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The effect of providing a greater freedom of movement through periodic exercise on the welfare and stress physiology of stall-housed gestating sows and on piglet behaviour

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Abstract

In Canada, the 2014 Code of Practice for the Care and Handling of Pigs proposed the continued operation of existing stall barns after 2024 on condition that bred sows be given access to periodic exercise. Therefore, this study evaluated the effects of periodic exercise on sow welfare. Sows (n = 180) were assigned to one of three treatments: stall-housed (Control: C); stall-housed and exercised weekly for 10 min (Exercise: E); and group-housed (Group: G). Sow postures and stereotypies were recorded once per week in early, mid and late gestation before (AM) and after (PM) exercise. Female piglets (n = 168 from C, E and G sows) underwent isolation and novel object tests at 19–22 days of age. Postures differed by treatment in AM with G sows lying more and sitting less than C and E sows, which did not differ. In PM, E sows sat more than G sows, with C sows being intermediate. In early gestation, G sows performed fewer stereotypies than E sows, with C sows being intermediate. In mid gestation, G sows performed fewer stereotypies than C and E sows, which did not differ. Piglets from C sows were more active in the novel object test than E and G piglets, which did not differ. Group housing improved sow comfort (indicated by postures) and reduced sow stress (indicated by stereotypies), but periodic exercise did not. Decreased activity level in piglets from sows given greater freedom of movement indicates that gestation housing can influence the behaviour of offspring.

Keywords: *animal welfare, gestation stall, hair cortisol, periodic movement, pig, prenatal stress*

Introduction

Confinement of sows in gestation stalls remains one of the major welfare concerns in the pork industry (Kim *et al* 2016). Gestation stalls are negatively perceived by society due to the restriction of sow movement, as well as foraging and social behaviour for prolonged periods of time (Tonsor *et al* 2009). Due to these circumstances, gestation stalls are being actively phased out around the world and replaced by group gestation systems. Previous studies indicate that stall-housed sows are motivated to leave the stall, and when out of the stall, they show a rebound response to prolonged confinement, spending a greater proportion of time in locomotion during their first opportunity to leave the stall in comparison to two subsequent consecutive opportunities within the same testing session (Tokareva *et al* 2021). These findings indicate the presence of an intrinsic behavioural need for movement, and it is likely considered that accommodating this need would lead to an improvement in sow welfare (Stolba & Wood-Gush 1984).

In Canada, the Code of Practice for the Care and Handling of Pigs (National Farm Animal Care Council [NFACC] 2014) proposed a grandfather clause that existing stall barns in good working order, constructed before 2014, could continue to house female pigs (*Sus scrofa*) in gestation stalls, if these animals are provided with opportunities for a greater freedom of movement, such as periodic exercise. Previous studies have shown some welfare benefits of group sow housing, which provides freedom of movement for sows. In particular, group-housed sows have lower levels of stereotypies, reduced restlessness and lower lameness scores than stall-housed sows (McGlone 2013). Previous research also indicated that providing intensive periodic exercise to stall-housed gestating sows can have physiological benefits (Schenck *et al* 2008; Harris *et al* 2013), in comparison to housing in stalls with no exercise. However, it is unknown if periodic exercise can improve sow welfare by reducing the stress of confinement, and how beneficial it is to provide a low level of exercise, which would be more achievable in intensive commercial barns. To understand how periodic

exercise could benefit gestating sows, a comparison of stall-housed sows receiving periodic exercise with stall- and group-housed sows is needed. Previous studies which looked at the effects of periodic exercise were primarily focused on physiological consequences for the sow and did not explore other welfare aspects such as chronic stress and how prenatal stress influences offspring.

When comparing housing systems that provide different levels of freedom of movement (stall- and group-housing with different space allowances), previous research has found differences in sow behaviour that suggests welfare changes (Weng *et al* 2009; Chapinal *et al* 2010). In particular, sows housed in stalls spent more time sitting in comparison to sows in groups, suggesting reduced comfort levels (Weng *et al* 2009), and restricted-fed sows performed a greater number of stereotypies (sham chewing and oronasofacial behaviours) when housed in stalls, than when in groups (Chapinal *et al* 2010), providing evidence that environmental restriction plays a role in the development of stereotypic behaviour.

If stall-housed, pregnant sows are experiencing stress, it is most likely to be chronic. Yet previous studies have focused on assessing acute stress responses, such as measuring levels of circulating cortisol (McGlone 2013). This has made evaluation of sow welfare in different housing systems quite challenging; when acute measures were assessed, no significant differences were found between group and stall systems (Karlen *et al* 2007). This may be because the type of stress experienced by gestating sows is different in these systems. Stressors experienced by the sow during gestation may alter the behavioural responses of piglets to stressors (Kranendonk *et al* 2007; Brajon *et al* 2017). Therefore, evaluating the effects of chronic prenatal stress, which affects both pregnant dams and their offspring, could be an alternate and more sensitive measure to evaluate how gestation housing affects sow welfare.

The objectives of this study were to determine the effects of providing periodic exercise to stall-housed sows throughout gestation on sow welfare, as evaluated through the measurement of sow behaviour, stress physiology and measures of prenatal stress, as measured in the behaviour of offspring. Additionally, these measures were compared to those obtained from stall-housed sows receiving no exercise and group-housed sows.

Materials and methods

All experimental procedures were approved by the University of Saskatchewan Animal Care Committee (#20170057). This experiment was conducted between February and November of 2019 at the Prairie Swine Centre, Saskatoon, Canada.

Study animals and husbandry

A total of 180 Camborough 42 sows (parities 0–7; mean [± SD] 2.42 [± 1.76]) were studied. Animals were housed in free-access stall gestation pens (Egebjerg INN-O-STALL® free access stalls, Egebjerg International A/S, Nykøbing Sjælland, Denmark). Each free-access stall pen contained 32 free access stalls, each 2.1 × 0.65 m (length × width), 16 stalls on adjacent sides of the pen, with a 3.0-m wide fully slatted loafing alleyway in between. The design of the free-access stall pens is described in detail in Rioja-Lang *et al* (2013). For experimental purposes, each gestation pen was divided into two halves with a central divider, so there were two rows of eight stalls in each half of the pen. Sows were moved to gestation pens on day 7–10 post breeding and remained in the gestation room until day 107–110 of gestation. A total of 12 experimental animals were randomly selected within one breeding week, forming one replicate block, with a total of 15 replicate blocks. Body condition score (BCS; Doyle *et al* 2015) was determined and recorded for each experimental animal. Sows were fed approximately 2.2 kg of a standard sow gestation diet containing 2.25 Mcal kg^{-1}, 11.96% crude protein and 4.55% crude fibre once per day at 0700h, and each individual stall and group loafing area were equipped with nipple drinkers.

On day 107–110 of gestation, sows were moved to standard farrowing crates equipped with electronic sow feeders and nipple drinkers. Farrowing was allowed to occur naturally, with limited intervention by trained personnel if a piglet birth interval was longer than 3 h. The piglets had access to a lit and heated location at the front of the farrowing crate, which was inaccessible to the sow. Cross-fostering occurred within two days after birth and was performed in accordance with the barn practices to maintain a litter size of 14 piglets per sow. Fostered piglets were not used for behavioural testing in the current study. Commercial husbandry procedures performed by the barn staff on the piglets included teeth clipping (one day of age), as well as ear notching, tail docking, iron injections and castration of male piglets (all at three days of age). Pain control (injectable meloxicam) was administered to both male and female piglets for processing at three days of age. Additionally, ear tagging was performed in those piglets that were selected for behavioural testing.

On day 1 after birth, three female piglets per sow from 17 control sows (distribution by parity group: young: n = 5; mid: n = 8; old: n = 4), 20 exercise sows (young: n = 2; mid: n = 14; old: n = 4) and 20 group sows (young: n = 7; mid: n = 12; old: n = 1) were selected for behavioural testing. The original aim was to test piglets from 20 sows per treatment, but three control sows had to be removed from the trial due to illness or not being pregnant. Out of 57 selected sows, three had only two female piglets, hence for these animals, two piglets were used for behavioural testing. Behavioural testing was performed on day 19–22 after birth, and a total of 168 piglets were tested.

