Effect of social ranks and gestation housing systems on oxidative stress status, reproductive performance, and immune status of sows

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ABSTRACT: Ninety-six multiparous sows were randomly assigned into 2 different gestation housing systems on d 35 of gestation: individual gestational crates ($n = 24$) or small groups with 3 sows in gestational pens ($n = 24$). Sows were classified into 4 treatments based on gestation housing systems and social ranks within each gestational pen: sows housed in individual gestational crates were in control treatment (CON), and sows destined to high, middle, or low social ranks within each pen were classified into high social rank treatment (HR), middle social rank treatment (MR), and low social rank treatment (LR). The social rank of sows within a pen was determined by their winning percentage during aggressive interactions observed for a 4-d period after mixing on d 35 of gestation. Plasma samples collected from each sow on d 35, 60, 90, and 109 of gestation and d 1 and 18 of lactation were used to determine concentrations of malondialdehyde (MDA), protein carbonyls, 8-hydroxy-deoxyguanosine (8-OHdG), IgG, and IgM. Sows in HR had higher ($P < 0.05$) body weight during gestation and lactation, smallest ($P < 0.05$) litter weight at birth, increased ($P < 0.05$) number of stillborn than sows in MR and LR, and tended to have decreased ($P = 0.073, P = 0.064$) number of born alive compared with sows in CON and LR. Sows in LR had lower farrowing rate compared with sows in MR. Plasma concentration of protein carbonyl in HR was higher ($P < 0.05$) than that in MR on d 3 of lactation. Plasma concentrations of 8-OHdG in LR was greater ($P < 0.05$) than that in HR on d 90 of gestation, d 3 and 18 of lactation, and greater ($P < 0.05$) than CON on d 18 of lactation. The reproductive performance of sows from all of the social ranks was related to their oxidative stress status during gestation and lactation. Collectively, the reproductive performance, oxidative stress status, and immune status did not differ between sows housed in gestational crates (CON) and pens (HR + MR + LR). Sows in CON and MR did not show inferior reproductive performance during gestation and lactation. Sows in HR and LR had increased oxidative damage during late gestation and lactation which could contribute to the reduced litter size and litter weight in HR and lower farrowing rate in LR.

Key words: oxidative stress, reproductive performance, social rank, sow

INTRODUCTION

Individual crates are widely used to manage pregnant sows. It is estimated that 60 to 70% of sows in the U.S. are housed in crates during gestation (Barnett et al., 2001). However, the use of gestational crates became a public concern as awareness of animal welfare increased. The European Union has banned the use of gestational crates from the fourth wk of pregnancy of sows since 2013 (Commission of the European Communities, 2001). McDonald’s also announced that it would require its U.S. pork suppliers to phase out the gestational crates (Pig Progress, 2012). Understanding the effect of changing from gestational crates to pens on the reproductive performance and biological changes of sows is essential to implement alternative gestational housing systems and assure the well-being of sows.
Studies showed that pregnant sows had elevated oxidative stress during late gestation and lactation, and heat stress could affect oxidative stress status of pregnant sows (Berchieri-Ronchi et al., 2011; Zhao et al., 2011; Kim et al., 2013). Mixing unfamiliar individuals may increase social stress and aggressive interactions which is shown to be associated with oxidative stress markers (Eskiocak et al., 2005), and cause a reduction of synthesizing circulating antibodies in birds (Siegel and Latimer, 1975) and mice (Vessey, 1964). Studies have focused on the effect of gestational housing systems and social ranks on the reproductive performance and social stress of sows (Mendl et al., 1992; Nicholson et al., 1993). However, it is not known if different gestational housing systems and social ranks affect oxidative stress and immune status of sows during gestation and lactation. Therefore, the objective of this study was to first determine if oxidative stress and immune status of sows during gestation and lactation could be affected by different gestational housing systems and social ranks and also examine the relationship between oxidative stress status and reproductive performance of sows.

