesis that the provision of a synthetic olfactory agonist can reduce aggression in adult sows at mixing.

The previously reported reduction in aggression and stress in young pigs, and increased reproduction in adult sows when dietary magnesium concentrations were elevated led us to hypothesise that this dietary manipulation would reduce aggression, injury and stress and so increase reproductive output in multiparous sows. The following three treatments were applied; 180 sows were fed a standard gestation diet (CON), 180 sows were fed the gestation diet mixed with 5kg/tonne of a commercially available marine algae extract (SUPP), and 180 sows were fed the gestation diet mixed with 2.84kg/tonne magnesium sulphate (MGSO4). Diets were fed to the sows at an allowance of 2.4kg and so sows from the SUPP and MGSO4 treatments received 1.4g elemental magnesium per day. There was little impact of the treatments on aggression, injury and reproduction. This experiment did identify a consistent seasonal effect on the level of scratches and injuries, with those mated during the warmer months displaying a higher score in early gestation, and those mated in cooler months recording increased scores in late gestation. Whilst magnesium does not appear to be an effective dietary manipulation to reduce aggression, further investigation into these seasonal effects on aggression are warranted.

Commercial use of group housing for lactating sows is limited but increased interest in reduced confinement housing and stimulation of ovulation during lactation has encouraged investigation of group lactation housing as a possible alternative mixing time. This study was designed to identify the optimum time to mix sows, with the aim of minimising aggression and stress during the formation of hierarchies, and reducing the effects of mixing on stress-sensitive reproductive milestones. One hundred and twenty multiparous sows were mixed into groups

of six after being allocated to one of four treatments: group-housed sows and litters from d21 of lactation (multi-suckle or MS), sows separated from litters in a group pen for 7h daily from d21 of lactation (SEP), sows mixed after weaning at d28 of lactation (WEAN) or mixed after insemination (MAI). On the day of mixing (M0) more fights per hour were observed in the SEP group (back-transformed mean; 0.5 fights/hr) when compared to all other treatments (average of other treatments; $P < 0.05$). The MS group recorded no fights on M0. The MS treatment increased the total number of piglets born at the subsequent farrowing ($P < 0.05$). Given these last two findings we can conclude that the benefits of multi-suckle housing are two-fold; an effective way to decrease aggression at the point of mixing as well as to increase reproductive output.

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1. Introduction

Two major changes are underway that will alter our future management of the breeding herd. These are the compulsory grouping of sows for the duration of gestation, and the adoption of lactational oestrus induction systems, these being likely to include elements of sow grouping prior to weaning

The change from housing pregnant sows in individual stalls to a group-housing system has several consequences that need to be addressed. Any group housing system must both satisfy the behavioural/welfare needs of the sow & the production needs of the pork producer. To this end the design & management of group housing systems requires that pregnant sows:

1. Are mixed into groups at the optimum time & in the optimum way to minimise aggression & thus minimise stress & it's negative consequences for farrowing rate & litter size

2. Are provided with adequate space, taking into account the group size, the parity mix of the group, season & whether or not individual feeding is to occur

The success or otherwise of mixing & housing strategies should be assessed using production parameters (e.g. farrowing rate & litter size) and behavioural, immune function & physiological measures (KPIs). Furthermore, it may be necessary to consider the consequences of dynamic v. static groups as many commercial enterprises are likely to attempt to maximise resource use by adding sows to groups as others are removed as a result of reproductive or other failure.

<u>Methods to ameliorate aggression at mixing</u>

Pen design & environmental enrichment

There is emerging evidence that aggression is inevitably associated with physiological & psychological stress (Spoolder *et al.* 2009) & this can lead to reductions in farrowing rate & litter size. One way to ameliorate this stress is to manage the mixing carefully, preferably using a specific mixing pen. We know that dominance-related fighting usually lasts 1-2 days (Arey and Edwards 1998) & thus that a mixing pen is only needed for 2-3d/group (Spoolder *et al.* 2009). We suggest that the key elements of a mixing pen may be:

a) Size - a large mixing pen allows adequate flight distances (Kay *et al.* 1999)
b) Shape -Docking *et al.* (2000) concluded that circular mixing pens with 9.3m^2/sow are ideal but rectangular pens with low stocking densities can also work
c) Internal fittings – provision of visual barriers & hide areas (Spoolder *et al.* 2009)
d) Enrichments - dominant sows may be distracted by 'enrichments' in a mixing pen on the first day & may thus be less aggressive (Elmore *et al.* 2011)

Summarising our current knowledge on mixing pens Spoolder *et al.* (2009) concluded that the key factors for mixing are likely to be gradual familiarisation of unfamiliar animals, plenty of space at initial mixing, minimising opportunities for dominant sows to steal food from subordinates, good flooring & bedding. However, much of our apparent knowledge of how to best mix unfamiliar animals is still extrapolated from studies on growing pigs & may thus not be appropriate for the breeding sow.

Use of a synthetic olfactory agonist (SOA)
Maternal secretions from nipples and milk have been shown to meet all the criteria for definition as a pheromone (Schaal *et al.* 2003), acting to attract and calm the offspring, as well as elicit teat seeking behaviour (Schaal 2010). Synthetic olfactory agonists commercially known as 'appeasing pheromones' mimic these maternally derived fatty acid secretions and have been shown to bind to both the nasal and vomero-nasal mucosae (Guiraudie *et al.* 2003). It has been known for some time that SOA usage reduces aggression in weaners at grouping (McGlone and Anderson 2002) & more recently it has been shown that SOA use alleviates social stress in adult pigs (Yonezawa *et al*. 2009). Thus, use of SOA at the time of mixing sows into gestation groups is ripe for investigation.

Including magnesium in the gestation diet
Circulating magnesium (Mg) concentrations and stress reactivity appear to be strongly linked. One published review of the literature examining magnesium deficiency and stress in humans reported that catecholamine and corticosteroid release *in vitro* and *in vivo* are increased in the presence of low Mg levels, high stress results in free fatty acid mobilisation which binds and inactivates Mg further reducing circulating Mg levels, and thus the author concluded that Mg supplementation may be of benefit to protect against stressful situations (Seelig 1994). There is increasing evidence that the provision of Mg may reduce indicators of stress in pigs. Under commonly experienced stressful situations such as transport, lairage and slaughter, grower and finisher pigs have been shown to exhibit reduced physiological (catecholamine and metabolite) and behavioural (activity levels and loin damage indicating aggression) responses under Mg supplementation regimes (Peeters *et al*. 2005; Peeters *et al*. 2006). This reduced stress response also results in some improvements in meat quality markers (D'Souza *et al*. 1998). In females specifically, a magnesium-rich marine product (AcidBuf) has been shown to reduce cortisol concentrations and injuries grower pigs (O'Driscoll *et al*. 2013). Despite this evidence, the effects of dietary supplementation of Mg in gestating sow diets remain to be elucidated.

<u>Timing of mixing</u>

It is well known that stressors will adversely affect early pregnancy, particularly if they lead to chronic elevation of cortisol. This can happen if sows are exposed to unfavourable social factors (aggressive sows), management (rough handling, low feed intake) &/or adverse climatic conditions (Soede *et al*. 2007; Spoolder *et al*. 2009). Current studies indicate that if sows must be mixed post-breeding the times to do this are either in the first 2 days after breeding or after day 21 post-breeding (Kirkwood and Zanella 2005; Geudeke 2008; Van Wettere *et al*. 2008; Spoolder *et al*. 2009).

Thus, for conventionally weaned sows – i.e. those not bred during lactation & weaned at 3-4 weeks post-partum - it would appear to be ideal to mix them into stable social groups at weaning so that stress is minimised during the sensitive period of early gestation. The disadvantage of this system is that oestrus detection & AI are not easily facilitated in large groups & thus the system can only be commercially adopted where gestating sows are to be housed in small groups.

Where lactational oestrus induction is adopted the system becomes more complex. Current belief is that this can be successfully achieved through daily boar contact starting at days 14-18 post-partum, preferably when the lactating sows are group-housed (either in follow-on, multisuckling accommodation or via the use of 'step-in-step-out' farrowing pens with a rear communal area for the sows). However, since most sows appear to attain oestrus 4-6 days after the start of boar contact, they are likely to be 1-2 weeks into gestation at the time of weaning. This would not be a problem if the stable social group established pre-weaning was to be maintained through early gestation. The problem would arise where weaned sows were transferred directly into large groups (40-200 sows) for the duration of gestation.

The studies described below address the issues of mixing by considering:

a. The development of specific mixing pens & protocols to facilitate establishment of the gestation group with minimal stress – to include studies on the value of the provision of high levels of space, environmental enrichment, synthetic agonists that behave like appeasing pheromones and dietary manipulations, to reduce aggression in gestating sows at the time of mixing

b. The impact of the timing of mixing on the above KPIs (N.B. this may be particularly important in systems where lactational oestrus is being stimulated or, conversely, may be ameliorated by a lactation housing system which encourages sow mixing)

2. Methodology

Experiment 1: The use of increased space in a mixing pen
The study used 132 multiparous (parity 1-7, 3.0 ± 1.3) Large White x Landrace sows that were weaned into stalls prior to mixing. The sows were exposed daily to boars until exhibition of oestrus, and then received three inseminations 24 hours apart. Following insemination, sows were mixed in groups of six into treatment pens which provided either $2m^2$/sow (LOW, 8 groups, 48 sows), $4m^2$/sow (MED, 7 groups, 42 sows) or $6m^2$/sow (HIGH, 7 groups, 42 sows) for days (d) 0, 1, 2 and 3 after mixing. On d4, the space allowance for all sows was standardised to $2m^2$/sow. With the exception of these varying space allowances, all sows were treated similarly (fed 2.4kg gestation sow diet daily in a single event, housed on partially slatted, concrete flooring, had access to two nipple drinkers). Video footage 6h in length was collected from 0700h until 1300h and included the feeding event (which occurred at 0730h). An ethogram describing the behaviours scored from this footage can be found in Table 1.

Table 1. Description of the behaviours scored for each of the six sows housed within a mixing pen during the 6h video recording period, and the type (continuous or point) of each of these behaviours.

Active, resting, eating and drinking	Sows were classed as eating if food was present and she was noted as collecting food from the floor, chewing and/or swallowing. The sow was drinking if her head was in the drinker and she could be seen to swallow and/ or actively manipulate the drinker nipple. If it was unclear what the sow was doing and if she was dog sitting, standing or walking she was considered active. Sows were considered resting if lying flat to the floor.	Continuous events
Exploring	Actively manipulating and exploring the surrounding environment, such as rooting, nosing the floor, moving drinkers and chewing fences	Continuous event
Displacement	Movement of one sow by another, from a valued resource such as food, drinker or lying space (if multiple knocks or bites are required, this is a fight)	Point event
Fighting	Aggression including three or more knocks or bites. Aggression can be reciprocal or non-reciprocal	Continuous event
Knock	One sow knocks another sow using her head and neck, contacting any part of the receiving sow	Point event
Bite	One single bite delivered from one sow to any part of another	Point event
Lunge	Sow lunges at another but does not make physical contact	Point event
Flee	Sow moves herself quickly and as far away as the can get from another sow, in response to an aggressive action	Point event
Mounting	One sow mounts another, with her front legs both over the back of the other animal	Point event
Non-aggressive sow-sow contact	Mutual contact between two sows which involves exploration of another animal with no aggressive outcomes (does not include lying with another sow)	Point event

Hierarchy was assessed using the number of successful displacements for each sow over all four days and the number of fights won and lost on the day of mixing. Displacements and fights were calculated as an overall or ‘global’ rank and not based on individual resource rank, such as displacements around food, water or space. For Fights (F) and displacements (D), the sows were separated into three groups and hierarchy was analyzed with both displacements and fights combined (i.e. 1D1F), allowing a possible nine hierarchy groups. For ease of discussion, our calculation of aggression and dominance will be referred to as hierarchy or rank. The ranking subgroups groups were as follows: 1D or 1F sows were involved in no fights or displacements, 2D or 2F sows lost more than they won and 3D or and 3F sows won more than they lost.

Skin lesion counts were recorded at 1400h on the day before mixing while they were still in the stalls (d-1), the day of mixing (d0) and d1, d3 and d4 post-mixing. A modification of the assessment described by Karlen *et al.* (2007) was used to describe lesion counts. Saliva samples were also collected on these days using cotton plugs (salivettes, Sarstedt Australia, SA, Australia) attached to plastic ties. Sampling began at 1330h on each sample day and concluded approximately one hour later. Samples were then centrifuged at 2012*g* for 10 min at room temperature and stored at -20ºC until analysis. The samples were sent to the School of Animal Biotechnology, University of Western Australia for analysis of salivary cortisol using a radioimmunoassay kit (Cortisol GammaCoat RIA kit CA-1549, Dia Sorin Inc., Stillwater MN, USA) (Beausoleil *et al.,* 2008). Limit of detection was 0.9nmol/L and the mean intra and inter-assay coefficients of variation were 2.7% and 5.1%, respectively. Pregnancy rate (measured by ultrasonography at approximately d28 of gestation), farrowing rate (measured by the number of sows that farrowed at full term) and total litter size were recorded.

Before analysis, data were checked for normality by examining the distribution of residual plots. This resulted in cortisol concentration data being square root transformed, and the majority of the behavioural measures being $\log_{10}$ transformed. When data is provided the transformation is specified. All data were analysed using the Statistics Package for the Social Sciences (SPSS) v20.0 (IBM, Armonk, New York, USA) using a general linear model. For the primary analysis pen was used as the unit with all measures taken as individual sow results and averaged across the pen. Replicate, day of measure and treatment were fitted as fixed effects and the d1 measure was also fitted as a covariate (with the exception of behavioural data). Linear mixed model analysis was also conducted with sow as the unit, and is referred to as the secondary analysis, in order to analyze the effect of social rank on their response to the treatment. In these cases sow was fit as a random effect, parity group as a fixed effect (parity 1, 2-3 and 4+). There was only one pen per treatment per replicate, thus adjusting for treatment and replicate also adjusted for the effects of pen. Where transformations occurred, non-transformed adjusted means are presented in parentheses. Data are expressed as least squares means ± the standard error of the mean (SEM) and a difference at $P < 0.05$ was deemed significant.