Treatments

Upon moving to gestation pens, sows were assigned to one of three treatments (four sows per treatment per replicate; n = 180 sows, 60 per treatment): sows housed in stalls throughout gestation (control: C); stall-housed sows given weekly exercise throughout gestation (exercise: E); sows housed in static groups after breeding (group: G). Efforts were made to balance treatments by parity. The method of randomisation within blocks was used, with experimental

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animals being blindly selected from the list of available sows, and then assigned to treatments based on their parity. All treatments were represented within the same gestation pen, with individual sow as the experimental unit. Two replicates were represented within each pen, as the central divider prevented two replicate groups from mixing.

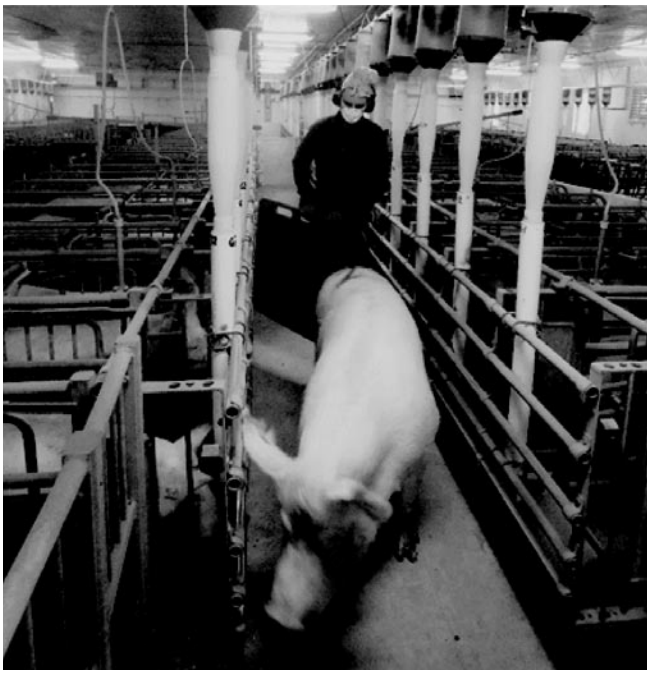
Sows from treatment C stayed locked in the free-access stalls throughout gestation. Sows from treatment E were also locked in stalls throughout gestation, except when removed for exercise. To provide exercise, E sows were backed out of their stalls once a week, walked out of the gestation pen and moved in a loop twice around the alleyways surrounding half of the gestation room (Figure 1). Exercise was performed between 1100 and 1300h on the same day each week. The distance travelled by each sow during one exercise session was approximately 160 m. Sows were encouraged to keep moving by the handler through vocal cues and, if needed, taps from the hand and use of a pig board. Sows were exercised one at a time before being returned to their stall. Sows from treatment G were locked out of the free access stalls for 6–7 h a day, remaining in the group loafing area (5.35 × 3.0 m [length × width]; 4.01 m^2 per sow) with concrete slatted floor, and having free access to the stalls and the loafing area during the rest of time. G sows were locked in the stalls once a week whilst E sows were being exercised.

Sow behavioural observations

A Pentax Optio W90 12.1 camera (Denver, CO, USA) programmed to take photos at 10-min intervals was mounted on the ceiling in the alleyway near each experimental pen to record the stalls containing C and E sows, as well as the loafing area containing G sows. The pictures were collected over two intervals on the same day that exercise provision occurred for E sows; AM data collection: 2 h in the morning before exercise from 0900 to 1100h; PM data collection: 1.5 h in the afternoon after exercise from 1300 to 1430h. Pictures were collected at three stages of gestation: early (week 2 post-breeding), mid (week 10 post-breeding) and late gestation (week 15 post-breeding). Prior to the start of recording, numbers were spray-painted on the back of each experimental sow for individual identification. The pictures were viewed by scan sampling (Martin & Bateson 1993), with the whole group of sows observed at consistent predetermined (10-min) intervals, at which point the postures of individual sows were recorded (Table 1).

Sows were also live-scored for stereotypic behaviours at three stages of gestation on the same days that recordings of sow postures took place. During the data collection, the observer was sitting quietly on a ladder above the level of sow stalls at the end of the aisle between pens, so all the observed animals were visible. Similarly to the posture data collection, sows were observed during two periods each day: a 1-h period in the morning before exercise and after feeding (0900 to 1000h, AM data collection), and a 1-h period in the afternoon after exercise (1330 to 1430h, PM data collection). A 2-min interval scan-sampling technique was used to record the presence of stereotypic behaviours (Table 1).

Figure 1



Sow exercising in the gestation room alleyway.

Chronic stress: hair cortisol analysis

To investigate the value of hair cortisol as a non-invasive, longer term marker of hypothalamic-pituitary-adrenal (HPA) axis activity, hair samples from sows in each treatment (C: n = 17; E: n = 20; G: n = 20), were collected and analysed. The original aim was to collect hair samples from 20 sows per treatment, but three control sows had to be removed from the trial due to illness or not being pregnant. Hair was shaved at weaning from the loin part of the dorsolumbar region from a maximum area of 100 cm^2 and then reshaved before sows entered the trial to achieve the maximal removal of hair growth from the previous lactation. At day 107–110 of gestation upon moving to the farrowing crate, the hair regrowth from the preshaved area was collected and processed for cortisol analysis. Hair was collected by shaving close to the skin with electric clippers, which were cleaned with a brush between sows. Once sampled, hair was stored at room temperature inside paper envelopes until it was analysed.

For hair sample preparation and cortisol extraction, the protocol developed by Macbeth *et al* (2010) was used. Methanol was used as wash solvent, as determined to be most appropriate for removing external contaminants from swine hair (Pollock *et al* unpublished). First, hair samples were mechanically cleaned with forceps to remove gross contaminants (manure, mud), and 100-mg samples of cleaned hair were weighed. Eleven samples were lighter than 100 mg, and these samples were also prepared for cortisol extraction as described below, with the volume of methanol added being reduced proportionally. Each sample was washed in 4 ml (for the 100-mg hair samples) of

Table 1 Sow postures and stereotypies recorded in early (week 2 post-breeding), mid (week 10 post-breeding) and late gestation (week 15 post-breeding) in each of three treatments: control sows housed in individual stalls throughout gestation (n = 53), stall-housed sows given exercise (10 min per week) during gestation (exercise; n = 58), and sows housed in groups throughout gestation (group; n = 58). (Adapted from Chapinal *et al* 2010 and Zhang *et al* 2017).

Category of behaviour	Description
Posture[†]	
Lying	The animal is lying down with or without a head in contact with the floor and non-weight-bearing on its limbs. Both lateral (lying on the side) and sternal (lying on the abdomen) lying were included
Standing	The animal is standing up with all four hooves in contact with the floor/other objects and weight-bearing on all four limbs
Sitting	The animal is sitting up with front legs extended and weight on the rump
Stereotypic behaviour[‡]	
Sham chewing	Continuous chewing with no substrate present in the mouth
Bar-biting	Nosing, rubbing, licking or biting any metal component of the stall other than the trough
Tongue-rolling	Extending the tongue out of the mouth and curling it to the side, without the tongue contacting any object
Trough manipulation	Nosing, rubbing, licking or biting the trough

[†] Recorded using scan sampling at 10-min intervals;
[‡] Recorded using scan sampling at 2-min intervals.

methanol three times. The washing procedure involved rotating the hair samples in the haematology mixer at 12 rpm for 3 min per wash, and then the samples were allowed to dry in plastic petri dishes at room temperature. After a minimum of 24 h drying, samples were ground into a fine powder with a Retsch MM 301 Mixer Mill (Retsch Inc, Newton, PA, USA) at 30 Hz. For the 100-mg samples, the grinding time was 0.03 min per mg of hair, and 10-ml stainless steel grinding jars with a 12-mm grinding ball were used; for samples lighter than 100 mg, the grinding time was 0.15 min per mg of hair, and 5-ml stainless steel grinding jars with a 7-mm grinding ball were used. After grinding, 25-mg samples of ground hair were weighed, transferred to 0.6-ml microtubes and stored at room temperature out of direct sunlight.