**MATERIALS AND METHODS**

Procedures used in this study were reviewed and approved by the North Carolina State University Animal Care and Use Committee.

**Experimental Design and Social Status**

Ninety-six multiparous sows (initial BW: 242.1 ± 5.5 kg; average parity: 5.4 ± 0.4; Yorkshire Landrace cross) from the North Carolina State University Swine Educational Unit (Raleigh, NC) were used in this study with a randomized block design. All sows used in this study were artificially inseminated 3 times after estrus onset, and pregnancy was detected and confirmed at d 30 postbreeding using an ultrasound scanner (VSS700 EZ Preg Checker; Veterinary Sales and Service Inc., Stuart, FL). Sows were divided into 8 blocks (12 sows/block) due to limited number of breeding sows in each week. On d 35 of gestation, sows within blocks were randomly assigned into 2 different housing systems: individual gestational crates (2.0 × 0.6 m; total n = 24) or small gestational pens (3.0 × 2.5 m, total n = 24) with 3 sows per pen (2.5 m²/sow). Each gestational pen had a group-pen area (5.52 m²) and 3 individual feeding areas (0.66 m² per each area) which were separated by 3 partial stalls (1.1 m). Sows were classified into 4 treatments. Sows housed in individual crates were coded as control (CON) whereas sows within a pen were classified into high social rank (HR), middle social rank (MR), and low social rank (LR) treatments.

Determination of social status of sows was based on their aggressive interactions and submissive behavior in pens (Andersen et al., 1999; Heo et al., 2005; Kranendonk et al., 2007; Hoy et al., 2009; Poletto et al., 2009). Color cameras connected to a digital video recorder (QSDF8204; Q-See Products, Anaheim, CA) were used to continually record behaviors of sows from d 35 to 39 of gestation right after sows were allocated to crates or pens. Cameras were ceiling-mounted above all gestational crates and pens to ensure animals were clearly recorded and the recording can cover the whole area of the pen or crate. Each gestational pen was recorded by 2 cameras. For observation purposes, sows within a pen were marked by 3 different colors on the back and flanks. The recording rate was 3 frames per second, and all the videos were downloaded and saved in a computer for subsequent behavioral analysis. An observer (always the same person) watched all the videos. Aggressive interactions and submissive behavior were predefined before the observation, including displacement, parallel pressing, butting, biting, and withdrawing (Jensen, 1982; Vargas et al., 1987) (Table 1). If a sow withdrew and quit fighting or was displaced by the opponent, it was considered the loser after the fight, and the opponent was a winner. The total frequency of aggressive interactions of each sow involved, the individual who initiated the aggressive interaction in the pen, and the fighting outcomes including the frequency of winning and losing of each sow during aggressive interactions in the 4-d period were registered and analyzed.

The winning percentage during aggressive interactions was adopted to determine the social rank of sows within a pen according to Heo et al. (2005) and Poletto et al. (2009, 2010). The percentage of winning interactions of a sow was calculated by the formula: (frequency of winning interactions of a sow/total frequency of aggressive interactions of the sow) × 100% (Kranendonk et al., 2007). Within a pen, a sow with the highest percentage of winning interactions was classified into the HR treatment. A sow with the middle range of winning

<table>
<thead>
<tr>
<th>Item</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aggressive interactions</td>
<td></td>
</tr>
<tr>
<td>Displacement¹</td>
<td>A sow takes another sow’s position at the feeding place.</td>
</tr>
<tr>
<td>Parallel pressing¹</td>
<td>Sows push their shoulder hard against each other.</td>
</tr>
<tr>
<td>Butting¹</td>
<td>Head to head or head to body knock.</td>
</tr>
<tr>
<td>Biting¹</td>
<td>One sow grasps and presses to another sow with the mouth.</td>
</tr>
<tr>
<td>Submissive behavior</td>
<td></td>
</tr>
<tr>
<td>Withdrawing¹</td>
<td>Sow moves away from another sow quickly.</td>
</tr>
</tbody>
</table>

¹Modified from Jensen (1982) and Vargas et al. (1987).
percentage was listed into the MR treatment, and a sow in the pen with the lowest winning percentage was classified into the LR treatment. Within these 24 pens, there were 2 sows in a pen that were listed into LR treatment because they had the same amount of winning percentage, and both of them did not initiate aggressive interactions during the 4-d observation. Only the third sow in the pen initiated all the aggressive interactions, which was listed into the HR treatment. Thus, there were 24, 23, and 25 sows in HR, MR, and LR, respectively.