<u>Experiment 2: The use of environmental enrichment in a mixing pen</u>
This study used 144 multiparous, Large White x Landrace sows, selected at weaning and kept in stalls prior to mixing. The sows were exposed daily to boars until the exhibition of oestrus, and then received up to three inseminations 24 hours apart. Sows were mixed into groups of 12 in treatment pens which provided a space allowance of $2m^2$/sow, were concrete partially slatted in design and contained two nipple drinkers. Six pens were each allocated to one of the following two treatments; STANDARD, containing no enrichment, or NOVEL,

containing two rubber mats, eight strands of 24mm-thick, untreated sisal rope and two yellow plastic discs (Swing, Slide, Climb, Sandelford Hardware Pty Ltd), all suspended from the internal roof beams. The enrichment hung to just below pig nose level, approximately 60-100cm from the floor. A diagrammatic representation of the enriched pen can be found in Figure 1.

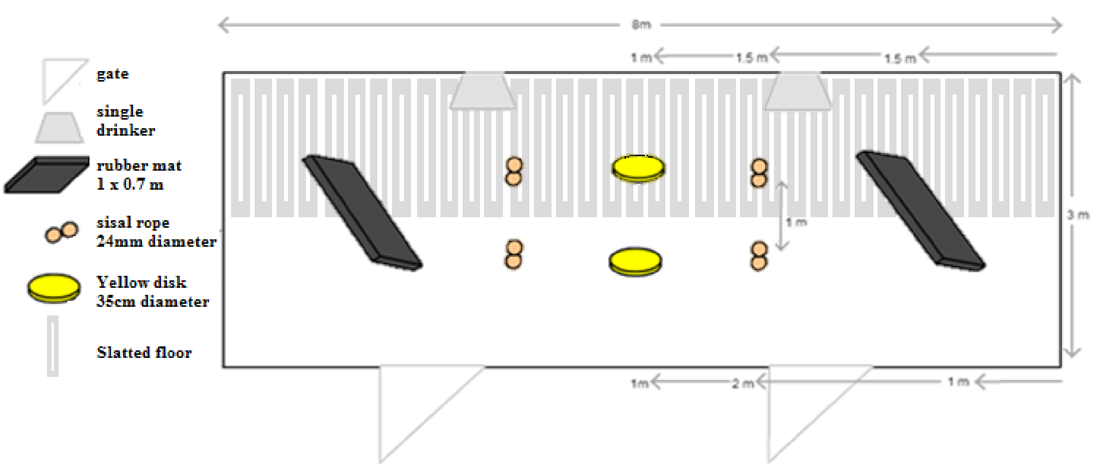

Figure 1. Placement of enrichment inside the novel treatment pen, which housed 12 gestating sows at a space allowance of 2 m^2/sow.

Salivary free cortisol concentration and total skin lesion count were measured d-1, d0, d1, d7 and d20 and behaviour on all days except d-1 in a similar manner to that outlined in Experiment 1. The ethogram outlined in Table 1 was expanded to include the additional behaviours defined in Table 2. Pregnancy rate, farrowing rate and total litter sizes, piglet weights and sex ratio of the litter were recorded. The saliva samples were sent to the School of Animal Biotechnology at The University of Western Australia for analysis of salivary cortisol using MP Biomedicals I^{125} RIA cortisol Kit (# 07-221106) (MP Biomedicals Australia, Seven Hills, NSW). Limit of detection was 0.25 µg/dL and the mean intra and inter-assay coefficients of variation were 5.8 % and 1.9 %, respectively.

Table 2. Description of the additional behaviours scored for each of the twelve sows housed within the mixing pen during the 6h video recording period, and the type (continuous or point) of each of these behaviours.

Exploration	Manipulating and exploring rope, swing or mat (and was also coded for exploration of the floor or pen work). Mainly chewing the material.	Continuous event
Play (excited play)	Excitedly and playfully interacting with an object in the pen. Including behaviours such as hopping, head tossing and pivoting whilst actively manipulating an element of the environment. Play could be coded for rope, swing, mat and play not centred on a specific object (other).	Continuous event
Guarding enrichment	Obvious defence to stop other sows from the use of enrichment, whether resulting in a fight or displacement from enrichment. This behaviour could fall under displacement or fight, but was specifically coded separately in order to allow analysis of any aggression surrounding the enrichment (sows could be coded as guarding individual enrichment items, mat, swing or rope).	Point event

Prior to analysis, data were checked for normality by examining the distribution of residual plots. This resulted in injury counts requiring square root transformation and the majority of the behaviours measured, and cortisol results, were log_{10} transformed. Where transformations occurred, non-transformed means have been presented in brackets and transformation type is specified either in tables or parentheses. Saliva samples were collected on all measurement days but only samples from d-1, d1 and d7 were analysed for cortisol levels. The data were analysed using SPSS 20.0 (IBM, USA) using a linear mixed model with pen as the unit, replicate, day of measure and treatment as fixed effects and pen by day as a repeated effect. With the exception of behavioural data, the d-1 measures were fit as covariates. Data are calculated as the average per sow in that day, and not a total within a pen. Data are expressed as least squares means ± the standard error of the mean (SEM) and a P value of < 0.05 was deemed significant. A linear mixed model analysis was also conducted with sow as the unit, and is referred to as the secondary analysis, in order to analyse the effect of social rank on their response to the treatment. In these cases, sow by day was fit as a repeated effect, parity group as a fixed effect (parity 1, 2-3 and 4+). There was only one pen per treatment per replicate, thus adjusting for treatment and replicate also adjusted for the effects of pen.

<u>Experiment 3: the use of a synthetic maternal olfactory agonist in a mixing pen</u>
Forty-eight Large White cross Landrace sows were weaned from their litters and housed in individual sow stalls. All sows were multiparous, were allocated to treatment in order to ensure an even parity distribution, mated when a standing reflex was observed during the back pressure test in the presence of a mature boar, and subsequently received three inseminations 24 hours apart. Four to five days after the first mating, sows were removed from stall housing and placed in group pens which marked the beginning of the experimental period. Sows were floor fed once daily 2.7 kg of a standard dry sow diet (13.0 MJ DE/kg) and had *ad libitum* access to water via three nipple drinkers.

This experiment was completed in two replicates, with four group pens investigated within each of the two replicates. The pens were partially slatted, with over half of the floor consisting of solid concrete, and were rectangular in shape (Figure 2). Each pen contained six sows, giving a space allowance of 2.4 m^2 per sow. Group pens were enclosed in two rooms that shared no ventilation, but were identical in design. Two control pens (CON) were located in the first room and no synthetic olfactory agonist was present in this room. The second room also housed two pens, and in this room, four diffuser blocks containing the synthetic olfactory agonist (Securepig® IRSEA, Quartier Salignan France) were installed to create the treated group (SOA). The diffusing blocks were placed inside the treatment room one day prior to the mixing event, and sows had no previous exposure to the treatment. The synthetic olfactory agonist contained lipid-soluble compounds found in skin secretions of sows using a formula described by Pageat (2001). The diffusing blocks consisted of slow-release macromolecular gelatin composed of water (> 90%), non-ionic surfactant (4%) a gelling gum (3%) and the active principle synthetic olfactory agonist (2%). The total weight of the block was 150g. The diffuser blocks were placed above the concrete flooring on which the sows were fed, and at the height of the ventilation window to ensure maximal circulation of the product (Figure 2). The treatments were reversed between the two replicates so that in the second replicate, the first room contained the synthetic olfactory agonist and the second room acted as the control. The two replicates were conducted four months apart after washing to ensure the rooms were free of any product residue.

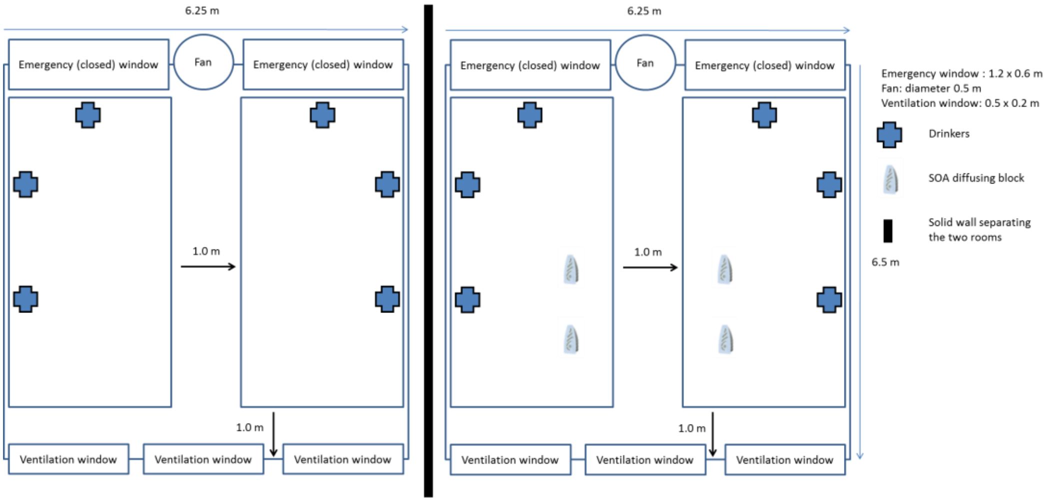

Figure 2. Diagrammatic plan of the two rooms utilised each containing a pair of group pens. The plan details the size of the rooms and pens, and the position of windows and ventilation, drinker lines and diffuser blocks containing the synthetic olfactory agonist (SOA).

On day 0, the sows were mixed into their respective treatment pens. At 1400h on day 7, the diffuser blocks were removed from the room containing the SOA treatment pens and emergency windows were opened overnight to remove agonist residue. The experimental period concluded on day 14.

Each of the four pens were monitored for behaviour of the sows using a digital video recorder (Legria HFR26, Canon, Sydney Australia). On recording days (d0, 1, 3, 7, 8 and 14), the four cameras were turned on at 0700h before floor feeding commenced (sows fed at 0730h). Pens were recorded for 6h, with cameras turned off at 1300h. Behavioural analysis of the recordings occurred in Observer XT v10.0 (Noldus Information Technology, Wageningen The Netherlands) and sows were scored for the behaviours outlined in Table 3. Behaviours were scored as mutually exclusive continuous variables, thus each sow had to be performing one of the listed behaviours at any given time. When a sow was observed to be performing the behaviour termed ‘aggression’, the occurrence of specific point variables (see Table 3 for descriptions) was also recorded. Successive bouts of the same behaviour were scored as two separate events when the interval was greater than 3 sec for continuous behavioural states (i.e. aggression), and this interval was reduced to 1 sec for point events (i.e. bite).

Table 3. Ethogram of continuous and point variable behaviours recorded for sows during the experimental period.

	Description of behaviours
Continuous behaviours scored	
Feeding	Sow forages ground with mouth and nose
Drinking	Sow places mouth or nose over nipple drinker
Walking	Sow walks around pen without performing another behaviour
Resting	Sow is standing stationary, sitting or lying
Eliminating	Sow passes urine or faeces

Aggression	Sow is involved in an aggressive interaction with another sow
Point event scored within aggression behaviour	
Bite	Sow bites another sow
Knock delivered	Sow knocks the head or body of another with closed snout
Displacement	Sow displaces another sow

At between 1300h and 1400h on d0, 1, 3, 7, 8 and 14, saliva was collected from each of the sows using Salivette ® swabs (Sarstedt, Adelaide Australia). Any sample not collected within two minutes was disregarded. The sampling order within each pen and time were recorded during collection. The samples were spun in a centrifuge for 10 minutes at 5000 rpm and then placed into storage tubes. Approximately 0.5 to 1 ml of saliva were collected by this method and stored at -20°C until analysis.

Cortisol analysis was conducted using a commercially available radioimmunoassay kit (DiaSorin Inc., USA) (Beausoleil *et al.* 2008) and modified for use in porcine saliva by adding 75 µL of buffer to 100 µL of saliva. Limit of detection was 0.9 nmol/L and the mean intra and inter-assay coefficients of variation were 2.5% and 4.8% respectively.

A skin lesion count was calculated after saliva collection on d 0, 1, 3, 7, 8 and 14 for each sow and lameness score allocated on the day prior to mixing and at the completion of the experimental period using previously outlined methods (Karlen *et al.* 2007). To summarise, examining skin lesions involved both sides of the sow being divided into 21 areas with each area inspected for the presence and number of scratches, abrasions, ulcers and cuts. These lesions were then summed to give a total lesion count. Lameness was scored whilst the sow walked (after at least a distance of 20 m) and a score of zero was given when ability to stand was unaffected and all legs bore weight similarly, one if the sow was not considered lame but movement was compromised, two if the sow was moderately lame and its ability to stand was obviously reduced, and three represented a severely lame sow whose ability to stand and move was severely restricted.

All data were analysed in the statistical package SPSS v20 (IBM Corporation, New York USA) and a P-value of < 0.05 was deemed significant. All not normally distributed data were transformed given the distribution of the residuals plots, resulting in $\log_{10}$ transformations for behavioural measures, and a square-root transformation for salivary cortisol and injury data prior to analysis. Where transformations occurred, back-transformed means are presented in parentheses for ease of interpretation. Sow weight and locomotion score were analysed using a general linear model with replicate, pen, parity and treatment fit as fixed terms and the day -1 measure fit as a covariate. Sow behaviours were analysed using a linear mixed model with replicate, pen, parity, day, treatment and the interaction between day and treatment fit as fixed terms, along sow identification as a random term. Cortisol concentration as well as skin lesion counts were analysed in a similar manner to sow behaviours with the exception that a covariate of the day -1 measure was included in the model. The model for cortisol concentration also included the covariates of time of sampling and order within the open in which the sample was collected. Chi-square analysis was conducted to examine the effects of treatment on conception rate.