For cortisol extraction, 0.5 ml of HPLC grade methanol was added to each sample, which was then vortexed for 10–15 s and placed on an automatic rotator at 18 rotations per min for 24 h. Afterwards, the samples were spun in a centrifuge at 4,500 rpm for 15 min. The entire supernatant was transferred to the bottom of a glass, 12 × 75 mm (diameter × length) test tube and dried under a gentle stream of nitrogen gas at 38°C. The extraction procedure was repeated for three collections in total to ensure that all steroids were recovered. Next, steroids were rinsed to the bottom of the test tubes with three consecutive methanol washes (0.4, 0.2 and 0.15 ml of methanol) and dried under a gentle stream of nitrogen gas at 38°C after each wash. Concentrated samples were reconstituted with 0.2 ml of phosphate buffer, vortexed on the lowest setting for 10 s, incubated at 4°C for 12 h, and repeatedly vortexed for 40 s. Samples were centrifuged for 2 min at 4,000 rpm and 20°C, transferred to 0.6-ml plastic vials and spun at 4,500 rpm for 5 min. The supernatants were collected and analysed with a commercially available EIA kit (Salimetrics® High Sensitivity Salivary Cortisol Enzyme Immunoassay kit; Salimetrics LLC, State College, PA, USA). The kit has previously been validated for use for the cortisol analysis in swine hair by Casal *et al* (2017) and presented good linearity with $R = 0.999$, and a recovery yield of 79.6 (± 3.2)% assessed by spiking the sample before extraction with pure cortisol. The measured hormone concentration in these samples correlated with the expected concentrations ($R = 0.999$), with the limit of detection being equal to 0.017 μg dL^{-1}, and the limit of quantification being equal to 0.05 μg dL^{-1}. Cross-reactivity of the antibody used for the EIA kit according to the manufacturer was: prednisolone (0.57%), cortisone (0.13%), 11-deoxycortisol (0.16%), dexamethasone (19.2%), corticosterone (0.21%), triamcinolone (0.09%). All other intermediates and hormones reported by the manufacturer exhibited cross-reactivity of ≤ 0.04%.

Piglet behavioural testing

As an additional measure of maternal stress, prenatal stress effects on the piglet behavioural response to stress were evaluated via isolation and novel object tests performed on a sub-sample of female piglets on days 19–22 after birth. For the testing, three experimental piglets from each sow were placed in a cart and moved from the farrowing room to the isolated waiting room equipped with a heat lamp; this room was adjacent to the test room. Each piglet was carried into the isolated test arena from the waiting room one at a time. The arena had solid concrete flooring measuring 2.95 × 2.36 m (length × width), and solid opaque walls, and was divided into 20 equal-sized squares using lines on the floor. Additionally, two circles with a radius of 0.50 and 1.00 m

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were drawn in the centre of the arena. The number of squares visited by a piglet, and the number of vocalisations within the 2-min isolation test were live-recorded by an experienced observer, blinded to treatment. A piglet was considered to have visited a square when the two front legs of the piglet were inside the square. Following cessation of the isolation test, there was a 1-min break when the piglet was left in the arena, following which a novel object (Bite-Rite toy, Ikadan System A/S, Ikast, Denmark) was placed in the centre of the test arena. The frequency of entering 1.00-m and 0.50-m circles around the novel object, the frequency of exiting the 1.00-m circle and remaining in the area bordering the perimeter of the pen, the number of squares visited, and the number of vocalisations during the novel object test were live-recorded by an experienced observer during the 2-min test. A piglet was considered to have visited a circle if the piglet changed its position from being outside of a certain circle with all four legs to having its two front legs inside this circle. The latencies to enter the 1.00-m and 0.50-m circles, latency to touch the novel object and the number of contacts were live-scored by another experienced observer during the 2-min period. Following these tests, the piglet was returned to the farrowing pen. Piglet behaviour throughout the tests was video-recorded with a Canon Vixia HF R800 camcorder (Canon Canada Inc, Brampton, ON, Canada) so the sessions could be re-watched if information needed to be verified.

Statistical analysis

For the statistical analysis, sows were assigned to one of three parity groups: young (parity 0–1; n = 49), mid (parity 2–4; n = 95) and old parity sows (parity 5–7; n = 24). To calculate the relative frequency of performing a certain behaviour, the number of times spent in this behaviour during one behavioural observation period (AM or PM data collection) was divided by the total number of behavioural events recorded in this observation. Each individual was considered as the statistical unit. Data were analysed using the statistical package SAS 9.4 (SAS Institute, Cary, NC, USA). The significance level was set at $P \leq 0.05$, and results with $P < 0.10$ were considered as statistical trends. Residuals of all dependent variables were examined for normality and homogeneity of variance, and the data were transformed as necessary. The least-square means (LSMEANS) of fixed effects with Tukey's adjustment were used to account for multiple comparisons. Results are presented as the mean and SEM from the raw (not transformed) data as computed from the final model. For the main effects, *P*-values and *F*-values are presented, and adjusted *P*-values are presented for *post hoc* comparisons.

Sow behaviour data

To compare postures of sows from control, exercise and group treatments, relative frequencies of lying, standing and sitting out of total observations per data collection period (AM or PM) were calculated per sow, and then analysed in separate models for each posture and each data collection period. Missing sow behaviour observations were considered as missing values for the sow behaviour analysis. To compare the levels of stereotypies performed in sows across different experimental treatments, relative frequencies of the total of recorded stereotypic behaviours (obtained from summing the frequencies of performing sham chewing, bar-biting, tongue-rolling and trough manipulation) were calculated and analysed in separate models for each data collection period (AM and PM). A mixed model (PROC MIXED procedure in SAS) with repeated measures of the stage of gestation and sow as a subject was used for analysing postures and stereotypy data, and simple correlation structure was used. The fixed effects of treatment, parity group and stage of gestation, as well as interactive effects of treatment and stage of gestation, and treatment and parity group were tested. The interactive effect of treatment and parity group was not significant in the posture models and hence was removed from these models but kept in the stereotypy models. The random effect of replicate was included in all models. The AM sow posture data required square-root transformation, and the PM sow posture data required arcsine square-root transformation. The AM stereotypy data required arcsine square-root transformation, and the PM stereotypy data required square-root transformation.

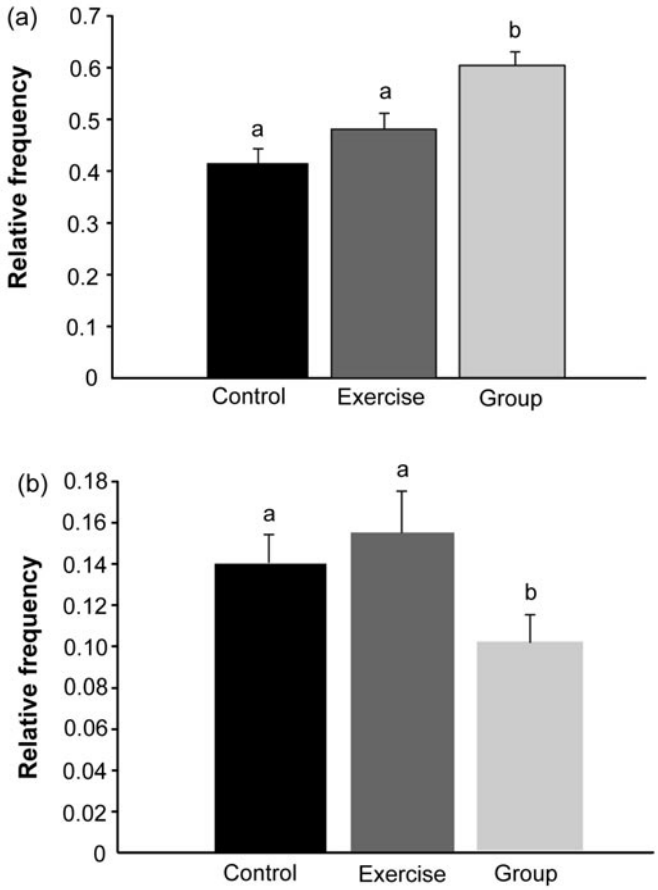
Hair cortisol data

A mixed model (PROC MIXED in SAS) was used to compare sow treatments for differences in hair cortisol, with treatment, parity group, BCS and interaction of treatment and parity group as main effects, and total litter size as a covariate. The values for hair cortisol were not normally distributed, and were log-transformed. The effects of parity group, BCS and interactive effects of sow treatment and parity group were not significant and these factors were removed from the model.

Piglet testing data

To evaluate the effects of sow treatment and parity group on piglet behaviour measures in the isolation test and novel object test, a mixed model (PROC MIXED) was used. If a piglet did not enter a certain circle or did not touch the novel object, it was assigned the maximal latency of 120 s for this measure. The mixed model was the model of choice for the piglet data, as using this model resulted in better fit statistics in comparison to PROC GLIMMIX model with Poisson distribution, which was also tested. Piglet nested within sow and replicate were included as random effects. Each of the behaviour variables of interest was analysed in a separate model, and all the variables required log-transformation. Parity group did not show a significant association in the isolation test, and it was removed from the final models for this test. The interaction of treatment and parity group did not have a significant effect for either the isolation test or the novel object test and was removed from all of the models.