**Animal Management**

Both gestational crates and pens were located in the same gestation building with slatted floors (15.2-cm concrete slat and 2.5-cm slot). The temperature of the gestation building was set at 22°C. Each pen was equipped with two nipple water drinkers. Sows had free access to water during gestation and lactation. Sows from gestational crates and pens were fed individually in the morning (between 0600 and 0800 h). Gestational diet was provided 2.2 kg daily, which contained 13.33% CP and 3.33 Mcal ME/kg, and was formulated to equal or exceed NRC (1998) nutrient requirements (Table 2). During the gestation period, all sows remained in their respective treatment to maintain the group hierarchy. On d 109 of gestation, sows were moved into individual farrowing crates (2.1 × 1.5 m) in an adjacent farrowing building. During the lactation period, sows were fed ad libitum with a typical lactation diet containing 17.14% CP and 3.42 Mcal ME/kg (NRC, 1998) (Table 2). Fresh feed was provided and the amount was recorded every day. At 0800 h, any feed left from the previous day was removed and weighed, and daily feed intake was calculated. Body weight of sows was measured on d 35 and 109 of gestation, and d 1 and 18 of lactation. Backfat thickness of sows was measured at the P 2 position (locate at left side of the 10th rib and 6 cm away from the spine) on d 1 and 18 of lactation using an ultrasound scanner (VSS700 EZ Preg Checker; Veterinary Sales and Service Inc., Stuart, FL). After farrowing, individual weight of piglet and litter size on d 1 (born alive, stillborn, and mummy) and d 18 of lactation were measured. The farrowing rate (number of sows with farrowing related to the number of sows confirmed pregnancy on d 30 of gestation) of sows was recorded.

**Sampling of Blood and Colostrum**

A single blood sample was collected from each sow 2 h after feeding by jugular venipuncture using a 9-mL syringe with potassium-EDTA (SARSTEDT Inc., Newton, NC) and a disposable 16-gauge × 0.1-mm hypodermic needle (Air-Tite Product Co. Inc., Virginia Beach, VA) on d 35, 60, 90, and 109 of gestation, and d 1 and 18 of lactation. Plasma samples were obtained by centrifugation (5810 R; Eppendorf AG, Hamburg, Germany) at 3,000 g, 15 min, 4°C, then allocated into 1.5-mL microcentrifuge tubes, kept in liquid nitrogen for 1 h, and stored at −80°C until analysis. Colostrum samples (20–30 mL) were collected from the first 3 pairs of anterior teats of all the sows within 10 h of post farrowing. Samples were then stored at −80°C and later analyzed for IgG and IgM concentration.

**Analysis of Oxidative Stress Parameters**

Plasma samples were used to measure concentrations of malondialdehyde (MDA), protein carbonyl, and 8-hydroxy-deoxyguanosine (8-OHdG). Thiobarbituric acid analysis kit (Cell Biolabs, San Diego, CA) was used to determine the plasma concentration of MDA according to the method described by Pialoux et al. (2009). Plasma samples and MDA standards were first