Experiment 4: the inclusion of dietary magnesium to reduce aggression in sows
This experiment was conducted at a large-scale commercial, pig breeder unit in South Australia, had approval from the PIRSA Animal Ethics Committee, and was conducted in weekly replicates from December (summer) until August (winter). The 360 sows were Large White x Landrace in breed and ranged in parity from two to seven. Sows were weaned into large group pens (40 sows/pen with six feeders and five water points per pen and a space allowance of 1.8m^2/sow) and checked for oestrus in the presence of a boar daily. Upon oestrus detection, each sow was removed from the group pen and placed in a mating station where it received two inseminations 24h apart.

Once a week, 30 multiparous sows mated on the same day were selected and randomly allocated to one of the three treatments. The day following the second insemination, the selected sows were moved to a gestation unit containing 60 group pens which were identical in design, with partially slatted flooring, two nipple drinkers and three drop feeders. Sows were mixed into groups of six at a space allowance of 1.9m^2/sow, marking the beginning of the experimental period. Sows remained in their respective treatment pens until d110 of gestation, at which point they were moved to an individual farrowing crate, where they gave birth without induction. In this pen, all sows received a standard lactation diet at an allowance of 2.4kg per day.

Sows were randomly allocated to one of the following treatment groups; 180 sows were fed a standard gestation diet (13.2 MJ/KgDE, 14.3% protein and 4.9% CF) in mash form (CON), 180 sows were fed the gestation diet mixed with 5kg/tonne of a commercially available marine algae extract (SUPP), and 180 sows were fed the gestation diet mixed with 2.84kg/tonne magnesium sulphate (MGSO4). The weights were balanced by altering the amount of a filler (bentonite) accordingly. Diets were fed to the sows once daily from three drop feeders located above each pen at an allowance of 2.4kg/sow/day. At this inclusion rate and feed allowance, sows from the SUPP and MGSO4 treatments received 1.4g elemental magnesium daily.

When housed individually in the mating stations, each sow was allocated a subjective scratch score for the head, body and rump. The sow received a score of zero if there were no scratches present, a score of one if there were one to five scratches, a score of two if there were six to ten scratches, and a score of three if there were greater than 11 scratches present for each of the three body sections. These scores were then summed together to give a total scratch score (out of nine). In addition to the scratch score, injuries other than scratches were counted. These injuries included abscesses, cuts, ulcers, mastitis, shoulder abscess and vulva bites. Given that the incidence of each of these injuries was low, they were summed together to give a total injury count. Scratch score and injury counts were collected once more on d 2, 31 and 102 of gestation. On all these measurement days, the sow was also classified as either lame or not lame. The severity of lameness was not determined, and the category included compromised movement through to inability to bear weight. The date of sow removal, outside failure to conceive or pregnancy loss, from excessive levels of aggression, poor health or euthanasia was noted.

A 10mL blood sample was collected from focal sows (n = 3 sows/pen) on d -2, 2, 31 and 102 of gestation. This sample was collected via jugular venipuncture after restraint by snare into lithium heparinised vacutainer tubes at 1300h. All blood samples were collected within two minutes of restraint, and were subsequently spun for harvest of plasma and stored at -20^{0}C. The plasma samples were assayed for cortisol in the Adelaide Research Assay Facility, School of Paediatrics and Reproductive Health, University of Adelaide in duplicate by radioimmunoassay (#07-221105, Immuchem, MP Biomedicals, Orangburg NY USA) according to the manufacturer's instructions using 50ul sample. The minimum detectable level was 10ng/mL. The intra-assay CV was less than 10%. The low inter-assay CV was 10% at 32ng/mL (expected range 25-39ng/mL), and high inter-assay CV was 7.2% at 125ng/mL (expected range 105-146ng/mL). Of the 1230 samples analysed, 216 recorded a cortisol concentration of less than 10ng/mL (below the detectable range), and these samples were allocated a concentration of half the detectable limit (5ng/mL).

Video cameras (GoPro Hero3 5MP, San Mateo CA USA) we suspended above 12 pens per treatment to record aggressive behaviour on d 1, 30 and 101 of gestation. Recording commenced at 0700h and continued until the battery was drained (approximately 2.5h). Aggressive behaviour was scored using Observer XT v11.5 software (Noldus Information Technology, Wagneningan The Netherlands). A definition of the aggressive behaviours scored is outlined in Table 4.

Table 4. Ethogram describing the aggressive behaviours scored within a pen.

Behaviour	Definition	State
Fight	A sow engages in aggression with another, being one sided or reciprocal, and is longer than 3 sec in duration	Continuous
Bite	A sow opens its mouth and delivers a bite to another	Point
Head knock	A sow knocks another with its head	Point
Body Knock	A sow knocks another with any other part of its body	Point
Displacement	A sow is displaced by the activities of another	Point

Conception rate was determined by ultrasonography at d28 of gestation, and farrowing rate as those giving birth to piglets at greater than d112 of gestation. A subset of 54 CON, 48 MGSO4, and 56 SUPP sows had measurements collected at the time of farrowing. The variance around gestation length was calculated as the deviation from an expected pregnancy of 116 days. At farrowing, the total number of piglets born, piglets born alive, and piglets born dead (stillbirths) were recorded. In addition to this, all piglets (born alive and dead) were weighed individually to calculate a total litter weight, lowest, highest, average, range and variance in piglet weight.

Data was tested for normality using Shapiro-Wilk value ($W > 0.80$ was considered normally distributed) before analysis performed in SPSS (IBM, New York USA). In order to normalize distribution, plasma cortisol concentration was square-root transformed, and behaviour data was $\log_{10}$ transformed. For measurements collected during gestation, a linear mixed model was applied to the data in. The model included the random terms of pen (one to 48) and animal identification, and fixed terms of month of insemination (December to May), parity (two to seven), day of measurement (-2, 0, 2, 31 and 102), treatment (CON, SUPP, MGSO4) and the interactions between month of insemination, parity and treatment with day of measurement. The binary traits of conception rate, incidence of lameness and removals were analysed in ASReml (VSN International, Hemptsed UK) by a generalised linear mixed model with logit transformation using the same model. Farrowing data were analysed using a general linear model with month of insemination, parity and treatment, and all significant two way interactions fit as fixed terms. The behavioural data was analysed using the same model with the exception that the total video recording duration was included as a covariate.

Experiment 5: the comparison of mixing sows during lactation, at weaning or post-breeding

The study utilized 120 multiparous (parity 2-7) Large White x Landrace sows, and was conducted over five replicates. Sows were mixed into groups of six and sorted into groups based on achieving an even parity mix across treatments (parity 3.7 ± 0.8). Two of the treatments involved sows being mixed during lactation:

1. From d21 lactation multisuckle (MS) sows were transferred from individual farrowing crates and mixed with into groups of six sows and their litters. MS sows (n=30) and their litters were housed allowing 4.7m^2 of sow accessible space, 1.1m^2 of creep per litter, or 5.7 m^2/sow and litter total area. Sows were then weaned on d28 of lactation into the same stable groups, and boar exposed and inseminated in these stable groups (space allowance was 2m^2/sow following weaning).
2. The SEP treatment comprised sows (n=30) that were separated from their litters for 7h per day from 0650h to 1350h and housed in groups of six at a space allowance of 2m^2/sow from day 21 of lactation. At the end of the 7h period, sows were then returned to their respective farrowing crate and litter for the remainder of the day. Weaning occurred on d28, and sows were then housed in the same group of six sows and inseminated in these stable groups (space allowance was 2m^2/sow following weaning).

The remaining two treatments were sows mixed at/after weaning:

3. Sows (n=30) were mixed at weaning (d28 lactation; WEAN) into group pens at the space allowance of 2m^2/sow pens and inseminated in the same stable groups.
4. Sows (n=30) were weaned into stalls and subsequently mixed into groups of six sows following insemination (MAI) at a space allowance of 2m^2/sow.

All sows received 20 min fence line boar exposure until exhibition of oestrus, and then received three inseminations 24 hours apart. The MS and SEP groups were exposed to boars from the day following allocation to treatment d21 of lactation, and exposure was performed daily until AI was complete. The WEAN and MAI groups were exposed to boars from the day following weaning until AI was complete.

Measures of salivary cortisol, total lesion counts and behavior for h daily (excluding -1 days) for all groups were taken on days M-1, M0, M1 and M6 in relation to the first mixing (M) event and then again on W-1, W0, W1 and W6 in relation to weaning (excluding MAI, which were measured only on W0 for cortisol and injury, as they were in stalls). Timing of all measures were analysed together and measures were also analysed in relation to mixing (M). MAI sows were also sampled on day W0, for comparison of crate levels of injury and cortisol and stress at weaning, when weaned into crates. Please see Experiment 1 methodology for more detail on measurement collected. The saliva samples were sent to the School of Animal Biotechnology, University of Western Australia for analysis of salivary cortisol using a MP Biomedicals I^{125} RIA cortisol Kit (# 07-221106) (MP Biomedicals Australia, Seven Hills, NSW). Limit of detection was 1.5 µg/dL and the mean intra- and inter-assay coefficients of variation were 5.2 and 9.1%, respectively.

Pregnancy rate, farrowing rate and total litter size were recorded. Day of first AI and weaning date were used in order to calculate average days to standing heat, incidence of lactation oestrus and number of sows in oestrus before weaning and after weaning per treatment. Lactation oestrus was determined as sows expressing oestrus behaviour prior to weaning on day 28 of lactation (WEAN and MAI groups), so was set at before or on the day after weaning (W+1) and the number of sows in oestrus before or on weaning (W0) was also analysed. Reproduction parameters were also analysed by sow, in order to separate lactation oestrus and non-lactation oestrus sows in each treatment. A blood sample by jugular venipuncture was taken on day 21 lactation from the treatments to be mixed in lactation and on W3 in sows that had not yet displayed oestrus, and analysed for progesterone. Blood plasma progesterone was determined in the Adelaide Research Assay Facility by coated tube radioimmunoassay (IM1188; Beckman Coulter, Brea, CA, USA) in duplicate. The minimum detectable limit of the assay was 0.08 ng/ml. The intra-assay coefficient of variation of the assay was 5.5%.

Before analysis, data were checked for normality by examining the distribution of residual plots. This resulted in the majority of the data being transformed. When data is provided, the transformation, square root (Sqrt), or Log_{10} (Lg_{10}), is specified. All data were analysed using the Statistics Package for the Social Sciences (SPSS) v20.0 (IBM, Armonk, New York, USA) using a general linear model. Pen was used as the unit with all measures taken as individual sow results and averaged across the pen. Replicate, day of measure and treatment were fitted as fixed effects. Pen by day was fitted as a repeated effect. Day -1 measures (effective baseline) were not fitted as covariates, but rather analysed as one of the treatment days, in order to compare responses, but also, as due to the span of the treatments several days for each treatment could have been implemented as a 'baseline day'. Two separate analyses were completed, one with 'all days' as a fixed effect and one with the measurement days arranged as 'mixing day' and fitted as a fixed effect. These two analyses are presented separately, to allow comparison of the specific effect of mixing within these housing systems, and then also the effects of weaning and the overall housing system. Reproduction was also analysed with sows as the unit, changing the model so that sow by day was a repeated effect, and adding parity group (sows grouped into parity 1, parity 2-3 and parity 4+). Where transformations occurred, non-transformed adjusted means are presented in parentheses. Data are expressed as least squares means ± the standard error of the mean (SEM) and a difference at $P < 0.05$ was deemed significant.

3. Outcomes

Experiment 1: The use of increased space in a mixing pen

The average number of fights per sow, duration of each individual fight and the percentage of overall time spent fighting were unaffected by treatment (P > 0.05). Displacement number was also not affected by treatment (P > 0.05). Treatment had no effect on the average number of bites received per sow, average knock number per sow, number of fleeing events and average number of lunges at other pigs (P > 0.05). Sows spent more time active in the HIGH pens than both the LOW and MED pens (P < 0.01, Table 5). The percentage of time spent resting was also affected by treatment (P < 0.005), with MED and LOW sows resting more compared to HIGH sows. Sows in the HIGH pens also spent a greater percentage of their time exploring their environment when compared to MED and LOW sows (P < 0.01). The number of non-aggressive sow-sow contact events was higher in the HIGH compared to the LOW treatment, with MED as an intermediary (P < 0.05). The number of times sows mounted other sows and the percentage of total time spent eating was not affected by treatment (P > 0.05). Free salivary cortisol concentration was greater in HIGH and MED than LOW (P < 0.01, Table 5) when samples were pooled across days. Both total and front injury numbers were not affected by treatment (P > 0.05).

Table 5. The effect of space allocation at mixing, in gestating sows on behaviours and free salivary cortisol concentrations.Transformed means ± SEM presented with non-transformed means in parentheses. Significant differences are highlighted using superscripts $^{a,\,b}$ within rows.