Figure 2



Mean (± SEM) relative frequency of (a) lying and (b) sitting during the AM data collection period (0900 to 1000h) for sows stall-housed throughout gestation (Control; n = 53), stall-housed sows exercised for 10 min once per week (Exercise; n = 56) and sows housed in groups from insemination to farrowing (Group; n = 58). Where superscripts differ; $P \leq 0.05$.

Results

In total, 12 sows were removed from the trial and their data were not included in the final statistical analysis, nine of these sows aborted (C: n = 5; E: n = 3; G: n = 1), and three were removed due to illness (C: n = 2; G: n = 1). Additionally, the posture data were missing for certain replicates over some data collection periods due to a secure digital card malfunction. Given these circumstances, the population of sows included in the analysed data was as follows: AM, early gestation: n = 146; AM mid gestation: n = 157; AM late gestation: n = 135; PM early gestation: n = 146; PM mid gestation: n = 156; PM late gestation: n = 134. For the main effects, *P*-values and *F*-values are presented, and adjusted *P*-values are presented for *post hoc* comparisons.

Sow behaviour

Postures

For sow postures in AM, the relative frequency of lying was influenced by sow treatment ($F_{2,411} = 6.55$; $P = 0.002$), parity group ($F_{2,424} = 7.05$; $P = 0.001$) and stage of gestation ($F_{2,422} = 18.42$; $P < 0.001$). Group sows lay more than C and E sows, which did not differ (Figure 2[a]). Young sows spent significantly more time lying, than mid ($P = 0.004$) and old parity sows ($P = 0.001$), for which the relative frequency of lying did not differ ($P = 0.112$; Young: 0.59 [± 0.04], Mid: 0.49 [± 0.03], Old: 0.45 [± 0.04], mean [± SEM]). Sows in mid gestation spent more time lying, than sows in early gestation ($P = 0.028$), and sows in late gestation lay more than sows in early ($P < 0.001$) and mid ($P < 0.001$) gestation (Early: 0.41 [± 0.03], Mid: 0.48 [± 0.03], Late: 0.63 [± 0.04]).

The relative frequency of sitting in AM was influenced by sow treatment ($F_{2,413} = 6.64$; $P = 0.001$) and stage of gestation ($F_{2,421} = 7.56$; $P = 0.001$). Group sows sat less than C and E sows, which did not differ (Figure 2[b]). Sows in early gestation spent less time sitting, than sows in mid ($P = 0.003$) and late ($P < 0.001$) gestation, which did not differ ($P = 0.398$; Early: 0.10 [± 0.02], Mid: 0.15 [± 0.02], Late: 0.15 [± 0.02]). There was no effect of parity group on the relative frequency of sitting (Young: 0.12 [± 0.02], Mid: 0.13 [± 0.01], Old: 0.14 [± 0.02]). There was also no interactive effect of treatment and stage of gestation on the relative frequency of lying and sitting in AM (Table 2).

For standing in AM, there was an effect of parity ($F_{2,417} = 4.22$; $P = 0.015$) and an interactive effect of treatment and stage of gestation ($F_{4,412} = 2.71$; $P = 0.030$). In early gestation, C sows tended to spend less time standing, than E sows, but did not differ from G sows, and E sows

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Table 2 Mean (± SEM) relative frequency of lying, sitting and standing during the AM (0900 to 1000h) and PM (1330 to 1430h) data collection periods for sows stall-housed throughout gestation (C, Control; n = 53), stall-housed sows exercised for 10 min once per week (E, Exercise; n = 56) and sows housed in groups from insemination to farrowing (G, Group; n = 58) in early (week 2), mid (week 10) and late gestation (week 15).

Posture	Stage of gestation									*F*-value	*P*-value
	Early			Mid			Late				
	Treatment										
	C	E	G	C	E	G	C	E	G		
	n = 53	n = 56	n = 58	n = 53	n = 56	n = 58	n = 53	n = 56			
AM											
Lying	0.37 (± 0.05)	0.34 (± 0.05)	0.52 (± 0.05)	0.41 (± 0.05)	0.51 (± 0.05)	0.51 (± 0.05)	0.55 (± 0.05)	0.63 (± 0.05)	0.72 (± 0.05)	1.73	0.143
Sitting	0.12 (± 0.03)	0.13 (± 0.03)	0.06 (± 0.03)	0.15 (± 0.03)	0.19 (± 0.03)	0.10 (± 0.03)	0.17 (± 0.03)	0.15 (± 0.03)	0.13 (± 0.03)	1.06	0.377
Standing	0.46 (± 0.04)^{a*}	0.54 (± 0.04)^{b*}	0.42 (± 0.04)^{a*}	0.43 (± 0.04)^{a*}	0.30 (± 0.04)^{b*}	0.37 (± 0.04)^{ab*}	0.28 (± 0.05)^{a}	0.23 (± 0.04)^{ab}	0.12 (± 0.05)^{b}	2.71	0.030
PM											
Lying	0.59 (± 0.05)	0.62 (± 0.05)	0.59 (± 0.05)	0.69 (± 0.05)	0.64 (± 0.05)	0.72 (± 0.05)	0.74 (± 0.05)	0.75 (± 0.05)	0.76 (± 0.05)	0.49	0.740
Sitting	0.08 (± 0.02)	0.10 (± 0.02)	0.03 (± 0.02)	0.08 (± 0.02)	0.14 (± 0.02)	0.04 (± 0.02)	0.09 (± 0.02)	0.12 (± 0.02)	0.06 (± 0.02)	0.46	0.766
Standing	0.26 (± 0.04)	0.26 (± 0.04)	0.32 (± 0.04)	0.21 (± 0.04)	0.22 (± 0.04)	0.20 (± 0.04)	0.18 (± 0.04)	0.13 (± 0.04)	0.16 (± 0.04)	0.84	0.498

Values with different superscripts differ significantly ($P \leq 0.05$), asterisks indicate tendency ($P < 0.1$).

tended to stand more, than G sows. In mid gestation, C sows tended to stand more than E sows, with G sows being intermediate. In late gestation, C sows stood more, than G sows, with E sows being intermediate (Figure 3). Also, C sows spent more time standing in early, than in late gestation ($P = 0.007$). In early gestation, E sows stood more than in mid and late gestation ($P < 0.001$). Exercised sows in mid gestation tended ($P = 0.054$) to stand more, than in late gestation. Group sows in late gestation stood less ($P < 0.001$), than in early and mid gestation.

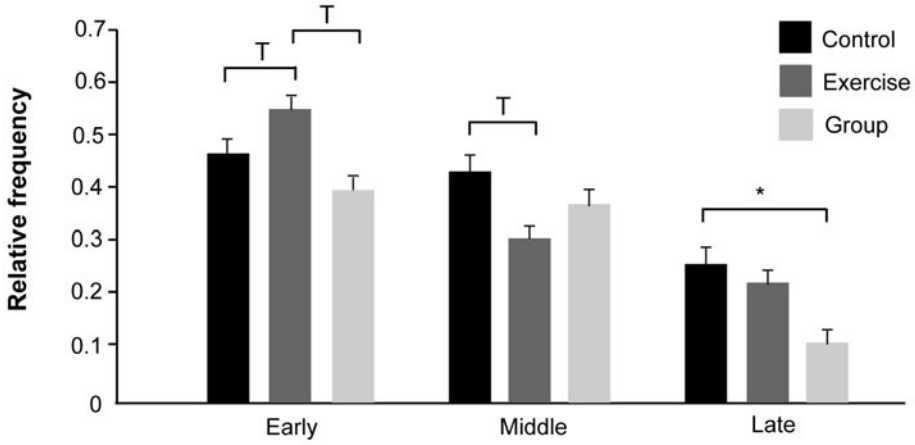
Young sows spent significantly less time standing in AM than mid ($P = 0.007$) and old sows ($P = 0.027$), for which the proportion of time spent standing did not differ (Young: 0.28 [± 0.03], Mid: 0.37 [± 0.02], Old: 0.39 [± 0.04]).