| Table 2. Composition of gestation and lactation diets (as-fed basis) |
|--------------------------|--------------------------|--------------------------|
| Item         | Gestation diet | Lactation diet |
| Corn, yellow | 81.30          | 69.00          |
| Soybean meal, 48% CP | 13.85          | 23.50          |
| Poultry fat  | 1.00           | 3.09           |
| Biolyxs1     | 0.00           | 0.25           |
| L-Thr        | 0.00           | 0.01           |
| Limestone    | 1.11           | 1.08           |
| Dicalcium phosphate | 2.05          | 2.38           |
| Salt         | 0.50           | 0.50           |
| Trace mineral premix2 | 0.15          | 0.15           |
| Vitamin premix3 | 0.04          | 0.04           |
| Total        | 100.00         | 100.00         |
| Calculated composition |
| DM, %        | 89.67          | 90.06          |
| ME, Meal/kg  | 3.330          | 3.422          |
| CP, %        | 13.33          | 17.14          |
| Lys, %       | 0.63           | 0.98           |
| Met, %       | 0.49           | 0.51           |
| Trp, %       | 0.14           | 0.17           |
| Thr, %       | 0.49           | 0.55           |
| Ca, %        | 1.03           | 1.01           |
| Total P, %   | 0.69           | 0.80           |
| Available P, % | 0.43          | 0.50           |

1 Biolyxs (Enovik Degussa, Kennesaw, GA) contains 50.7% of L-Lys.
2The trace mineral premix provided per kilogram of complete diet: 3.96 mg of Mn as manganous oxide; 16.5 mg of Fe as ferrous sulfate; 16.5 mg of Zn as zinc sulfate; 1.65 mg of Ca as copper sulfate; 0.30 mg of I as ethylenediamine dihydroiodide; and 0.30 mg of Se as sodium selenite.
3The vitamin premix provided per kilogram of complete diet: 8228 IU of vitamin A as vitamin A acetate; 1173 IU of vitamin D3; 47 IU of vitamin E; 0.03 mg of vitamin B12; 5.88 mg of riboflavin; 23.52 mg of D-pantothenic acid as calcium pantothenate; 35.27 mg of niacin; 0.24 mg of biotin; 1.76 mg folic acid; 3.88 mg menadione.
incubated and reacted with thiobarbituric acid at 95°C; after incubation and butanol extraction, samples and MDA standards were read at 532 nm. The concentration of MDA in plasma was determined by comparing with the MDA standard curve which was drawn by the concentration and absorbance of standards. The detecting limit of MDA analysis was 0.98 µM.

Protein concentration in plasma samples were measured using bicinchoninic acid protein assay (Thermo Fisher Scientific Inc., Rockford, IL). All plasma samples were then diluted with 1 × PBS to reach protein concentration at 10 µg per milliliter before analyzing protein carbonyl. The concentration of protein carbonyl was measured according to the method described by Neretti et al. (2009) by using an ELISA kit (Cell Biolabs, San Diego, CA). The protein carbonyl presented in the sample or standard was derivatized to dinitrophenyl (DNP) hydrazine and probed with an anti-DNP antibody, then incubated with a secondary antibody. Samples and standards were read at 450 nm, and the protein carbonyl concentration in the sample was determined by comparing with the protein carbonyl standard curve which was determined by the concentration and absorbance of standards. The detecting limit for protein carbonyl was 0.375 nmol/mg.

An ELISA kit (Cell Biolabs, San Diego, CA) that utilizes an anti-8-OHdG monoclonal antibody to recognize 8-OHdG was used to determine the concentration of 8-OHdG in the plasma sample according to the method described by Pialoux et al. (2009). Briefly, plasma samples and 8-OHdG standards were first added into a 96-well plate. Then an anti-8-OHdG monoclonal antibody was added, followed by adding a secondary antibody. The plate was read at 450 nm, and the concentration of 8-OHdG in the plasma sample was calculated against the standard curve which was determined by the concentration and absorbance of standards. The detecting limit of 8-OHdG was 0.078 ng/mL.