	LOW	MED	HIGH	Transform	P value
Time spent active, %	1.5 ± 0.02^{a} (33.7)	1.5 ± 0.02^{a} (36.5)	1.6 ± 0.02^{b} (43.4)	Lg10	< 0.01
Time spent resting, %	1.8 ± 0.01^{a} (66.1)	1.8 ± 0.01^{a} (63.3)	1.7 ± 0.01^{b} (56.5)	Lg10	< 0.005
Time spent exploring, %	1.8 ± 0.1^{a} (3.5)	2.0 ± 0.1^{a} (4.0)	2.3 ± 0.1^{b} (5.7)	Lg10	< 0.01
Sow-sow contact, number	0.3 ± 0.09^{a} (2.2)	0.4 ± 0.07^{ab} (3.2)	0.5 ± 0.07^{b} (4.5)	Lg10	< 0.05
Free salivary cortisol, ng/ml	2.5 ± 0.1^{a} (6.5)	2.9 ± 0.1^{b} (9.1)	3.1 ± 0.1^{b} (10.1)	Sqrt	< 0.05

The pregnancy rate was equal to the farrowing rate as no pregnancy loss occurred after d28 of gestation, and both measures were not affected by treatment (LOW = 79.5 ± 5.1, MED = 88.1 ± 5.4 and HIGH = 81.0 ± 5.4, P > 0.05). The total number of piglets born was also not affected by treatment [LOW = 3.5 ± 0.1 square root transformed total piglets (12.2 piglets), MED = 3.4 ± 0.1 (11.8 piglets), HIGH = 3.4 ± 0.1 (11.6 piglets), P > 0.05].

Irrespective of treatment, there were more fights on d0 than on all other days (P < 0.01, Table 6). The average duration of individual fights (P < 0.001) and total percentage of time spent fighting (P < 0.01) were also affected by day, with significantly longer fights and higher percentage of total time spent fighting on d0, with fight duration halving and percentage of total time spent fighting reducing by 75% on the day following mixing. There was no increase in any of the aggressive behaviors analyzed when space was standardized to 2m^2/sow on d4 following mixing (P > 0.05). A higher bite number was observed on d0 than all other days (P < 0.005), along with higher knock number (P < 0.01), number of fleeing events (P > 0.005) and the number of mounting events (P < 0.005). The number of lunges was not altered by day after mixing (P > 0.05). Displacements were similarly affected by day, with a higher number of displacements on d0 than all other days (P < 0.01). The number of non- aggressive sow-sow contact events was also affected by day, with a lower number of social contact events not resulting in aggression on d0 compared to all other days (P < 0.00005).The percentage of time spent active and resting differed across day (P < 0.001), with pigs being more active and resting less on d0 compared to all other days. Eating time varied across day, with a lower percentage of time spent eating on d0 than all other days and on d1 than on d4 and d6 (P > 0.00005). Free salivary cortisol concentrations were also affected by day, with higher cortisol levels on d1 than on d4 (P < 0.01). Lesion counts were unaffected by day of mixing (P > 0.05).

Table 6. The effect of day after mixing on behavioural and physiological factors associated with sow aggression and stress. Transformed means ± SEM presented with non-transformed means in parentheses. Significant differences are highlighted using superscripts $^{a, b}$ within rows. NOTE All pens were standardised to $2m^2$/sow on d4.

	D0	D1	D3	D4	Transform.	P value
Fight number	1.0 ± 0.1^a (13.8)	0.4 ± 0.1^b (4.2)	0.7 ± 0.1^b (5.3)	0.7 ± 0.1^b (5.5)	Lg10	< 0.005
Fight duration	4.3 ± 0.3^a (20.5)	2.1 ± 0.3^b (6.5)	2.5 ± 0.3^b (8.0)	2.4 ± 0.3^b (7.8)	Sqrt	< 0.000005
Time spent fighting, %	-1.0 ± 0.1^a (0.2)	-1.6 ± 0.1^b (0.04)	-1.4 ± 0.1^b (0.07)	-1.5 ± 0.1^b (0.04)	Lg10	< 0.0005
Displacement, number	4.5 ± 0.3^a (23.9)	3.4 ± 0.3^b (12.6)	3.2 ± 0.3^b (10.7)	3.5 ± 0.3^b (12.7)	Sqrt	< 0.005
Bite number	1.0 ± 0.1^a (14.9)	0.4 ± 0.1^b (3.8)	0.5 ± 0.1^b (4.5)	0.6 ± 0.1^b (4.6)	Lg10	< 0.005
Knock number	1.1 ± 0.1^a (14.3)	0.7 ± 0.1^b (9.1)	0.9 ± 0.1^{ab} (10.1)	1.1 ± 0.1^a (15.5)	Lg10	< 0.01
Flee number	0.7 ± 0.1^a (6.7)	0.3 ± 0.1^b (2.6)	0.2 ± 0.1^b (1.8)	0.3 ± 0.1^b (1.5)	Lg10	< 0.005
Mount number	0.8 ± 0.1^a (3.3)	0.8 ± 0.1^b (1.4)	-0.6 ± 0.1^b (0.1)	-0.1 ± 0.1^b (0.5)	Lg10	< 0.005
Time spent active, %	1.7 ± 0.02^a (46.9)	1.5 ± 0.02^b (32.7)	1.6 ± 0.02^b (36.9)	1.5 ± 0.02^b (34.9)	Lg10	< 0.0005
Time spent resting, %	1.7 ± 0.02^a (52.8)	1.8 ± 0.02^b (67.1)	1.8 ± 0.02^b (63.2)	1.8 ± 0.02^b (64.7)	Lg10	< 0.0005
Time spent eating, %	5.8 ± 0.6^a	7.3 ± 0.5^b	9.1 ± 0.5^c	9.8 ± 0.6^c	N.A.	< 0.00005
Sow-sow contact, number	0.9 ± 0.09^a (9.9)	0.4 ± 0.1^b (1.6)	0.3 ± 0.1^b (1.0)	0.007 ± 0.1^c (0.6)	Lg10	< 0.00005
Free salivary cortisol, ng/ml	2.9 ± 0.1^a (9.4)	3.2 ± 0.1^a (10.8)	2.6 ± 0.2^b (7.6)	2.5 ± 0.2^b (6.5)	Sqrt	< 0.05

There was no significant interaction between calculated hierarchy rank and space treatment for any of the behavioral parameters measured (P > 0.05) including aggression traits. There was no significant effect of hierarchy within space treatments for salivary cortisol concentration on any of the days investigated (P > 0.05). A significant interaction between hierarchy rank and treatment was identified for lesion counts with the most submissive group (1D1F) displaying higher injuries than all other hierarchies when housed in the LOW treatment (P < 0.05, Figure 3).

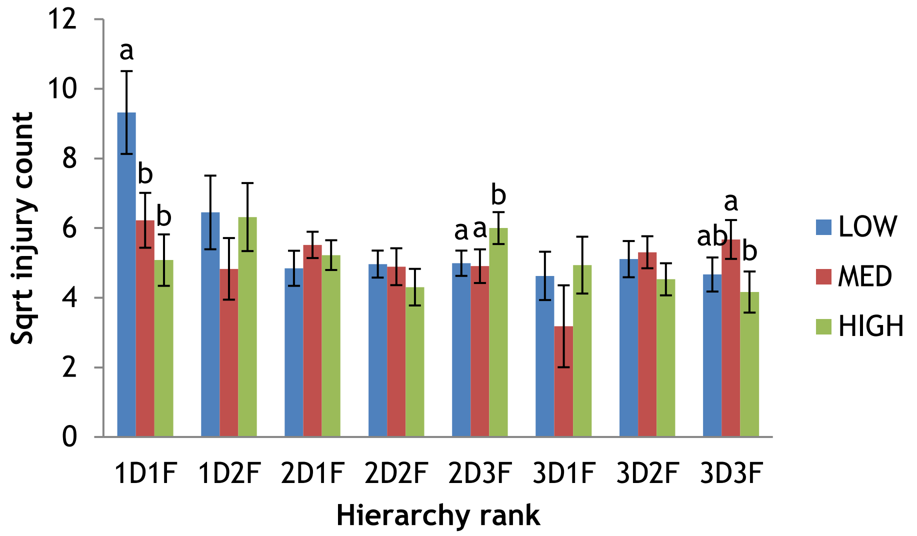

Figure 3. The impact of calculated hierarchy rank on injury count for sows housed at $2m^2$ (LOW), $4m^2$ (MED) or $6m^2$ (HIGH) per sow. Significant differences within rank indicated by different superscripts, [a,b] P < 0.05)

Experiment 2: The use of environmental enrichment in a mixing pen

The presence of novel materials had no effect on the number of fights per sow, on the duration of individual fights or on the percentage of total time spent fighting (P > 0.05). Fight number was affected by the number of days following mixing, with more fights recorded on d0 than any other day (P < 0.0001). Similarly fight duration was longer on d0 than any other day (P < 0.0001). The overall percentage of time spent fighting was greater on d0 compared to other days, and greater on d1 compared to d0 (P < 0.0001, Table 7). The number of displacements was not affected by the presence of novel materials (P > 0.05). The number of days after mixing affected displacements, with more displacements occurring on d0 than all other days and more displacements occurring on d1 and d4 compared to d7 and d20 (P < 0.0001). Bite number was unaffected by treatment (P > 0.05) but was impacted by day after mixing, with higher bite number on d0 than all other days and lower bite number on d20 than on d1 and 4 (P < 0.0001). The overall number of knocks delivered was not altered by treatment (P > 0.05) but was by day, with increased knock number on d0 than all other days and lower knock number on d20 compared to d4 and d7 (P < 0.001). The interaction of treatment by day was not significant for knock number (P < 0.05). There was no aggression as a direct result of the novel objects recorded, or enrichment guarding, across any of the days examined.

The number of injuries sustained by sows in groups was not altered by treatment (P > 0.05). When injury scores were grouped into severity (0-5) there was also no difference across treatments (P > 0.05). The number of injuries was shown to differ across day of measurement (P < 0.005), with lower injuries on d0 than d1, d4 and d7 and lower injuries on d20 compared to all other days (Table 7).

Table 7. The effect of day after the mixing event on parameters linked to aggression, including different behaviours and injury counts per sow in sows housed in groups of 12 from the day of mixing to day 20 following mixing. Significant differences within row are indicated by superscripts $^{a, b, c}$ and non-transformed means are presented in brackets.

Day	0	1	4	7	20	Tran.	P value
Fight number	0.4 ± 0.1^{a} (3.4)	-0.4 ± 0.1^{b} (0.3)	-0.5 ± 0.1^{b} (0.3)	-0.7 ± 0.1^{b} (0.2)	-0.5 ± 0.2^{b} (0.1)	Log^{10}	< 0.001
Fight duration, sec	1.9 ± 0.2^{a} (127.6)	0.9 ± 0.2^{b} (13.0)	0.8 ± 0.2^{b} (10.8)	0.7 ± 0.3^{b} (14.9)	0.6 ± 0.3^{b} (9.7)	Log^{10}	< 0.001
Time spent fighting,%	-0.4 ± 0.2^{a} (0.5)	-1.4 ± 0.2^{b} (0.06)	-1.8 ± 0.2^{b} (0.03)	-2.1 ± 0.3^{b} (0.1)	-1.9 ± 0.3^{b} (0.1)	Log^{10}	< 0.001
Displacement number	2.0 ± 0.1^{a} (4.2)	1.3 ± 0.1^{bc} (1.9)	1.4 ± 0.1^{b} (2.2)	± 0.1^{c} (1.3)	1.1 ± 0.1^{c} (1.1)	Sqrt	< 0.001
Bite number	1.3 ± 0.1^{a} (25.1)	0.6 ± 0.1^{b} (4.9)	0.6 ± 0.1^{b} (4.5)	0.4 ± 0.1^{c} (2.9)	0.3 ± 0.1^{c} (2.3)	Log^{10}	< 0.001
Knock number	1.1 ± 0.1^{a} (14.5)	0.6 ± 0.1^{bc} (4.0)	0.7 ± 0.1^{b} (5.4)	0.7 ± 0.1^{b} (4.6)	0.5 ± 0.1^{c} (3.4)	Log^{10}	< 0.001
Injury count	4.9 ± 0.2^{a} (24.7)	5.5 ± 0.2^{b} (31.6)	5.5 ± 0.2^{b} (31.3)	5.4 ± 0.3^{b} (30.9)	4.1 ± 0.2^{c} (17.9)	Sqrt	< 0.05

The standard pen recorded no play events across all days examined, with the novel material group displaying a greater percentage of time spent playing on all days ($P < 0.0001$). The percentage of time spent playing was increased within the novel materials treatment across days, with significantly higher percentage of time spent playing on d4, d7 and d20, compared to d0 and d1 ($P < 0.005$, Figure 4).

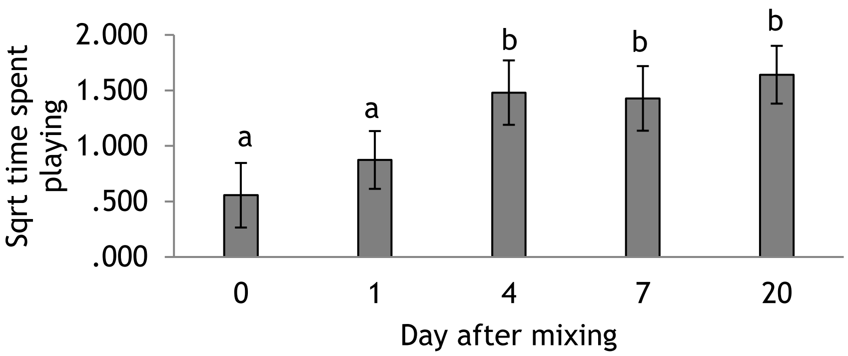

Figure 4. The effect of novel housing on mean ± SEM percent of total time spent in excited play behaviour and overall time spent interacting with enrichment, of group housed sows following mixing (d0) in (significant differences are indicated by different superscripts, ^{a,b} $P < 0.05$).

The percentage of time spent exploring materials, floor and penwork was not affected by treatment ($P > 0.05$). However, the percentage of time spent exploring the enrichment alone was 0.9% (non-transformed mean, Sqrt transformed mean = 0.84 ± 0.1%) pooled across days which, as expected, was significantly more exploration of enrichment than the standard group (0%; P > 0.0001). Overall, the rope was the most highly used enrichment (Table 8).