Within PM measurements, the relative frequency of lying did not differ by treatment (Table 3), and there was no interaction between treatment and stage of gestation (Table 2). Parity group tended ($F_{2,424} = 2.49$; $P = 0.084$) to affect the relative frequency of lying, with old sows lying less than young sows, and mid sows being intermediate (Young: 0.72 [± 0.04], Mid: 0.68 [± 0.03], Old: 0.63 [± 0.05]). The relative frequency of lying in PM was affected by the stage of gestation ($F_{2,423} = 7.40$; $P = 0.001$). Sows in early gestation lay less than in mid ($P = 0.040$) and late gestation ($P < 0.001$), and there was a tendency ($P = 0.053$) for sows in late gestation to lie more than in mid gestation (Early: 0.60 [± 0.04], Mid: 0.68 [± 0.03], Late: 0.75 [± 0.04]).

There was an effect of treatment on the relative frequency of sitting in PM ($F_{2,424} = 10.93$; $P < 0.001$). E sows sat more than C ($P = 0.025$) and G sows ($P < 0.001$), and C sows sat more, than G sows ($P = 0.021$; C: 0.08 [± 0.01], E: 0.12 [± 0.01], G: 0.04 [± 0.01]). There was no effect of parity group on the relative frequency of sitting in PM (Young: 0.07 [± 0.01], Mid: 0.07 [± 0.01], Old: 0.10 [± 0.02]). There was also no effect of the stage of gestation (Table 3) and no interactive effect of treatment and stage of gestation on the relative frequency of sitting in PM (Table 2).

There was no effect of treatment on the relative frequency of standing in PM (Table 3), but it was influenced by sow parity group ($F_{2,424} = 3.98$; $P = 0.019$) and by the stage of gestation ($F_{2,423} = 5.84$; $P = 0.003$). Young sows stood significantly less, than mid parity ($P = 0.008$) and old sows ($P = 0.036$), for which the relative frequency of standing did not differ (Young: 0.16 [± 0.03], Mid: 0.24 [± 0.03], Old: 0.25 [± 0.04]). Sows stood less in late gestation than in early ($P = 0.001$) and mid gestation ($P = 0.051$) and, for sows in early and mid gestation, the relative frequency of standing did not differ (Early: 0.28 [± 0.03], Mid: 0.21 [± 0.03], Late: 0.16 [± 0.03]). There was no interactive effect of treatment and stage of gestation on the relative frequency of standing in PM (Table 2).

Figure 3



Mean (± SEM) relative frequency of standing during the AM data collection period (0900 to 1000h) for sows stall-housed throughout gestation (Control; n = 53), stall-housed sows exercised for 10 min once per week (Exercise; n = 56) and sows housed in groups from insemination to farrowing (Group; n = 58) in early (week 2), mid (week 10) and late gestation (week 15). Brackets connect treatments with significant differences. * $P \leq 0.05$; T: Tendency, $P < 0.1$.

Table 3 Mean (± SEM) relative frequency of lying, sitting and standing during the PM (1330 to 1430h) data collection period for sows stall-housed throughout gestation (C, Control; n = 53), stall-housed sows exercised for 10 min once per week (E, Exercise; n = 56) and sows housed in groups from insemination to farrowing (G, Group; n = 58) in early (week 2), mid (week 10) and late gestation (week 15).

Posture	Treatment			*F*-value	*P*-value	Stage of gestation			*F*-value	*P*-value
	C	E	G			Early	Mid	Late		
	n = 53	n = 56	n = 58			n = 167	n = 167	n = 167		
Lying	0.67 (± 0.04)	0.67 (± 0.04)	0.69 (± 0.04)	0.14	0.867	0.60 (± 0.04)[a]	0.68 (± 0.03)[b]*	0.75 (± 0.04)[c]*	7.40	0.001
Sitting	0.15 (± 0.02)[a]	0.16 (± 0.02)[b]	0.10 (± 0.02)[c]	10.93	< 0.001	0.10 (± 0.02)	0.15 (± 0.02)	0.15 (± 0.02)	0.94	0.392
Standing	0.22 (± 0.30)	0.21 (± 0.03)	0.23 (± 0.03)	0.44	0.641	0.28 (± 0.03)[a]	0.21 (± 0.03)[a]	0.16 (± 0.03)[b]	5.84	0.003

Values with different superscripts differ significantly ($P \leq 0.05$), asterisks indicate tendency ($P < 0.1$).

Stereotypic behaviour

There was an interactive effect of treatment and stage of gestation on the relative frequency of stereotypies in AM ($F_{4,456} = 2.51$; $P = 0.041$). In early gestation, C sows tended to perform more stereotypies than G sows, but did not differ from E sows, which performed significantly more stereotypies than G sows. In mid gestation, G sows performed fewer stereotypies than C and E sows, which did not differ. However, in late gestation, the relative frequency of performing stereotypies did not differ across all three treatments (Figure 4). In early gestation, C sows performed fewer stereotypies than in mid ($P < 0.001$) and late gestation ($P = 0.019$), which did not differ. Similarly, E sows in early gestation performed fewer stereotypies than in mid ($P = 0.001$) and late gestation ($P = 0.017$), which did not differ. Sows from the group treatment in late gestation performed significantly more stereotypies than in early ($P < 0.001$) and mid gestation ($P = 0.005$), for which the relative frequency of performing stereotypies did not differ.

There was also an interactive effect of treatment and parity group on the relative frequency of performing stereotypies in AM ($F_{4,446} = 3.06$; $P = 0.017$). Young C sows performed more stereotypies than young E and G sows ($P = 0.016$), which did not differ. Mid parity E sows performed more stereotypies than mid C and G sows ($P = 0.019$), which did not differ. Old G sows performed fewer stereotypies than old C and E sows ($P = 0.035$), which did not differ. Young C sows performed fewer stereotypies in AM than old control sows ($P = 0.003$), and they tended ($P = 0.058$) to perform fewer stereotypies than mid parity C sows. Mid C sows tended ($P = 0.089$) to perform fewer stereotypies than old C sows. Young E sows performed significantly fewer stereotypies than mid and old E sows ($P < 0.001$), which did not differ. Similarly, young G sows performed fewer stereo-

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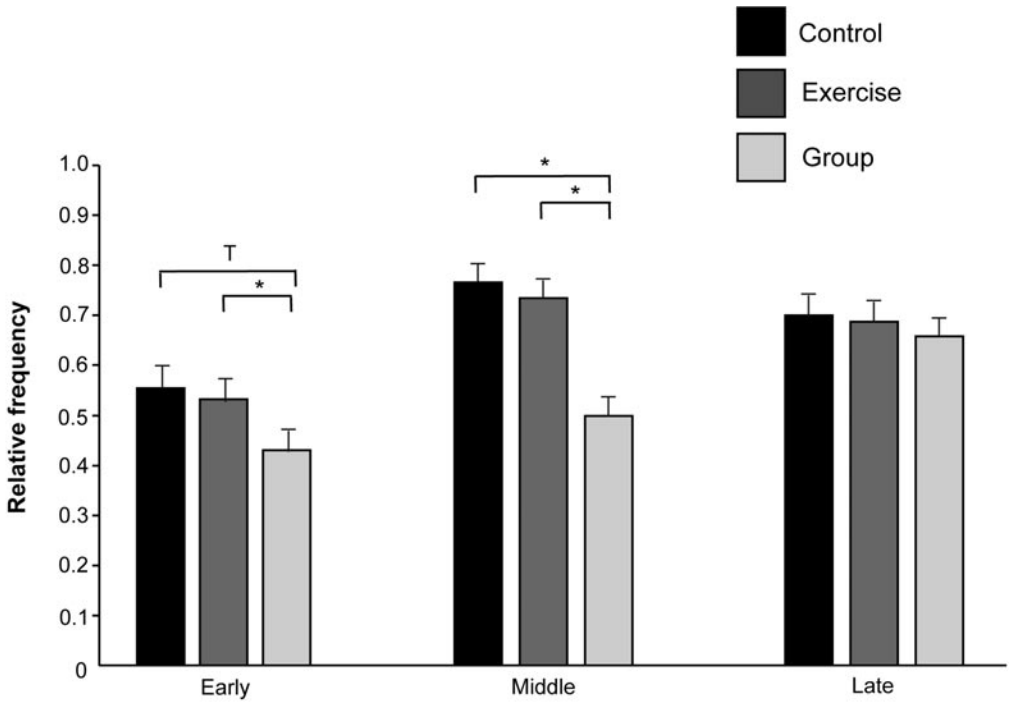
Figure 4



Mean ± (SEM) relative frequency of performing stereotypies (total of sham chewing, bar-biting, tongue-rolling and trough manipulation) during the AM data collection period (0900 to 1000h) for sows stall-housed throughout gestation (Control; n = 53), stall-housed sows exercised for 10 min once per week (Exercise; n = 56) and sows housed in groups from insemination to farrowing (Group; n = 58) in early (week 2), mid (week 10) and late gestation (week 15). Brackets connect treatments with significant differences. * $P \leq 0.05$; T: Tendency, $P < 0.1$.