**Immunoglobulin Evaluation**

Concentrations of IgG and IgM in colostrums and plasma samples from d 109 of gestation and d 3 and 18 of lactation were measured by ELISA kits (Bethyl Laboratories Inc., Montgomery, TX) according to the method described by Chaytor et al. (2011). The goat antipig IgG or goat antipig IgM was used as a capture antibody to coat wells. Plasma samples and colostrums were diluted to 1:100,000 for IgG and IgM measurement. Horseradish peroxidase goat antipig IgG or IgM was used as the detection. The plate was read at 450 nm. The concentration of the sample was quantified against the standard curve which was drawn from the concentration and absorbance of standards. The detecting limit was 7.8 ng/mL for IgG and 15.6 ng/mL for IgM, respectively.

| Table 3. Aggressive interactions of sows from different social rank treatments1 |
|-----------------------------|----------------|----------------|----------------|-------------|
| Item                        | HR2            | MR2            | LR2            | SEM         | P-value    |
| n                           | 24             | 23             | 25             |             |            |
| Frequency of aggressive interactions3 No. | 21.2          | 16.2           | 15.8           | 3.0         | 0.340      |
| Frequency of initiation of fighting4 No. | 16.7a          | 6.8b           | 3.2b           | 2.1         | <0.001     |
| Winning interactions5 %     | 67.9a          | 22.4b          | 10.3c          | 2.9         | <0.001     |

1 Means within a row with different superscripts differ (P < 0.05).
2 Sows in crates did not have aggressive interaction with others; therefore, no data was presented.
3 HR = sows in high social rank treatment; MR = sows in middle social rank treatment; LR = sows in low social rank treatment.
4 Total frequency of aggressive interactions during the 4-d observation.
5 Frequency of initiated fighting during the 4-d observation.
6 The percentage of winning interactions of a sow calculated from the formula: (frequency of winning interactions of a sow/total frequency of aggressive interactions of the sow) × 100%.

**Statistical Analysis**

This experiment was a randomized block design. All data sets were checked for outliers and normality before being analyzed using the MIXED procedure (SAS Inst. Inc., Cary, NC) with individual sow as the experimental unit. Treatment was a fixed effect. Block and treatment by block interaction were random effects. For the oxidative stress markers, data on d 35 of gestation were used as a covariance. Least significant difference (LSD) procedure was used for pairwise comparisons of means among treatments. Orthogonal contrast was used to determine differences in means of reproductive performance, oxidative stress status, and immune status between CON and HR + MR + LR. Correlation between reproductive performance and oxidative stress indicators within each treatment were analyzed by the CORR procedure of SAS. Results of farrowing rate were analyzed by Chi-square. Mean differences were determined as statistically significant when probability values were less than 0.05, and probability less than 0.1 and equal or greater than 0.05 was considered as a tendency.

**RESULTS**

**Aggression**

Sows in HR had the highest (P < 0.05) percentage of winning interactions (67.9%), and the percentage of winning interactions in MR (22.4%) was higher (P < 0.05) than that of LR (10.3%). The frequency of initiated fighting of sows in HR was greater (P < 0.05) than sows in MR and LR (Table 3). Sows in crates were not observed in aggressive interactions; therefore, no data was presented.
Reproductive Performance of Sows

There were no differences in reproductive performance between CON and HR + MR + LR (Table 4). However, body weight of sows in HR was greater (P < 0.05) than sows in CON on d 35 of gestation, and it was also greater (P < 0.05) than sows in MR and LR through gestation and lactation (Table 4). The backfat thickness of sows in HR tended to be greater (P = 0.093) than that in CON on d 1 of lactation and was greater (P < 0.05) compared with sows in LR on d 18 of lactation (Table 4). Sows in HR had increased (P < 0.05) number of stillborn piglets than sows in MR and LR and also tended to have decreased (P = 0.073, P = 0.064) number of born alive and smaller (P < 0.05) litter size on d 18 of lactation compared with sows in CON and LR (Table 4). Sows in HR had smaller (P < 0.05) litter weight than sows in CON, MR, and LR on d 1 of lactation and LR on d 18 of lactation. Sows in HR also tended to have decreased (P = 0.072) litter weight gain compared with sows in LR during lactation (Table 4). The piglet’s ADG during lactation in HR was greater (P < 0.05) compared with the piglet from CON (Table 4). The weights of piglets from LR sows was lower (P < 0.05) than the weights of piglets from HR on d 18 of lactation. Three CON sows did not farrow, and 10 sows housed in gestational pens did not farrow (2, 1, and 7 sows in HR, MR, and LR, respectively).