Table 8. The Sqrt transformed proportion of use for each enrichment provided (rope, mat and swing) and the amount of play and exploration of the materials calculated per sow in the novel treatment.

Behaviour	Total time in play, %	Total time exploring, %
Rope	± 0.1 (2.4)	0.9 ± 0.2 (1.1)
Mat	0.2 ± 0.1 (0.3)	0.5 ± 0.3 (0.5)
Swing	0.3 ± 0.1 (0.1)	0.1 ± 0.1 (0.1)

The percentage of time spent eating was not affected by treatment (P > 0.05) or day (P > 0.05). The percentage of total time spent resting was affected by day (P < 0.0001), with higher percentage of time spent inactive or 'resting' on d0 (45.1 ± 2.5) compared to all other days and d20 (56.5 ± 2.3) compared to d1 (64.3 ± 2.3), d4 (61.5 ± 2.6) and d7 (65.5 ± 2.6).

Salivary cortisol concentration was not affected by treatment (P > 0.05, novel 1.8 ± 0.02, 80.5 ng/ml and standard 1.8 ± 0.02, (80.6 ng/ml)) but was affected by day [P < 0.01, d 1 = 1.7 ± 0.3 (65.1 ng/ml), d 7 = 1.9 ± 0.3 (95.9 ng/ml)]. The interaction of day by treatment was not significant (P > 0.05).

There was no effect of treatment on the change in weight from d-1 to d20 after mixing (P > 0.05). Due to problems with on-farm fertility, pregnancy rate was low (72.4 ± 9.1 %), but was unaffected by treatment (novel = 68.0 ± 3.6 %, standard = 77.8 ± 3.6 %, P > 0.05). The total number of piglets born per litter (novel = 12.2 ± 0.7, standard = 13.4 ± 0.7), piglet weight (novel = 1.4 ± 0.05, standard = 1.4 ± 0.05) and sex ratio of the litters (percentage of the litter female, novel = 44.7 ± 2.7, standard = 51.0 ± 2.7) were also not affected by treatment (P > 0.05).

<u>Experiment 3: the use of a synthetic maternal olfactory agonist in a mixing pen</u>

Sows were observed to eat less frequently on day 1 ($\log_{10}$ mean ± SEM 0.93± 0.05; back-transformed mean 8.4 events, P < 0.001) when compared with all other days (day 0: 1.13 ± 0.04; 13.5 events, day 3: 1.21± 0.04; 13.2 events, day 7: 1.10± 0.04; 12.6 events, day 8: 1.10 ± 0.04; 12.7 events and day 14: 1.04 ± 0.04; 11.0 events). There was also an overall effect of treatment identified for eating behaviour, with sows from the SOA treatment exhibiting an increased $\log_{10}$ mean eating duration (3.62 ± 0.03; 69.6 min; P < 0.01) and frequency (1.12 ± 0.04; 13.2 events; P < 0.05) per sow over the six hour recording period compared with CON (3.52 ± 0.03; 54.7 min and 1.02 ± 0.04; 10.4 times respectively; P < 0.05) when averaged across days. The day by treatment interaction was not significant (Table 9; P > 0.05). The $\log_{10}$ duration of walking differed across days, with sows walking for less time of day 1 (2.65 ± 0.08; back-transformed mean 2.8 min) than days 0, 7, 8 and 14 (2.65 ± 0.08; 7.5 min, 2.69 ± 0.08; 7.9 min, 2.51 ± 0.08; 5.3 min; 2.70 ± 0.09; 8.2 min respectively, with day 3 being intermediate (2.48 ± 0.08; 4.9 min; P < 0.001). The number of walking events differed between treatments across days with sows observed to walk more frequently in the SOA than the CON group on day 0 (Table 9; P < 0.001). The length of time a sow spent resting was influenced by day (P < 0.001), and treatment (P < 0.01), and the interaction between day and treatment (P < 0.001; Table 9). SOA sows spent a longer time engaged in resting behaviour on day 0, no difference was identified on day 1, whilst CON sows rested for longer on the remainder of the experimental days. Similarly, the mean number of times a sow rested was dependent on day ($P < 0.001$) and the interaction between day and treatment ($P < 0.001$; Table 9). Sows from the SOA treatment performed a resting behaviour more often on day 0, whilst CON sows rested more frequently on day 14. Day, treatment and the interaction between the two terms was not significant for the behaviours drinking and eliminating ($P > 0.05$).

Table 9. Log_{10} transformed mean ± SEM non-agonistic sow behaviours (duration and frequency) on the days following mixing for control sows (CON) and those in the presence of the synthetic olfactory agonist (SOA) until day 7, at which point the treatment was removed. Means within days with different superscripts are significantly different from one another. Back-transformed means are presented in parentheses. *Back-transformed means for duration have been converted to mins.

	CON						SOA						
Day	0	1	3	7	8	14	0	1	3	7	8	14	P value
*Log_{10} behaviour duration (sec)**													
Eating	3.49 ± 0.04 (51.5)	3.48 ± 0.04 (50.3)	3.47 ± 0.04 (49.2)	3.58 ± 0.04 (63.4)	3.56 ± 0.04 (60.5)	3.52 ± 0.04 (55.2)	3.54 ± 0.05 (57.8)	3.66 ± 0.05 (76.2)	3.57 ± 0.05 (61.9)	3.63 ± 0.05 (71.1)	3.67 ± 0.05 (78.0)	3.65 ± 0.05 (74.4)	ns
Walking	2.53 ± 0.11 (5.6)	2.18 ± 0.12 (2.5)	2.31 ± 0.11 (3.4)	2.62 ± 0.11 (6.9)	2.53 ± 0.11 (5.6)	2.82 ± 0.11 (11.0)	2.78 ± 0.11 (10.0)	2.27 ± 0.13 (3.1)	2.64 ± 0.11 (7.3)	2.74 ± 0.11 (9.2)	2.48 ± 0.11 (5.0)	2.57 ± 0.13 (6.2)	ns
Resting	4.10 ± 0.02^a (209.8)	4.22 ± 0.02 (276.6)	4.26 ± 0.02^a (303.3)	4.23 ± 0.02^a (283.0)	4.24 ± 0.02^a (289.6)	4.23 ± 0.02^a (283.0)	4.14 ± 0.02^b (230.1)	4.20 ± 0.02 (264.1)	4.22 ± 0.02^b (276.6)	4.20 ± 0.02^b (264.1)	4.20 ± 0.02^b (264.1)	4.16 ± 0.02^b (240.9)	< 0.001
Log_{10} behaviour frequency													
Eating	1.06 ± 0.05 (11.5)	0.85 ± 0.05 (7.1)	1.08 ± 0.05 (12.0)	1.07 ± 0.05 (11.7)	1.06 ± 0.05 (11.5)	1.00 ± 0.05 (10.0)	1.20 ± 0.05 (15.8)	1.00 ± 0.06 (10.0)	1.16 ± 0.05 (14.5)	1.23 ± 0.05 (17.0)	1.14 ± 0.05 (13.8)	1.08 ± 0.06 (12.0)	ns

Walking	0.64 ± 0.07[a] (4.4)	0.41 ± 0.08 (2.6)	0.65 ± 0.07 (4.5)	0.69 ± 0.07 (4.9)	0.64 ± 0.07 (4.4)	0.79 ± 0.07 (6.2)	1.07 ± 0.07[b] (11.7)	0.43 ± 0.07 (2.7)	0.77 ± 0.07 (5.9)	0.75 ± 0.07 (5.6)	0.62 ± 0.07 (4.2)	0.67 ± 0.09 (4.7)	< 0.001
Resting	1.16 ± 0.04[a] (14.5)	1.12 ± 0.04 (13.2)	1.20 ± 0.04 (15.8)	1.25 ± 0.05 (17.8)	1.18 ± 0.04 (15.1)	1.30 ± 0.04[a] (20.0)	1.35 ± 0.05[b] (22.4)	1.14 ± 0.05 (13.8)	1.22 ± 0.05 (16.6)	1.23 ± 0.05 (17.0)	1.13 ± 0.05 (13.5)	1.13 ± 0.05[b] (13.5)	$= 0.001$

The $\log_{10}$ number of aggressive interactions per sow observed was reduced ($P < 0.05$) in SOA sows when compared with CON (Figure 5). SOA sows fought less frequently on days 0 (back-transformed mean 5.6 events), 1 (1.7 events), 3 (2.9 events), 7 (2.1 events), and 8 (4.0 events) than CON sows (day 0; 10.6 events, day 1; 3.3 events, day 3; 6.6 events, day 7; 7.9 events and day 8; 5.9 events respectively). By day 14, there was no difference in the frequency of aggression between the two treatments ($P > 0.05$).

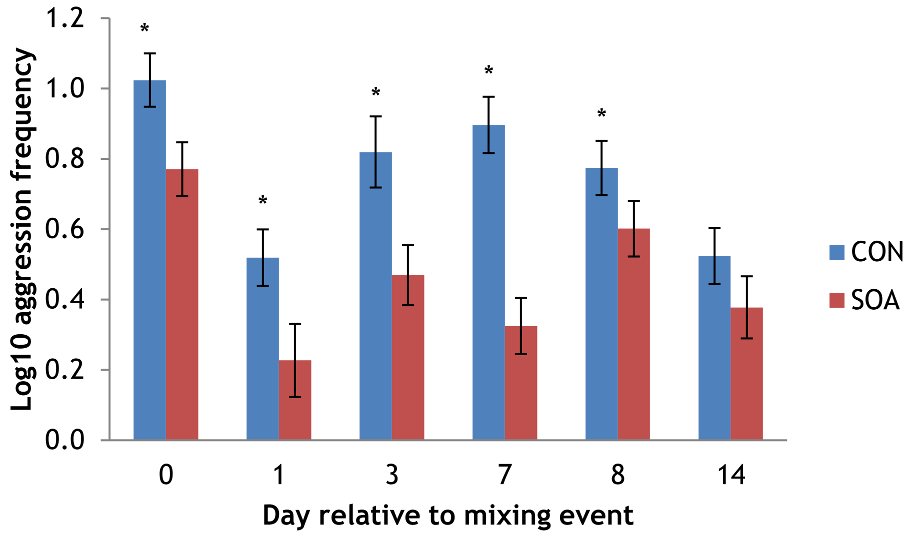

Figure 5. $\log_{10}$ mean ± SEM number of aggressive interactions observed on the days following mixing for control sows (CON) and those in the presence of a synthetic olfactory agonist (SOA) until day 7, at which point the agonist was removed. * Means within days are significantly different from one another ($P < 0.05$).

No effect of treatment on any day was observed when the total duration of aggressive behaviour per sow over the six hours was investigated ($P > 0.05$). There was, however, an effect of day (Table 10; $P < 0.001$), the longest duration of fights observed on day 0, lowest on days 1 and 14, with days 3, 7 and 8 being intermediate. The number of bites, knocks and displacements did not differ with treatment ($P > 0.05$). Bites and knocks were higher on day 0 (Table 10; $P < 0.05$), but the number of displacements did not differ across days ($P > 0.05$).

Table 10. $\log_{10}$ aggressive behaviours scored on the days following mixing for all sows. Means with different superscripts are significantly different ($P < 0.05$). Back-transformed means are presented in parentheses. *Back-transformed means for duration have been converted to mins.

Day	0	1	3	7	8	14	P-value
Total fight duration (sec)*	2.45 ± 0.08^{a} (14.0)	1.65 ± 0.09^{b} (2.2)	1.93 ± 0.08^{c} (4.3)	2.07 ± 0.08^{c} (5.9)	1.93 ± 0.09^{c} (4.3)	1.65 ± 0.09^{bc} (2.3)	< 0.001
Bites (events)	0.37 ± 0.07^{a} (2.3)	0.08 ± 0.08^{b} (1.2)	0.23 ± 0.06^{ab} (1.7)	0.25 ± 0.07^{ab} (1.8)	0.19 ± 0.07^{ab} (1.9)	0.15 ± 0.07^{b} (1.4)	< 0.05

Knocks (events)	0.69 ± 0.05^{a} (4.9)	0.11 ± 0.07^{b} (1.3)	0.36 ± 0.06^{c} (2.3)	0.31 ± 0.06^{bc} (2.0)	0.41 ± 0.06^{bc} (2.6)	0.20 ± 0.07^{bc} (1.6)	< 0.001
Displacements (events)	0.18 ± 0.06 (1.5)	0.03 ± 0.10 (1.1)	0.06 ± 0.09 (1.1)	0.00 ± 0.08 (1.0)	0.13 ± 0.08 (1.3)	0.00 ± 0.12 (1.0)	ns

There was an effect of treatment (P < 0.05) in changes in aggressive behaviour between days 7 and 8 when the agonist diffuser blocks were removed from the SOA treatment (Table 11). The mean number of aggressive interactions that a sow was involved in was reduced in the CON treatment (-0.38 ± 1.0 events) between days 7 and 8, whilst for SOA sows this value was increased (2.2 ± 1.1 events).

Table 11. Changes in aggressive behaviour variables scored between days 7 and 8 after mixing when the synthetic olfactory agonist was removed from the treatment (SOA) pens. Means with different superscripts are significantly different (P < 0.05).

	CON	SOA	P-value
Change in behaviour between days 7 and 8			
Duration of aggressive interaction (sec)	-67.6 ± 65.1	48.9 ± 68.2	P = 0.1
Number of aggressive interactions	-0.38 ± 1.0^{a}	2.2 ± 1.1^{b}	P < 0.05
Bites	-0.28 ± 0.7	-0.24 ± 0.7	ns
Head knocks	0.28 ± 1.0	0.68 ± 1.1	ns
Body knocks	0.03 ± 0.1	0.06 ± 0.1	ns
Displacements	-0.13 ± 0.2	0.21 ± 0.2	ns

Time of collection and sampling order were shown to have no effect over salivary cortisol concentration (P > 0.05). Additionally salivary cortisol did not differ across day or by treatment (P > 0.05).