Table 4 Mean (± SEM) relative frequency of performing stereotypies (a total of sham chewing, bar-biting, tongue-rolling and trough manipulation) during the PM (1330 to 1430h) data collection period for sows stall-housed throughout gestation (C, Control; n = 53), stall-housed sows exercised for 10 min once per week (E, Exercise; n = 56) and sows housed in groups from insemination to farrowing (G, Group; n = 58) in early (week 2), mid (week 10) and late gestation (week 15).

Stage of gestation	Treatment		
	C	E	G
	n = 53	n = 56	n = 58
Early	0.32 (± 0.05)	0.37 (± 0.05)	0.21 (± 0.05)
Mid	0.47 (± 0.05)	0.50 (± 0.05)	0.31 (± 0.05)
Late	0.36 (± 0.05)	0.33 (± 0.05)	0.35 (± 0.05)

$F_{4,459} = 1$; $P = 0.136$.

typies, than mid parity and old ($P = 0.012$) G sows, which did not differ (Young C: 0.58 [± 0.04], Mid C: 0.67 [± 0.03], Old C: 0.77 [± 0.05]; Young E: 0.41 [± 0.05], Mid E: 0.77 [± 0.03], Old E: 0.78 [± 0.06]; Young G: 0.41 [± 0.04], Mid G: 0.60 [± 0.03], Old G: 0.59 [± 0.06]).

In PM, the relative frequency of performing stereotypies was influenced by the stage of gestation ($F_{4,446} = 3.06$; $P = 0.006$). In mid gestation, sows performed more stereotypies, than in early ($P = 0.005$) and late gestation ($P = 0.007$), for which the levels of performing stereotypies did not differ (Early: 0.30 [± 0.03], Middle: 0.43 [± 0.03], Late: 0.34 [± 0.03]). There was no interactive effect of treatment and stage of gestation on the relative frequency of performing stereotypies in PM (Table 4).

There was an interactive effect of treatment and parity group on the relative frequency of performing stereotypies in PM ($F_{4,471} = 4.47$; $P = 0.002$). Young C sows performed more stereotypies than young E sows ($P = 0.007$), and they

Table 5 Results of isolation and novel object tests in piglets (n = 168; 2 to 3 piglets per sow), born from sows that were stall-housed throughout gestation (Control; n = 17, 49 piglets), sows stall-housed and walked 10 min around the gestation room once per week (Exercise; n = 20, 59 piglets) and sows housed in groups from insemination to farrowing (Group; n = 20, 60 piglets), (mean [± pooled SEM]).

Variable	Control (n = 49)	Exercise (n = 59)	Group (n = 60)	Pooled SEM	*F*-value	*P*-value
Isolation test						
Number of squares visited	23.52	18.31	19.18	3.74	1.63	0.199
Number of vocalisations	80.51[a]*	65.14[b]*	78.70[a]*	12.24	3.02	0.052
Novel object test						
Number of squares visited	23.10[a]	17.64[b]	18.67[b]	3.80	3.14	0.046
Number of vocalisations	61.05	48.41	57.12	12.24	1.55	0.216
Frequency of entering the 1.00-m circle	4.99	4.03	4.23	0.83	1.65	0.196
Frequency of entering the 0.50-m circle	2.64[a]*	1.86[b]*	2.01[ab]*	0.48	2.94	0.056
Frequency of exiting the 1.00-m circle and remaining in the area bordering the perimeter of the pen	2.45	1.98	2.27	0.45	0.98	0.379
Latency to enter the 1.00-m circle (s)	30.54	41.78	37.51	12.59	0.50	0.606
Latency to enter the 0.50-m circle (s)	40.95	56.53	47.13	13.63	0.40	0.670
Latency to touch the novel object (s)	45.03	61.67	51.33	13.65	0.47	0.623
Number of contacts with the novel object	3.21	2.32	2.19	0.83	0.92	0.400

Values with different superscripts differ significantly ($P \leq 0.05$), asterisks indicate tendency ($P < 0.1$).

tended to perform more stereotypies than young G sows ($P = 0.067$). Young E sows did not differ in their levels of performing stereotypies from young G sows. Mid parity G sows performed fewer stereotypies than mid C and E sows ($P = 0.004$), which did not differ. Old E sows performed more stereotypies, than old C sows ($P = 0.017$), and they tended to perform more stereotypies than old G sows ($P = 0.054$), but the relative frequencies of performing stereotypies in old C and old G sows did not differ. Young C sows did not differ in their performance of stereotypies from mid and old C sows, but mid control sows tended ($P = 0.067$) to perform more stereotypies than old C sows. Young E sows performed fewer stereotypies than mid and old exercised sows ($P < 0.001$), which did not differ. For G sows, the levels of performing stereotypies did not differ across different parity groups (Young G vs Mid G: $P = 0.749$, Mid G vs Old G: $P = 0.703$, Young G vs Old G: $P = 0.577$; Young C: 0.39 [± 0.05], Mid C: 0.44 [± 0.04], Old C: 0.32 [± 0.07]; Young E: 0.19 [± 0.06], Mid E: 0.49 [± 0.04], Old E: 0.54 [± 0.07]; Young G: 0.27 [± 0.05], Mid G: 0.29 [± 0.04], Old G: 0.32 [± 0.08]).

Hair cortisol analysis

The intra-assay coefficients of variation for high and low hair concentration samples were 6.37 and 13.75%, respectively. The inter-assay coefficients of variation for high and low cortisol concentration were 13.82 and 7.06%, respectively. The coefficients of variation between duplicate samples were not higher than the accepted cut-offs (15%). However, the final results need to be interpreted with caution, as some of the intra-assay coefficients of variation were higher than the accepted cut-off of 10%, which might reflect some pipetting errors, for example.

There was no effect of treatment on sow hair cortisol levels (C: 37.24 [± 5.81] pg mg^{-1}; E: 29.34 [± 5.41] pg mg^{-1}; G: 35.83 [± 5.41] pg mg^{-1}; $F_{2,53} = 0.91$; $P = 0.410$).

Piglet behavioural response to stress tests

During the isolation test, piglets from C and G sows had a tendency to vocalise more than piglets from E sows (Table 5), with the numbers of vocalisations from piglets of C and G sows being no different.

During the novel object test, piglets from C sows visited significantly more squares than piglets from E and G sows which did not differ (Table 5). Piglets from C sows also had a tendency to come within 0.50 m of the novel object more frequently than piglets from E sows, with piglets from G sows being intermediate. All other results of the isolation test and the novel object test did not differ across treatments (Table 5).

In the novel object test, the latency to enter the 1.00-m circle tended to be influenced by parity group ($F_{2,112} = 2.41$; $P = 0.095$): piglets from old parity sows tended to have lower latency to enter the 1.00-m circle, than piglets from

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Table 6 Results of the novel object tests in piglets (n = 168; 2 to 3 piglets per sow), born from sows belonging to young (parity 0–1; n = 14; 41 piglets), mid (parity 2–4; n = 34; 101 piglets), and old (parity 5–7; n = 9; 26 piglets) parity groups (mean [± pooled SEM]).

Variable	Young (n = 41)	Mid (n = 101)	Old (n = 26)	Pooled SEM	*F*-value	*P*-value
Number of squares visited	19.42	20.43	19.52	4.58	0.10	0.909
Number of vocalisations	58.44	52.57	55.57	14.96	0.59	0.554
Frequency of entering the 1.00-m circle	4.30	4.33	4.63	0.95	0.47	0.629
Frequency of entering the 0.50-m circle	2.10	2.10	2.30	0.55	0.08	0.925
Frequency of exiting the 1.00-m circle and remaining in the area bordering the perimeter of the pen	2.10	2.19	2.44	0.55	0.53	0.589
Latency to enter the 1.00-m circle (s)	39.76a*	41.66a*	28.41b*	14.85	2.41	0.095
Latency to enter the 0.50-m circle (s)	50.13	52.62	41.86	16.36	1.17	0.312
Latency to touch the novel object (s)	52.69	56.13	49.22	16.46	0.86	0.428
Number of contacts with the novel object	2.57	2.81	2.34	1.02	0.06	0.940

Values with different superscripts differ significantly ($P \leq 0.05$), asterisks indicate tendency ($P < 0.1$).

young and mid parity sows, which did not differ (Young; n = 14: 39.76 [± 8.45], Mid; n = 34: 41.66 [± 5.65], Old; n = 9: 28.41 [± 9.64]). All other results of the novel object test did not differ across parity groups (Table 6).