Table 4. Reproductive performance of sows from different housing systems and social ranks

<table>
<thead>
<tr>
<th>Item</th>
<th>Treatments</th>
<th>SEM</th>
<th>P-value</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>CON 24</td>
<td>HR 24</td>
<td>MR 23</td>
<td>LR 25</td>
</tr>
<tr>
<td>Parity</td>
<td>5.3</td>
<td>5.9</td>
<td>5.1</td>
<td>5.1</td>
</tr>
<tr>
<td>Body weight of sows, kg</td>
<td>240.5b</td>
<td>256.3a</td>
<td>237.6b</td>
<td>233.8b</td>
</tr>
<tr>
<td>d 35 of gestation</td>
<td>282.1a</td>
<td>289.1a</td>
<td>260.4b</td>
<td>262.9b</td>
</tr>
<tr>
<td>d 109 of gestation</td>
<td>272.0a</td>
<td>283.2b</td>
<td>256.2b</td>
<td>258.2b</td>
</tr>
<tr>
<td>d 18 of lactation</td>
<td>263.4ab</td>
<td>275.2a</td>
<td>252.2b</td>
<td>247.9b</td>
</tr>
<tr>
<td>Backfat thickness</td>
<td>16.2A</td>
<td>18.0B</td>
<td>17.3AB</td>
<td>16.4AB</td>
</tr>
<tr>
<td>d 1 of lactation</td>
<td>15.3ab</td>
<td>17.1a</td>
<td>16.8ab</td>
<td>14.9b</td>
</tr>
<tr>
<td>Change from d 1 to 18</td>
<td>-0.9</td>
<td>-0.9</td>
<td>-0.5</td>
<td>-1.5</td>
</tr>
<tr>
<td>ADFI of sows during lactation, kg</td>
<td>4.7</td>
<td>4.3</td>
<td>4.8</td>
<td>4.3</td>
</tr>
<tr>
<td>Litter size, pig No./litter</td>
<td>13.4</td>
<td>12.6</td>
<td>11.8</td>
<td>12.8</td>
</tr>
<tr>
<td>d 1, total born</td>
<td>11.1B</td>
<td>9.6A</td>
<td>10.3AB</td>
<td>11.2B</td>
</tr>
<tr>
<td>d 1, stillborn</td>
<td>1.9ab</td>
<td>2.6a</td>
<td>1.2b</td>
<td>1.4b</td>
</tr>
<tr>
<td>d 1, mummy</td>
<td>0.5</td>
<td>0.5</td>
<td>0.3</td>
<td>0.3</td>
</tr>
<tr>
<td>d 18</td>
<td>9.1b</td>
<td>7.2a</td>
<td>8.2ab</td>
<td>9.6c</td>
</tr>
<tr>
<td>Change from d 1 to 18</td>
<td>-2.0</td>
<td>-2.5</td>
<td>-2.1</td>
<td>-1.6</td>
</tr>
<tr>
<td>Litter weight, kg</td>
<td>16.8a</td>
<td>13.6b</td>
<td>16.3a</td>
<td>16.6a</td>
</tr>
<tr>
<td>d 18</td>
<td>50.0ab</td>
<td>43.1a</td>
<td>47.8ab</td>
<td>51.8b</td>
</tr>
<tr>
<td>Gain from d 1 to 18</td>
<td>33.3AB</td>
<td>29.5A</td>
<td>31.5AB</td>
<td>35.3B</td>
</tr>
<tr>
<td>Piglet weight, kg</td>
<td>1.53</td>
<td>1.47</td>
<td>1.58</td>
<td>1.50</td>
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<tr>
<td>d 17</td>
<td>5.61ab</td>
<td>6.11a</td>
<td>5.86ab</td>
<td>5.47b</td>
</tr>
<tr>
<td>ADG from d 1 to 18, g/d</td>
<td>204.3a</td>
<td>233.3b</td>
<td>211.7ab</td>
<td>207.0ab</td>
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<tr>
<td>Farrowing rate, %</td>
<td>87.5ab</td>
<td>91.7ab</td>
<td>95.7a</td>
<td>72.0b</td>
</tr>
</tbody>
</table>