Treatment had no effect on the total number of skin lesions (P > 0.05). Overall, lesions were increased (P < 0.01) from day 1 (6.08 ± 0.26; back-transformed mean 37.0 lesions) to day 14 (6.84 ± 0.26; 46.8 lesions) with all other days being intermediate (day 3 6.24 ± 0.26; 38.9 lesions, day 7 6.38 ± 0.26; 40.7 lesions and day 8 6.45 ± 0.26; 41.5 lesions).

No treatment effects were observed for sow weight or lameness score recorded at the end of the experimental period, or conception rate measured by ultrasonography at 35 days gestation (P > 0.05).

Experiment 4: the inclusion of dietary magnesium to reduce aggression in sows

The weight of sows did not differ on day 0 (P < 0.05), but by day 102 sows from the CON group weighted more than those from the MGSO4 and SUPP treatments (P < 0.05; Figure 6). Weight change during gestation differed with treatment (P < 0.001), as CON sows gained more weight (46.9 ± 4.4) than those from the MGSO4 (36.4 ± 4.2) and SUPP (31.5 ± 4.4) sows.

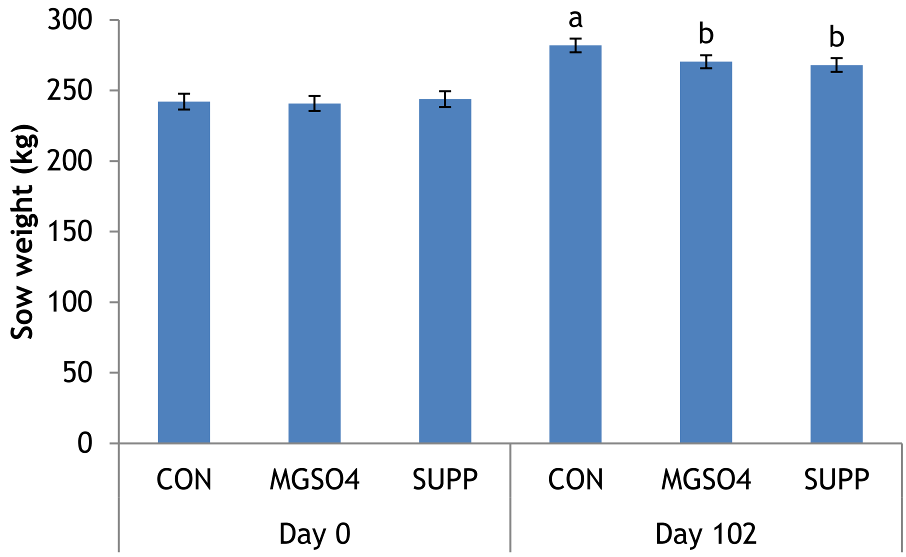

Figure 6. Mean ± SEM weight on days 0 and 102 of gestation from control (CON), magnesium sulphate treated (MGSO4), and magnesium rich supplemented (SUPP) sows. Means with differing superscripts [a,b] are significantly different ($P < 0.05$) from one another within day of measure.

There was also an effect of parity ($P < 0.001$) and the month in which mating occurred ($P < 0.01$) on sow weight that differed across day of gestation (Table 12).

Table 12. Mean ± SEM weight on days 0 and 102 of gestation from sows raging in parity from two to seven, and mated during the months of December to May. Means with differing superscripts [a,b,c,d,e] are significantly different ($P < 0.01$) from one another within day of measure.

		Day 0	Day 102	Sig.
Parity				< 0.001
	2	207 ± 7[a]	252 ± 7[a]	
	3	223 ± 2[b]	271 ± 2[b]	
	4	241 ± 2[c]	277 ± 2[c]	
	5	250 ± 3[d]	281 ± 3[c]	
	6	250 ± 3[d]	283 ± 4[c]	
	7	263 ± 9[e]	274 ± 9[bc]	
Mating month				0.002
	December	238 ± 6[a]	276 ± 6	
	January	237 ± 6[a]	273 ± 5	
	March	240 ± 6[ab]	273 ± 5	
	April	245 ± 6[ab]	273 ± 5	
	May	250 ± 6[b]	273 ± 5	

An issue with data integrity meant that total number of fights, total fight duration and mean bout duration could not be analysed. There was a significant effect of treatment on the frequency of bites on day 1 of gestation ($P < 0.05$), with pens housing MGSO4 sows delivering fewer (back-transformed mean; 32.3 bites), SUPP sows delivering more (56.5 bites), with CON sows intermediate (42.4 bites; Figure 7). However, on days 31 and 102 of gestation, this treatment effect had disappeared.

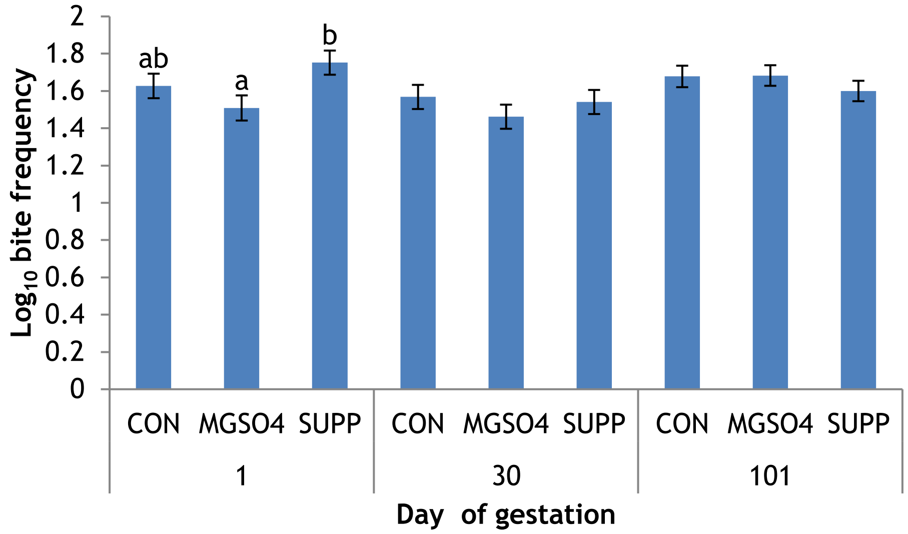

Figure 7. Mean ± SEM $\log_{10}$ number of bites observed within a pen on days 1, 30 and 101 of gestation from control (CON), magnesium sulphate treated (MGSO4), and magnesium rich supplemented (SUPP) sows. Means with differing superscripts ^{a,b} are significantly different ($P < 0.05$) from one another within day of measure.

The number of body knocks recorded within a pen followed a similar pattern to bites on day 1 (MGSO4 sows 5.5 knocks, SUPP sows 9.5 knocks, and CON sows 8.2 knocks; Figure 8), however by day 30, there was a higher body knock frequency in the MGSO4 sows (6.8 knocks) compared to the SUPP sows (3.4 knocks), with CON sows intermediate (4.7 knocks; $P = 0.05$). There was no effect of treatment on body knocks on day 101.

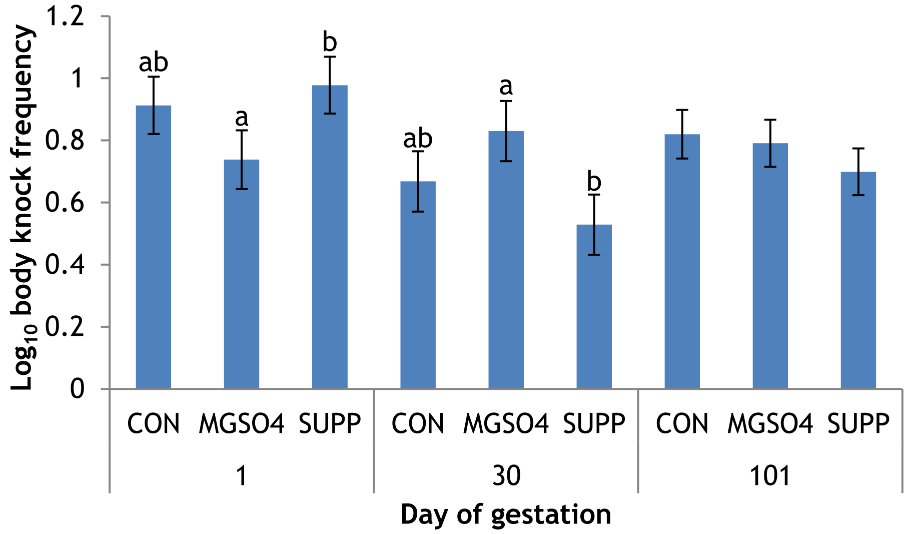

Figure 8. Mean ± SEM $\log_{10}$ number of body knocks observed within a pen on days 1, 30 and 101 of gestation from control (CON), magnesium sulphate treated (MGSO4), and magnesium rich supplemented (SUPP) sows. Means with differing

superscripts [a,b] are significantly different (P = 0.05) from one another within day of measure.

For the remaining behaviours scored, the interaction between treatment and day was insignificant. The main effect of day was however significant for both head knock and displacement frequency, with fewer head knocks, but higher displacements on day 1 than on day 30 and 101 (P = 0.01; Table 13). The main effect of treatment was also significant for number of head knocks, with fewer observed in MGSO4 pens than SUPP pens, with CON intermediate (P = 0.05).

Table 13. Mean ± SEM log10 head knock and displacement frequency across day of gestation (1, 20 and 101) and treatment (control (CON), magnesium sulphate treated (MGSO4), and magnesium rich supplemented (SUPP) sows). Means with differing superscripts [a,b] are significantly different (P < 0.05) from one another. Back-transformed means are presented in parentheses.

	Day				Treatment			
	1	30	101	Sig.	CON	MGSO4	SUPP	Sig.
Log_{10} behaviour frequency								
Head knock	0.24 ± 0.06[a] (1.7)	0.40 ± 0.05[b] (2.5)	0.46 ± 0.05[b] (2.9)	0.01	0.36 ± 0.05[ab] (2.3)	0.29 ± 0.05[a] (1.9)	0.45 ± 0.05[b] (2.8)	0.05
Displacement	0.31 ± 0.05[a] (2.0)	0.18 ± 0.04[b] (1.5)	0.18 ± 0.05[b] (1.5)	0.01	0.22 ± 0.05 (1.7)	0.23 ± 0.05 (1.7)	0.22 ± 0.05 (1.7)	0.992

Treatment exerted no effect on plasma cortisol concentration, but this measure did differ with day (P < 0.001). Levels were highest on days -2 (4.8 ± 0.1; back-transformed mean 22.8ng/mL) and 102 (4.6 ± 0.1; 21.3ng/mL) when compared to days 2 (3.2 ± 0.1; 10.5ng/mL) and 31(3.4 ± 0.1; 11.2 ng/mL). There was no impact of treatment on scratch score or injury count, both within a day and when averaged across all measurement days (Table 14).

Table 14. Scratch score allocated to the head, body and rump, as well as total scratch score (the sum of these three regions) and total injury count for control (CON), magnesium sulphate treated (MGSO4), and magnesium rich supplemented (SUPP) sows.

		CON	MGSO4	SUPP	Sig.
Scratch score					
	Head	2.0 ± 0.1	1.9 ± 0.1	1.9 ± 0.1	0.225
	Body	2.0 ± 0.1	1.9 ± 0.1	1.9 ± 0.1	0.924
	Rump	1.5 ± 0.1	1.4 ± 0.1	1.4 ± 0.1	0.213
	Total	4.9 ± 0.2	4.5 ± 0.2	4.7 ± 0.2	0.100
Injury count					
	Total	0.41 ± 0.05	0.38 ± 0.04	0.42 ± 0.05	0.695

Scratch scores were shown to differ across day according to the month in which sows were mated (Figure 9). To generalize, Sows received lower scores on all regions of the body during the cooler months in early gestation, but this was revered in late gestation, with those mated during warmer months recording lower scores.

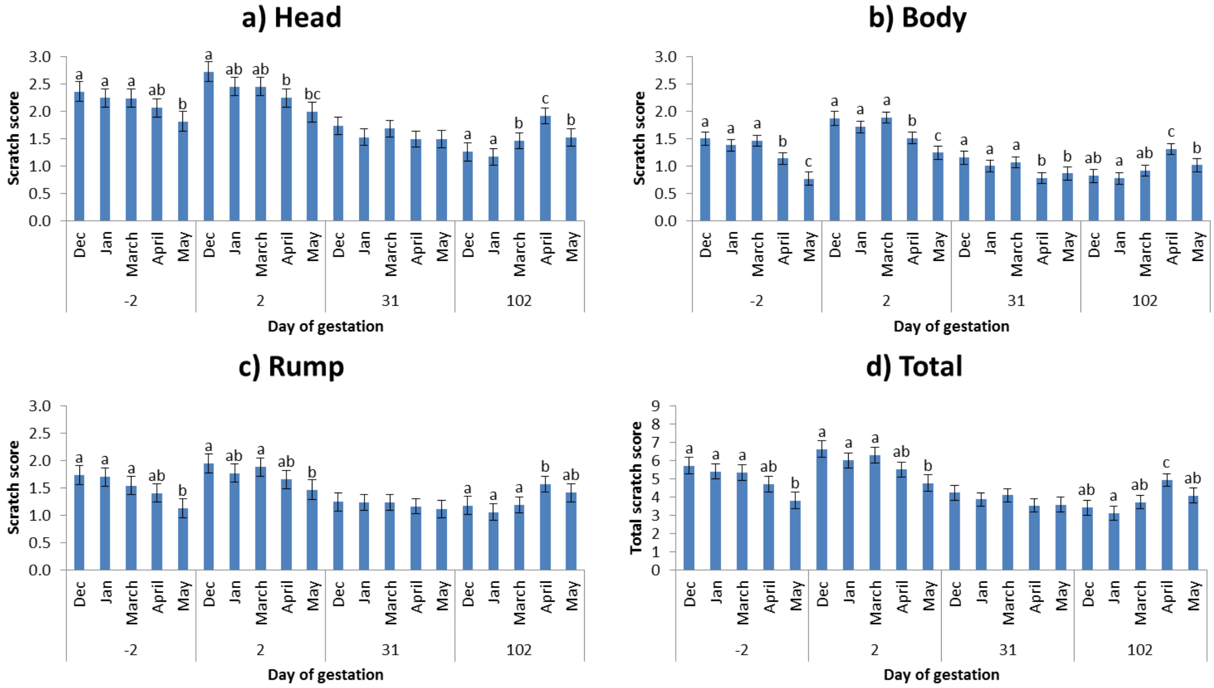

Figure 9. Mean ± SEM scratch score (on a scale of zero to three) for a) head, b) body, and c) rump as well as d) total scratch score of sows collected on days -2, 2, 31, and 102 of gestation for those mated during the months of December to May. Means with differing superscripts [a,b,c] are significantly different ($P < 0.001$) from one another within day of measure.