Discussion

In the present study, the analysis of postures showed that group-housed sows laid more (in AM) and sat less (in AM and PM) than stall-housed sows with or without access to periodic exercise. Also, group-housed sows stood less in AM in late gestation than control sows. These results are in agreement with the findings of Weng *et al* (2009) and Chapinal *et al* (2010), who reported a greater proportion of sows lying when housed in groups than in stalls. Weng *et al* (2009) also reported that stalled sows spent more time sitting and standing than group-housed sows. If the increased time standing is interpreted as being more restless, and increased lying is interpreted as being less restless, then sows which were standing more can be considered as having lower welfare. Previous research has found that sows show lower activity when housed in tether stalls, and compared to sows in groups, tethered sows had a higher density of μ opioid receptors in the frontal cortex, which means that in a chronic stress situation these animals would have higher levels of circulating endogenous opioids, diminishing the impact of stress (Zanella *et al* 1996; Drolet *et al* 2001), which in turn could be responsible for the reduced activity levels observed (increased percentage of time lying, sitting or standing idle; Zanella *et al* 1996), further suggesting improved sow welfare. Odberg (1987) demonstrated that frustration, induced by unresolved conflicts (such as an unsatisfied need for exploration in animals housed in the barren environment) can elevate the levels of arousal and lead to increased general activity, which could also explain the higher relative frequency of standing in the current study. At the same time, the type of housing system and space allowance need to be considered when interpreting these results. For instance, an increased proportion of time spent lying and reduced time standing was observed in sows housed in narrow stalls, which suggests that severe space restrictions increase the difficulty of standing (Li & Gonyou 2007). Sitting, as an intermediate posture between lying and standing, is suggested to be an indicator of difficulty to change posture, for example, in a narrow stall (Li & Gonyou 2007), which can explain the decrease in the relative frequency of sitting in group-housed sows. In the current study, the lower performance of lying behaviour and increased performance of standing in stall-housed (control and exercised) sows is possibly linked to lower levels of lying comfort and increased arousal, resulting in restlessness. Arousal, defined as non-specific internal effects that modulate the expression of specific motivational states by affecting the general activity of the animal (Lawrence & Terlouw 1993), could be induced by frustration related to prevention of foraging in feed-restricted sows, which is highly motivated behaviour (Odberg 1978), especially in the presence of a feed trough in the stall. This idea is supported by the fact that the majority of the posture differences were observed in AM, in the hours following the sows finishing their meal. Reduced level of comfort in the stall, which can be related to restricted space and lack of control over the environment, such as inability to separate dunging, feeding and lying areas, inability to adjust location in response to ambient temperature, lack of opportunities to establish a social relationship and inability to avoid aggressive sows or fear-eliciting stimuli, has been proposed to reduce the relative frequency of lying in stalled sows (Rhodes *et al* 2005).

Control and periodically exercised sows did not differ in their relative frequency of lying and sitting, and also standing in late gestation, as opposed to the study of Harris *et al* (2013), which found that stall-housed gilts sat longer, stood less, and tended to lie more in comparison to exercised gilts. This difference might be due to the more intensive exercise schedule used in the study of Harris *et al* (2013), which required gilts to be exercised for 30 min three times per week from mid to late gestation. This finding suggests that periodic exercise provided at a low level, as examined in the current study, does not influence the sow postural repertoire, but housing in groups does.

The relatively small body size of young sows, which allows fitting in conventional stalls more comfortably, may explain the increased relative frequency of lying in AM, decreased standing in AM and PM, as well as the tendency for increased lying in PM in young parity sows. These results are in agreement with the findings of Broom *et al* (1995), who reported an increase in general activity (as determined by the time spent in standing, sitting and moving) in fourth-parity sows in comparison to first parity sows in stalls and groups, with the most dramatic increase being observed in stall-housed sows. Similarly, Zhang *et al* (2017) found that stall-housed pregnant gilts (starting from day 55 of gestation) stood less than parity 3–4 sows and that parity 0–1 sows lay laterally more than older sows (parity 2 and 5), suggesting that younger and smaller animals experience more comfort in standard stalls due to a proportionally lower space restriction, than older and larger sows.

The increase in the relative frequency of lying (in AM and PM) and sitting (in AM) and decrease in the relative frequency of standing in AM and PM as gestation advanced, is consistent with previous findings. For example, Marchant-Forde and Marchant-Forde (2004) reported a progressive decline in the proportion of standing and a corresponding increase in lying behaviour (from 54 to 73% of the time) over gestation in group-housed gilts. These results could be related to an increase in metabolic requirements and weight of actively growing fetuses, which promotes a reduction in physical activity. The observed shift in activity levels also could happen due to the changes in sow size, with larger sows during the advanced stages of gestation finding it more difficult to move.

It was found that in early and mid gestation, group-housed sows performed fewer stereotypies in AM compared to stall-housed sows (with or without having opportunities to exercise periodically), but for late gestation in AM, and for all stages of gestation in PM, the level of stereotypies did not differ across treatments. The AM results suggest that housing in barren group pens can temporarily relieve stress, related primarily to frustration post-feeding due to ingestion of an insufficient amount of feed (Chapinal *et al* 2010) during the first two trimesters of sow gestation, but it is not effective during the last trimester. No differences in performing stereotypies in PM suggests that group housing does not reduce the performance of stereotypies which are less associated with restricted feeding.

Interestingly, the performance of stereotypies in AM in sows from all treatments increased with age and with the stage of gestation. These findings are in agreement with the results of Broom *et al* (1995), who reported that fourth-parity sows spent a greater proportion of time sham chewing in comparison to primiparous sows. Zhang *et al* (2017) also reported results comparable to the current study: the authors demonstrated a gradual increase in the frequency of sham chewing from day 25 to day 100 of gestation in stall-housed sows, and a significantly higher frequency of sham chewing in parity 5 sows in comparison to younger sows. Both of these effects may be due to a cumulative effect of confinement in stall-housed sows (Zhang *et al* 2017), and hence sows that experience the longest confinement durations (older animals and animals in the last trimester of gestation) could have a higher incidence of stereotypies compared to sows which were confined for a shorter period of time. For the group-housed sows, the increase in performance of stereotypies may be due to a cumulative effect of stress experienced by sows in groups, possibly because of the lack of opportunities to express the full repertoire of innate behaviours, such as rooting and nest-building, in the barren pen environment, and also due to social stress related to an inability to avoid aggressive pen-mates and competition over preferred lying areas (Spoolder & Vermeer 2015). The increase in stereotypies in older sows may also be related to bigger stomach size in large animals. Feed restriction in these sows results in lower gut fill, which leads to increased feeding motivation (Holt *et al* 2006), developing higher levels of frustration and performing more stereotypies in response, in comparison to younger sows that have a smaller stomach size.

The present study showed that young and old group-housed sows performed fewer stereotypies than both control and exercised young sows, and mid-parity, group-housed sows performed fewer stereotypies than exercised sows in AM. Similarly, in PM, young, group-housed sows tended to perform fewer stereotypies than control sows, mid-parity sows from the group treatment performed fewer stereotypies than stall-housed sows (with or without access to periodic exercise), and old, group-housed sows tended to perform fewer stereotypies than exercised sows. These results demonstrate a general trend of reduction in performing stereotypic behaviour in group-housing settings by sows of all ages. These findings are in agreement with the results of Broom *et al* (1995) and Chapinal *et al* (2010), who demonstrated that stall-housed sows had a higher level of performing stereotypies than sows in group-housing systems. Given that the development of stereotypies is promoted by the lack of opportunities to exhibit a full repertoire of animals' innate behaviours (Fraser *et al* 1997) and is considered to be an indicator of stress, it can be concluded that group-housed sows in the current study experienced lower levels of stress in comparison to stall-housed animals and providing periodic exercise to stall-housed sows did not help to reduce this stress.