a,bMeans within a row with different superscripts differ (P < 0.05).
A,BMeans within a row with different superscripts show a tendency to differ (0.05 ≤ P < 0.10).
1CON = sows housed in gestational crates; HR = sows in high social rank treatment; MR = sows in middle social rank treatment; LR = sows in low social rank treatment.
2Comparison among treatments.
3Comparison between CON and HR + MR + LR.
4Measured at the P2 position (locate at left side of the 10th rib and 6 cm away from the spine).
5Difference between d 1 and 18 of lactation.
6Litter weight at birth was based on the total number of piglets born alive.
7Average birth weight of piglet was based on the total number of piglets born alive.
8There were 3 sows that did not farrow in CON, and 2, 1, and 7 sows that did not farrow in HR, MR, and LR, respectively.
Oxidative stress in sows

The farrowing rate of sows in LR (72%) was smaller ($P < 0.05$) compared with sows in MR (95.7%).

**Oxidative Stress Parameters**

Plasma concentrations of MDA, protein carbonyl, and 8-OHdG on different days of gestation and lactation did not differ between CON and HR + MR + LR. Plasma concentrations of MDA did not differ among treatments on different days of gestation and lactation (Table 5). There were no differences in plasma concentration of protein carbonyl among treatments on d 60 and 109 of gestation and d 18 of lactation. However, plasma concentration of protein carbonyl in HR tended to be greater ($P = 0.072$) than CON on d 90 of gestation and was greater ($P < 0.05$) than the concentration in MR on d 3 of lactation (Table 5). Plasma concentrations of 8-OHdG in LR was greater ($P < 0.05$) than concentrations in HR on d 90 of gestation and d 3 and 18 of lactation, and greater ($P < 0.05$) than CON on d 18 of lactation (Table 5).

**Correlation Analysis: Oxidative Stress and Reproductive Performance**

There were several significant correlations between reproductive performance and oxidative stress parameters within each treatment, and $r$-values are reported in Tables 6–8 and Fig. 1. Plasma MDA concentration on d 109 of gestation was found to be negatively correlated ($P < 0.05$) with the BW of sows on d 35 and 109 of gestation and d 1 and 18 of lactation in both MR and LR (Table 6). Plasma protein carbonyl concentration on d 60 of gestation showed negative correlations ($P < 0.05$) with the number of piglets born alive, litter size on d 18 of lactation, litter weight gain, and litter weight on d 1 and 18 of lactation in LR (Table 7). Plasma 8-OHdG concentration on d 109 of gestation were negatively correlated ($P < 0.05$) with the litter size on d 18 of lactation, litter weight on d 1 and 18 of lactation in LR (Table 7), and number of piglets born alive in HR (Fig. 1A). Backfat thickness on d 18 of lactation in HR was negatively correlated ($P < 0.05$) with plasma 8-OHdG concentration on d 60 of gestation (Fig. 1B). Piglet’s BW on d 1 of lactation in LR showed a negative correlation ($P < 0.05$) with protein carbonyl concentrations on d 60 and 90 of gestation (Table 8). Besides, piglet’s BW on d 18 of lactation in MR was found to be negatively correlated ($P < 0.05$) with MDA concentration on d 109 of gestation and protein carbonyl concentrations on d 109 of gestation and d 18 of lactation. The number of stillborn in MR was positively correlated with protein carbonyl concentration on d 90 of gestation (Table 8). Other oxidative indicators were not significantly correlated with these reproductive performance measurements.