The count of injuries on a sow was affected by day of measure, with more injuries counted on days -2 (0.56 ± 0.05) and 2 (0.56 ± 0.06) than on days 31 (0.26 ± 0.06) and 102 (0.21 ± 0.06; $P = 0.001$). None of the fixed effects examined were shown to influence the incidence of lameness (1.4 ± 0.2%) and removals (0.9 ± 0.2%).

Treatment had no effect on almost all of the reproductive parameters measured. The only exception was an increase in piglet birth weight variance in SUPP sows compared to CON sows, with MGSO4 intermediate (Table 15).

Table 15. Reproduction and piglet weight variables from control (CON), magnesium sulphate treated (MGSO4), and magnesium rich supplemented (SUPP) sows.

	CON	MGSO4	SUPP	Sig.
Reproduction				
Conception rate (%)*	98.0 (95-100)	97.1 (93-100)	98.7 (96-100)	0.464
Farrowing rate (%)*	95.5 (89-100)	95.1 (09-100)	94.6 (89-100)	0.465
Gestation†	1.0 ± 0.3	0.6 ± 0.3	1.0 ± 0.3	0.193
Total born	12.9 ± 1.0	12.9 ± 1.0	12.7 ± 1.0	0.960
Born alive	11.1 ± 0.8	10.9 ± 0.8	11.1 ± 0.8	0.947
Stillborn (%)	12.1 ± 2.8	13.1 ± 2.8	11.3 ± 2.8	0.632
Piglet weight				

Total litter	18.3 ± 1.2	17.9 ± 1.1	18.3 ± 1.1	0.864
Lowest	0.8 ± 0.1	0.8 ± 0.1	0.8 ± 0.1	0.480
Highest	1.9 ± 0.1	1.9 ± 0.1	1.9 ± 0.1	0.688
Average	1.4 ± 0.1	1.2 ± 0.1	1.2 ± 0.1	0.631
Range	1.1 ± 0.1	1.2 ± 0.1	1.2 ± 0.1	0.631
Variance	0.11 ± 0.02[a]	0.12 ± 0.02[ab]	0.15 ± 0.02[b]	0.020

*95% confidence intervals are presented in parentheses as means have been back-transformed after logit transformation.
†Deviation from an expected gestation length of 116 days.

Experiment 5: the comparison of mixing sows during lactation, at weaning or post-breeding

Anlaysed with respect to day of mixing: Total and front injuries were higher in the SEP group on M0 and M1, than all other treatments (P < 0.0001; Table 16). The MS group had significantly fewer total injuries on day M1 and M6 than other treatments, fewer front injuries on M0 and M1 than MAI and SEP and fewer front injuries on M6 than all other treatments. There were more fights per hour in the SEP group than any other treatment on M0 (P < 0.005). In the MS group, M1 and M6 had no fights coded. The MAI group had significantly lower fight number on M0 than WEAN and SEP, with WEAN fight number per hour decreasing on M6, and so significantly less than both SEP and MAI (P < 0.05).

Table 16. The effect of day relative to the mixing event (M) for treatments (MS= multi-suckle, SEP= sow separation, WEAN= sows grouped at weaning, MAI= sows grouped after insemination) on the measures of total and front lesions and number of fights per hour. Means with differing superscripts ^{a,b} are significantly different (P < 0.05) from one another. Back-transformed means are presented in parentheses.

Mixing day	M-1				M0				M1				M6				
Treat.	MS	SEP	WEAN	MAI	MS	SEP	WEAN	MAI	MS	SEP	WEAN	MAI	MS	SEP	WEAN	MAI	P valu
Sqrt Total lesion, number	2.4 ± 0.4 (5.6)	2.7 ± 0.4 (6.7)	1.9 ± 0.4 (4.4)	2.0 ± 0.4 (4.4)	3.7 ± 0.4^{a} (14.6)	5.6 ± 0.4^{b} (32.3)	4.0 ± 0.4^{a} (16.3)	4.4 ± 0.4^{a} (19.7)	3.4 ± 0.4^{a} (11.4)	5.8 ± 0.4^{b} (34.4)	4.5 ± 0.4^{c} (20.7)	4.6 ± 0.4^{c} (21.6)	2. 9 ± 0.4^{a} (9.5)	5.9 ± 0.4^{b} (36.1)	6.1 ± 0.4^{b} (39.1)	6.3 ± 0.4^{b} (40.9)	< 0.0001
Sqrt Front lesion, number	1.5 ± 0.3 (2.0)	1.7 ± 0.3 (2.5)	1.2 ± 0.3 (1.8)	1.5 ± 0.3 (2.1)	2.5 ± 0.3^{a} (6.7)	3.9 ± 0.3^{b} (15.1)	2.6 ± 0.3^{a} (7.2)	3.3 ± 0.3^{b} (11.2)	2.2 ± 0.3^{a} (5.1)	4.0 ± 0.3^{b} (15.9)	3.3 ± 0.3^{a} (10.8)	3.8 ± 0.3^{b} (14.3)	1.9 ± 0.3^{a} (4.1)	4.2 ± 0.3^{b} (18.2)	4.4 ± 0.3^{b} (20.3)	4.9 ± 0.3^{b} (24.0)	< 0.0001
Lg^{10} Fights per hr, number					-0.6 ± 0.1^{a} (0.27)	-0.4 ± 0.1^{b} (0.48)	-0.7 ± 0.1^{a} (0.27)	-0.6 ± 0.1^{a} (0.20)		-0.7 ± 0.1^{a} (0.10)	-0.8 ± 0.1^{b} (0.06)	-1.1 ± 0.1^{b} (0.06)	0	-1.0 ± 0.1^{a} (0.08)	-0.2 ± 0.2^{b} (0.13)	-1.0 ± 0.1^{a} (0.06)	< 0.05

Treatment also affected the number of bites and knocks delivered, with more bites delivered when pooled across the M days in the SEP group compared to all others ($P < 0.05$) and more knocks by the SEP group than the MS group ($P < 0.05$, The number of displacements per hour was not affected by treatment when analysed in relation to mixing. Cortisol was not significantly affected by treatment or M day or the interaction between the two ($P > 0.05$).

Analysed with respect to all measurement days: SEP sows had significantly higher injury numbers through lactation compared to MS sows, but also exhibited higher injuries on W0 (the day of weaning) and W1 compared to all other treatments. The WEAN group had significantly higher injuries on W6 than both other treatments measured on that day (Figure 10).

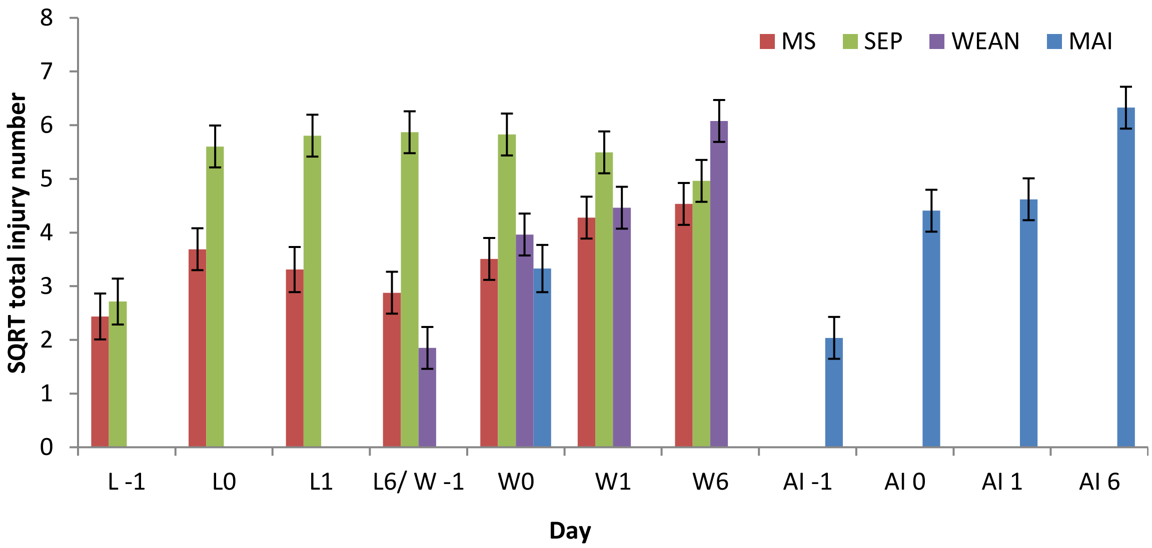

Figure 10. Total injury number (square-root transformed) for MS (multi-suckle), SEP (sow separation), WEAN (grouped at weaning) and MAI (grouped after insemination) across days where L0 is the day of grouping for MS and SEP treatments, W0 is the day of grouping for WEAN treatment, and AI0 is the day of grouping for MAI treatment.

The day by treatment interaction was insignificant but the main effect of treatment had a significant effect on the free salivary cortisol concentration, with increased cortisol in the SEP group [1.4 ± 0.1 (32.4 ng/ml)] and MS [1.3 ± 0.1 (31.8 ng/ml)] group compared to MAI group [1.1 ± 0.1 (24.8 ng/ml), $P < 0.05$] when pooled across all days, with the WEAN group intermediate [1.2 ± 0.1 (30.9 ng/ml)].

Fight number, duration, bite number and knock number were affected by treatment when pooled across all days. There was a greater number of fights on average in the WEAN group over all other groups and greater fight duration in WEAN and MAI than the lactation treatments (Table 17, $P < 0.005$). There were a greater number of bites in the SEP group than all other groups and a greater number of knocks in the SEP group compared to the MS group ($P < 0.005$).

Table 17. Aggressive behaviour variables and reproductive parameters for MS (multi-suckle), SEP (sow separation), WEAN (grouped after weaning), and MAI (grouped after insemination) sows. Means with differing superscripts [a,b] are

significantly different (P < 0.05) from one another. Back-transformed means are presented in parentheses.

Behaviour	MS	SEP	WEAN	MAI	P value
Lg^{10} Fight number per hr	-0.9 ± 0.1^{a} (0.05)	-0.8 ± 0.1^{a} (0.1)	-0.6 ± 0.1^{b} (0.2)	-0.9 ± 0.04^{a} (0.1)	< 0.005
Lg^{10} Mean fight duration	0.9 ± 0.1^{a} (2.0)	0.7 ± 0.1^{a} (8.1)	1.2 ± 0.2^{b} (16.8)	0.8 ± 0.2^{b} (16.5)	< 0.005
Lg^{10} Bite number	-0.6 ± 0.1^{a} (0.3)	-0.2 ± 0.1^{b} (1.2)	-0.3 ± 0.1^{b} (1.0)	-0.4 ± 0.1^{b} (0.9)	< 0.005
Lg^{10} Knock number	-0.6 ± 0.1^{a} (0.4)	-0.1 ± 0.1^{b} (1.3)	-0.3 ± 0.1^{ab} (1.1)	-0.3 ± 0.1^{ab} (0.7)	< 0.01
Pregnant, %	93.3 ± 7.0	90.0 ± 7.0	91.7 ± 7.0	80.0 ± 7.0	> 0.05
Sqrt Total born	3.7 ± 0.1^{a} (13.8)	3.5 ± 0.1^{ab} (12.2)	3.5 ± 0.1^{ab} (12.3)	3.3 ± 0.1^{b} (11.0)	< 0.05
Number of days from weaning to oestrus	1.0 ± 0.7^{a}	1.0 ± 0.7^{a}	4.3 ± 0.7^{b}	4.9 ± 0.7^{b}	< 0.005
Sqrt Lactation oestrus, %	7.0 ± 1.0^{a} (50.7)	7.0 ± 1.0^{a} (61.7)	0.0 ± 1.0^{b} (0.0)	0.0 ± 1.0^{b} (0.0)	< 0.0001
Sqrt Oestrus before weaning, %	6.8 ± 0.9^{a} (46.7)	6.0 ± 0.9^{a} (46.7)	0.0 ± 0.9^{b} (0.0)	0.0 ± 0.9^{b} (0.0)	< 0.0001

There was no difference in pregnancy rate between treatments (P > 0.05, Table 17). However, the total number of piglets born in the subsequent litter was affected by treatment, with increased total number of piglets (including lactation oestrus and non-lactation oestrus animals) born in the MS treatment than in the MAI treatment (P < 0.05). The MS and SEP treatments had fewer average days from weaning to first standing heat (P < 0.005), higher level of lactation oestrus (determined as before or on the day after weaning (W+1); P < 0.0001) and a greater number of sows in oestrus before weaning (and the number of sows in oestrus before or on weaning (W0); P < 0.0001) than the WEAN and MAI sows. However, there was no significant difference in these three measures between the MS and SEP groups.