To assess for the presence and intensity of chronic stress experienced by pregnant sows during gestation in the current study, sow hair cortisol was measured. The obtained

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values of hair cortisol concentration are within the previously reported range for gestating sows (Bacci *et al* 2014; Roelofs *et al* 2019; Everding *et al* 2020). Hair cortisol concentrations were not affected by sow treatment, which implies that the levels of chronic stress did not depend on the level of access to a greater freedom of movement. In contrast, Everding *et al* (2020) demonstrated that stall-housed sows had higher hair cortisol levels during gestation than those housed in groups of 10–15 sows. The rapid rise of cortisol levels during the third trimester of pregnancy is more a physiological response of the organism to impending labour (Dorr *et al* 1989) than an indicator of stress and, theoretically, this pre-parturient increase in cortisol concentration could obscure the differences between treatments in the current study. However, the differences in group space allowance, number of animals in the group, feeding system, group pen design and flooring could be the factors that contributed to detectably decreased levels of hair cortisol in group-housed sows compared to those housed in stalls in the study of Everding *et al* (2020), as well as compared to the current study.

The current study demonstrated rather ambiguous effects of providing a greater freedom of movement to the sow on piglets' behavioural response to stress tests. In a social isolation test, the number of visited squares did not differ across treatments. This result is in agreement with the study of Brajon *et al* (2017), in which piglets from group-housed sows exposed to the social stress of mixing in mid gestation and from non-stressed, group-housed sows did not differ in the number of squares visited. The authors of the latter study suggest that the stress due to social isolation could be stronger than prenatal stress, and hence the differences caused by prenatal stress could have been masked.

The number of vocalisations in the social isolation test was higher in piglets from control and group-housed sows than in piglets from exercised sows. Similarly, Sorrells *et al* (2006) demonstrated that piglets from stall-housed sows grunted more than those from sows group-housed throughout gestation; however, the frequency of squealing during the isolation test did not differ between piglets from stall- and group-housed sows. Brajon *et al* (2017) reported that piglets from group-housed sows exposed to the social stress of mixing in mid gestation had a lower frequency of low calls in comparison to piglets from non-stressed sows; however, prenatally stressed piglets did not differ in the total number of vocalisations from piglets of non-stressed, group-housed sows. Considering that grunting may be interpreted rather as an indicator of exploration than as a sign of stress (Sorrells *et al* 2006), it can be assumed that piglets from non-stressed sows in the latter study performed more exploration than piglets from socially stressed sows. Hence, the previous literature suggests that being exposed to prenatal stress may affect the number of vocalisations in the social isolation test. However, the amplitude of sound should be taken into account, and in the current study only the number of vocalisations was recorded. Additionally, piglet behavioural responses can be influenced not only by the prenatal environment but also by maternal behaviour during lactation which, in turn, can be influenced by previous life experiences of sows. However, the latter effects were not assessed in previous literature on piglet behavioural stress responses and in the current study and therefore results should be interpreted with some caution. Based on the presented results and considering the above-mentioned limitations, it can be suggested that in the current study piglets from sows stall-housed throughout gestation and group-housed sows may experience similar levels of prenatal stress and providing periodic exercise to stall-housed sows may relieve the effects of this stress. However, for a better understanding of the levels and nature of stress experienced by piglets from different treatments, a separate analysis of vocalisations of low and high amplitude, as well as analysis of sow behaviour during lactation may be helpful.

In the novel object test, piglets from control sows visited more squares in comparison to piglets from exercised and group-housed sows and tended to have the higher number of times spent in close proximity to the novel object (0.50 m), which in previous literature was interpreted as an indicator of lower anxiety in these piglets (Kranendonk *et al* 2007). However, in the current experimental conditions it was subjectively noticed, that in those piglets that had increased locomotor activity, the patterns of movement were fairly chaotic, suggesting that these animals were rather fearful of the presence of the novel object. A similar explanation may be applied to the higher number of times spent near the novel object in piglets from control sows, which may be a result of increased activity due to anxiety and hence higher frequency of sporadic approaches to the novel object. However, to confirm that increased activity is indeed related to higher anxiety in the novel object test, more detailed analyses of piglet movement patterns and the amplitude of vocalisations should be performed. Weaver *et al* (2000) found that boars neonatally stressed by handling for the first 14 days of life entered more inner squares of the pen in an open field test at seven months of age in comparison to non-stressed boars. The authors reported that it was not a reflection of increased activity, as the number of visited outer squares was reduced proportionally; therefore, it was concluded that this alteration in behaviour indicated reduced anxiety levels in neonatally stressed boars. However, unlike in the study of Weaver *et al* (2000), in the current study the levels of activity were increased when the novel object, which probably was the main source of anxiety, was present in the testing arena. These discrepancies suggest that piglets from control sows in the current study were more active due to being more anxious.

Interestingly, in the current experiment, the results of the NO test did not differ for piglets from sows, receiving some exercise during gestation (exercised and group-housed sows), while being different for piglets from control sows. This suggests that providing a greater freedom of movement may positively affect piglet behavioural response to stress, considering that exercised sows were more adapted to 'life changes' than sows which were restrained in stalls throughout gestation.

Such variables as the number of vocalisations and the number of contacts with the novel object in the NO test did not differ across treatments, suggesting that these variables were not influenced by the maternal level of access to a greater freedom of movement. These results are in agreement with the study of Kranendonk *et al* (2006), who found no difference in these parameters between piglets from control and prenatally stressed sows. However, Kranendonk *et al* (2006) used oral administration of hydrocortisone acetate as opposed to the naturally induced stress applied in the current study. Responses to these stressors cannot be compared accurately due to the difference in physiological changes, and also due to exposing animals to additional stress during hormone administration (Lay *et al* 2008). On the other hand, similarly to the isolation test, the stress caused by the NO test could have been masking more subtle effects of prenatal stress. In contrast, Tatemoto *et al* (2019) found that female piglets born from sows that had access to straw during pregnancy, spent more time interacting with the novel object than female piglets from sows housed in the barren environment throughout gestation, which suggests that piglets from sows having access to environmental enrichment were less fearful. However, the piglets used in the study of Tatemoto *et al* (2019) were tested at 41 days of age, whereas piglets from the current study were tested on day 19–22 of age. This implies that the effects of prenatal stress on piglet behavioural response to stress tests may be developing with a delay.

The latency to touch the NO did not differ across treatments in the current study. In contrast, Kranendonk *et al* (2007) found that piglets from the sows with a high social rank, which presumably (based on their tendency to have lower salivary cortisol concentration in week 13 of gestation in comparison to low social ranking sows) experienced lower levels of stress during gestation, had a lower latency to touch the novel object, suggesting a more confident piglet with lower fear in comparison to piglets from the sows that had a low social rank, which are presumed to have experienced higher levels of stress during gestation. That a social rank effect was found by Kranendonk *et al* (2007) but no effect of gestation housing on piglet response to the NO in the present study provides an example of how different gestational stressors may influence piglet development differently. Further work on how the gestational management of sows impacts offspring development and behaviour is warranted.

Animal welfare implications

The findings of this study support the idea that providing a greater freedom of movement can bring some welfare benefits to the stall-housed sow and imply that group housing appears to be the most effective way of providing a greater freedom of movement to stall-housed gestating sows, rather than exercising stall-housed sows. It is also important to consider the fact that a group-housing environment provides not only freedom of movement, but also social contact and opportunities to make choices and have control over the environment, and the influence of these factors on gestating sow welfare was not explored in our study. Sow gestation housing was found to influence offspring characteristics, and this should be studied in greater depth as producers make the transition to group housing and new farming and husbandry methods are being developed globally.

Conclusion

Providing periodic exercise to stall-housed gestating sows at the low level used in the current study did not improve sow comfort, as indicated by similar relative frequencies of standing, sitting and lying in stall-housed and periodically exercised sows. Similarly, the relative frequencies of performing stereotypies by periodically exercised sows were comparable to those recorded in sows that were stall-housed throughout gestation. However, housing in groups was shown to improve sow comfort and reduce the performance of stereotypies compared to stall-housed sows (with or without access to periodic exercise), which is considered to be linked to lower levels of gestational stress in group-housed sows. Sow hair cortisol analysis was unable to identify any difference in the levels of chronic stress experienced by pregnant sows with different levels of access to a greater freedom of movement, possibly due to taking an approach of measuring average cortisol levels for the whole gestation cycle, which needs to be considered. In piglets, the results of behavioural testing demonstrated some effects of sow gestation treatment on piglets' behavioural response. In particular, the novel object test indicated that the lack of access to greater freedom of movement in stall-housed sows resulted in more proactive behavioural response in offspring.

Declaration of interest

None.

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