When analysed with sow as the unit and split into non-lactation oestrus (oestrus after W1) and lactation oestrus (on W1 or before), there were significantly higher piglet numbers in subsequent litters in MS sows in the non-lac group than both MAI and WEAN and also in the SEP group than WEAN (Figure 11).

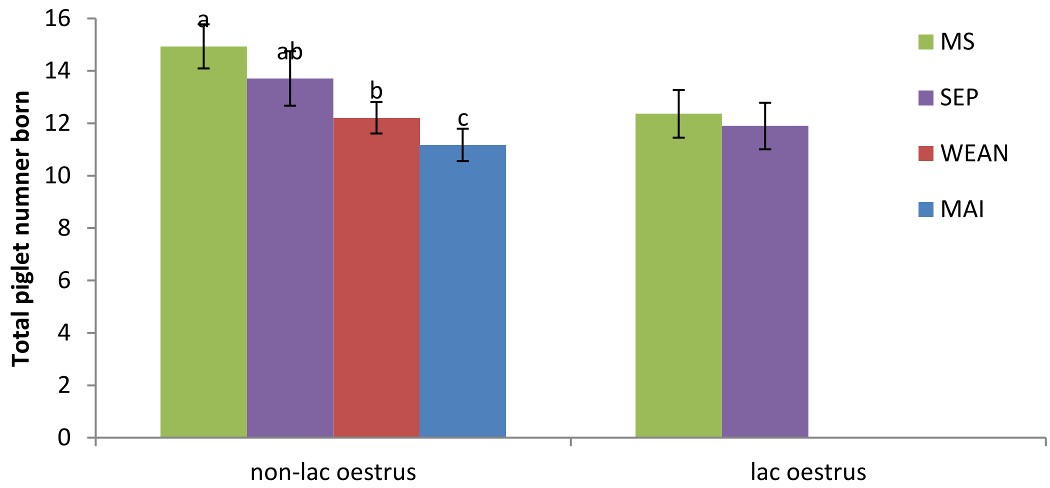

Figure 11. The total number of piglets born to MS (multi-suckle), SEP (sow separation), WEAN (grouped after weaning) and MAI (grouped after insemination) sows and that were not mated in lactation (non-lac oestrus) and mated in lactation (lac oestrus).

The days between farrowing and oestrus were not significantly affected when analysed with pen as the unit. With sow as the unit there were fewer days between farrowing and oestrus in SEP (28.7 ± 0.7 days) compared to the MAI (31.5 ± 0.6 days) group ($P < 0.005$). There was no difference between the WEAN (31.3 ± 0.6 days) and MAI (31.5 ± 0.6 days) sows or the SEP (28.7 ± 0.7 days) and MS sows (29.2 ± 0.6 days; $P > 0.05$). The days from farrowing to oestrus were also affected by lactation oestrus, with lactation oestrus sows coming into heat 25.8 ± 0.6 days after farrowing, versus sows that did not come onto heat in lactation, which came into oestrus 31.4 ± 0.3 days after farrowing ($P < 0.0001$).

Circulating progesterone concentration was not altered by treatment ($P > 0.05$). When sows were grouped as that would not stand (WNS, showed no standing heat response at boar exposure) and those which were mated and found either pregnant or not, progesterone was significantly higher in WNS sows on day W3 [pregnant = -0.3 ± 0.1 Lg_{10} transformed mean and SEM (0.8 ng/ml non-transformed), not pregnant = -0.4 ± 0.3 (0.2 ng/ml), WNS = 1.2 ± 0.2 (17.4 ng/ml)], but was no different in these animals on day 21 lactation [bleed 1, pregnant = -0.3 ± 0.1 (1.4 ng/ml), not pregnant = -0.2 ± 0.3 (1.1 ng/ml), WNS = -0.1 ± 0.2 (0.8 ng/ml)].

4. Application of Research

Whilst the investigators understand that space is an expensive resource, hierarchy establishment is thought to occur quickly, and so the premise behind the first experiment was to establish whether a high space allowance could be provided in the short term to decrease sow aggression. The primary analysis revealed there was little impact of relatively large amounts of space (4-6m^2/sow) on sow aggression when examined at a pen level. This was unexpected given the findings of Weng *et al.* (1998) who showed reduced aggression and injury at generous space levels, but these animals were not newly mixed, rather in stable groups when treatments were applied. Additionally, the LOW space allowance (2m^2/sow) used in this experiment was still generous considering the Australian code currently recommends a minimum allowance of 1.4m^2/sow. What was identified by the secondary analysis at the individual sow level was that injuries were fewer in low ranking animals at these high space allowances. This would suggest that whilst there may be no overall benefit, it is those who are lower in social rank that are assisted by high space allowances. Based on their findings of increased levels of injury and cortisol levels, Verdon *et al.* (2016) suggested that subdominant and submissive sows would benefit from additional resources. Our findings would support the use of high space allowances in a mixing pen to improve the welfare of subordinate sows. This could be of benefit to producers that are unable to segregate based on parity or size (based on recommendations by Li *et al.* (2012) i.e. those younger, smaller sows are more likely to be low ranking). Interestingly, there was no rise in aggression when pen sizes were standardized and so it would appear that three days is an adequate period for this extra space provision. In fact, most aggression had subsided one day after mixing which is lower than the two to ten days cited in a review by Greenwood *et al.* (2014), and so duration spent inside a mixing pen could be reduced further but this requires more investigation.

The second environmental manipulation of a mixing pen that was investigated involved the use of toy-like enrichments and barriers, but these fixtures were ineffective at reducing aggression levels in sows. They were originally selected as there is a wide body of research that demonstrates their effectiveness in young pigs. It would therefore be tempting to argue that the older sows were simply not interested in the enrichment devices selected, and so there was no impact on aggression. However, the materials were apparently valued by the sows (discussed later) so the unaltered aggression might be explained by the level of motivation for different limiting resources. In an interesting study in operant conditioning, Pedersen *et al.* (2002) investigated how hard a pig would work for certain resources (by pressing a button with their snout) both in isolation and with the presence of a companion pig. The experiment found that in the presence of a companion pig, the motivation for food increased but the motivation for straw was not increased. Given that establishing the hierarchy (and so aggression) is mostly centered on feeding, this may help to explain why no effect of the novel environment was observed. We demonstrated quite clearly that the sows did engage with the enrichment, and in fact, this interest did not decline over time, as was expected. Whilst the sows housed within the enriched pens failed to show reduced aggression, they were encouraged to perform other positive aspects of behaviour. Specifically, sows housed in pens containing the novel materials spent more time engaged in play. Based on these findings, we believe that the provision of these novel materials is not an effective method of reducing aggression in mixed sows, but the increased exhibition of behaviours commonly cited as indicating a positive affect (play), may suggest that the welfare of sows housed mixing with novel materials is improved. We suspect, given the available literature (Jensen *et al.* 2000), that a more species specific enrichment, such as straw, will have a higher impact on sow aggressive behaviour, and so are investigating this on a commercial breeder unit currently (Pork CRC project 1C-116).

The novel findings from the third experiment provided the first evidence that synthetic olfactory agonists can be successfully applied to reduce the aggression that is common amongst commercial sows when first mixed into groups. Previous studies into the use of 'synthetic pheromones' on pig behaviour involved repeat administration via aerosol, making its application difficult under commercial conditions. However, the unique diffusing methodology employed in the present investigation resulted in a slower release of the compound which allowed for the study of its impacts on longer (days rather than hours) behavioural stressors such as sow aggression. In electronic sow feeding systems (ESF), the frequency and duration of queuing events are positively associated with aggression (Anil *et al.* 2006), and so diffusing blocks placed in proximity to the ESF may act to alleviate these problem behaviours. In addition to the changes in agonistic behaviours, sows from the SOA treatment spent longer eating following feeding, in agreement with the work conducted in piglets (McGlone and Anderson 2002). In sows, an increase in the time spent foraging after feed delivery may represent a reduction in the perception of competition. These behavioural findings may be of relevance to the apparent risk of an increase in weight variation under group housing conditions, especially in floor fed systems (Brouns and Edwards 1994). If submissive sows are allowed access to feed for longer periods of time, they would be more likely to consume close to daily requirements reducing weight disparities. Further work examining the impacts of these behavioural findings on sow production parameters should be undertaken.

A recent investigation that increased the magnesium concentration of gestating and lactating sow diets (Zang *et al.* 2014) sowed improvements in reproductive parameters (wean to oestrus interval, number of piglets born) and we hypothesised this may be due to the reported lower aggression, injuries and stress observed in younger, female pigs (O'Driscoll *et al.* 2013). Experiment 4 showed little evidence to support the notion that increasing the levels of magnesium in a gestating sow diet reduced aggression, injuries and stress, and so improved reproduction. The commercial farm on which the experiment was conducted was unable to wean sows into stalls and so sows were weaned into large groups in the mating unit. After insemination, sows were moved to a separate gestation unit mixed into smaller groups and fed the treatment diets. We suspect that the fact effectively this was a re-mix investigation, and so sows may have been familiar with one another, may be one explanatory variable. Additionally, the diets were not fed prior to the mixing event, and so would have had little impact at the time of mixing. Finally, the reproductive parameters reported in this experiment were exceptional (conception rates ~98%, farrowing rates ~95%, and total born ~13.0 piglets) suggesting that the animals utilized by this experiment were well managed and so it would be difficult to further improve things. An interesting finding though, was that there appeared to be a seasonal effect on the number of scratches and injuries found on sows. To generalize, these were higher in summer months early in gestation, but higher in winter months later in gestation. It is tempting to conclude that the lighter weights at grouping (presumably due to reduced feed intake in lactation), may have caused increased competition over feed, but this was not measured. If true, this would support the recommendation of allowing sows higher feed allowances at grouping and in early gestation. There may also be a temperature effect on sow activity and so aggression levels but this warrants further study.

With significant interest in alternate lactation accommodation, as well and the induction of ovulation during lactation, we saw an opportunity to examine the effects of ways to mix sows both in lactation as well as after weaning. If sows could be mixed during lactation the advantages may be two-fold; lactation may dampen the stress response (reported in sheep by Ralph and Tilbrook (2016), and the mixing event occurs prior to the risky time of breeding and implantation. What is abundantly clear from our results is that mixing sows into multi-suckle groups in the presence of piglets resulted in no aggression, and subsequently, improved the number of piglets born in the next litter. The treatment involving mixing during a separation event of the sow from her litter did not yield similar results to the multi-suckle group which would suggest that constant suckling from piglets is required in order to achieve this dampening of stress and so reduced aggression in lactation. In addition to the effect of the presence of piglets, there were also some other confounding factors between these two treatments that need mentioning, namely space ($2m^2$/sow versus $4.7m^2$/sow) and feeding (constant access versus ration delivery). The SEP sows also experienced two stressors at once; piglet separation and mixing with unfamiliar sows, which may have acted to increase aggression. It could be argued that the WEAN treatment was effectively the same at M0 (first day of mixing), but aggression was lower in these sows. There is a lot of evidence that shows the effects of weaning age on piglets behaviour (Weary and Fraser 1997; Weary *et al.* 1999; Worobec *et al.* 1999) and on subsequent sow reproductive performance (Xue *et al.* 1993), but this might be the first investigation that reports the effects on aggression and so is a very interesting finding. Maternal attachment may be higher at d21 of lactation that d28, and so the added stress of piglet separation in addition to the mixing with unfamiliar pen-mates could act to increase aggression but this requires further investigation. The findings from this small study were inconclusive with regards to whether it is better to mix sows pre or post breeding but this has been investigated and reported in other Pork CRC funded projects (1C-111 and 1C-112).

The salivary cortisol concentrations were significantly higher in Experiment 2, despite the same laboratory being used for all analyses. Mean values of greater than 90 ng/ml were obtained during the anlaysis and this is exceptionally high given the media (saliva) and expected value (other experiments within this report range from 10 ng/ml to 30 ng/ml; for comparison, plasma samples collected from piglets after weaning averaged 70 ng/ml in 1B-103). The investigators will re-analyse samples from Experiment 2 for salivary cortisol concentration prior to the publication of this experiment in a scientific journal.

5. Conclusion

Aspects of a dedicated mixing pen have the ability to improve sow welfare during the highly stressful mixing period. Whilst aggression at a pen level was not reduced, the welfare of individuals within the pen was improved through the provision of high space allowances (namely those classified as submissive), and a more positive welfare state (through the observation of play behaviour) was induced through the provision of toy-like enrichments. Aggression frequency was successfully reduced when sows were housed in the presence of a synthetic olfactory agonist that behaved similarly to 'appeasing pheromones'. The dietary manipulation (increased magnesium concentration), failed to impact on aggression, injury, stress and reproduction, but this study did identify a seasonality to injury that requires further work. Lastly, multi-suckle housing of sows in stable groups is an attractive option to ameliorate aggression in sows and increase reproductive output.

6. Limitations/Risks

Almost all the experiments reported in this document have involved housing sows in small, stable groups. Given that sow aggression is highly dependent on the type of system (stable versus dynamic; group size; feed delivery, frequency and amount, etc), care should be taken when extrapolating these findings under different conditions.

7. Recommendations

The following recommendations can be made based on the findings from the experiments conducted within this project:

1. The provision of high space allowance in a dedicated mixing pen should be adopted where the effects of aggression are detrimental on low ranking sows
2. Enriching the mixing environment with toy-like fixtures and barriers can be implemented to induce a positive welfare state in sows
3. Incidence of aggressive events can be successfully reduced when sows are housed in the presence of diffusers containing a synthetic olfactory agonist
4. Dietary magnesium is ineffective at reducing aggression during gestation, but further work into seasonal influences on aggression is warranted
5. In addition to reducing the level confinement during lactation, and the increased incidence of lactational oestrus, multi-suckle housing is an effective way to ameliorate mixing aggression and improve subsequent reproductive performance

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