### Table 5. Oxidative stress status of sows from different housing systems and social ranks

<table>
<thead>
<tr>
<th>Item</th>
<th>Treatments</th>
<th>CON vs. HR+MR+LR</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n</td>
<td>CON HR MR LR SEM</td>
</tr>
<tr>
<td>Malondialdehyde, µmol/L</td>
<td></td>
<td></td>
</tr>
<tr>
<td>d 60 of gestation</td>
<td>24</td>
<td>24</td>
</tr>
<tr>
<td>d 90 of gestation</td>
<td>5.50</td>
<td>5.00</td>
</tr>
<tr>
<td>d 109 of gestation</td>
<td>5.79</td>
<td>5.89</td>
</tr>
<tr>
<td>d 3 of lactation</td>
<td>6.22</td>
<td>5.42</td>
</tr>
<tr>
<td>d 18 of lactation</td>
<td>6.13</td>
<td>6.35</td>
</tr>
<tr>
<td>Protein carbonyl, nmol/mg</td>
<td></td>
<td></td>
</tr>
<tr>
<td>d 60 of gestation</td>
<td>1.34</td>
<td>1.18</td>
</tr>
<tr>
<td>d 90 of gestation</td>
<td>0.95&lt;sup&gt;A&lt;/sup&gt;</td>
<td>1.37&lt;sup&gt;B&lt;/sup&gt;</td>
</tr>
<tr>
<td>d 109 of gestation</td>
<td>1.66</td>
<td>1.53</td>
</tr>
<tr>
<td>d 3 of lactation</td>
<td>0.40&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>1.48&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>d 18 of lactation</td>
<td>1.28</td>
<td>1.10</td>
</tr>
<tr>
<td>8-hydroxy-deoxyguanosine, ng/mL</td>
<td></td>
<td></td>
</tr>
<tr>
<td>d 60 of gestation</td>
<td>0.72</td>
<td>0.66</td>
</tr>
<tr>
<td>d 90 of gestation</td>
<td>0.69&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>0.60&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>d 109 of gestation</td>
<td>0.95</td>
<td>1.00</td>
</tr>
<tr>
<td>d 3 of lactation</td>
<td>0.74&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>0.68&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>d 18 of lactation</td>
<td>0.36&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.36&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>a,b</sup>Means within a row with different superscripts differ ($P < 0.05$).

<sup>A,B</sup>Means within a row with different superscripts show a tendency to differ ($0.05 \leq P < 0.10$).

<sup>1</sup>CON = sows housed in gestational crates; HR = sows in high social rank treatment; MR = sows in middle social rank treatment; LR = sows in low social rank treatment.

<sup>2</sup>Comparison among treatments.

<sup>3</sup>Comparison between CON and HR + MR + LR.
**Immunoglobulin Evaluation**

Immunoglobulin G and IgM in plasma and colostrum were used as indicators of immune status of sows in this study. There were no differences of IgG and IgM concentrations in plasma and IgM concentration in colostrum among treatments on different days of gestation and lactation (Table 9). However, IgG concentration in colostrum tended to be greater \( (P = 0.069) \) in HR compared with sows in MR (Table 9).

**DISCUSSION**

**Group Housing and Social Rank Determination**

The increase in consumer awareness about animal welfare issues has led producers to begin the implementation of group housing of sows after breeding. Gestational pens with partial stalls are designed for use with small groups of sows (Boyle, 2005) and lend themselves well for units being converted from individual gestational crates into a group housing system. In this study, gestational pens with a floor space of 3.0 × 2.5 m were used based on the space allowance suggested by the European Union (Mul et al., 2010). Three sows were housed in one gestational pen allowing 2.5 m² per sow. The advantage of using small pens with partial stalls is that they reduce competitions between sows for accessing to feed (Pedersen et al., 1993; Boyle, 2005). Therefore, small groups tend to be static, whereby the level of aggression is low once the dominance hierarchy is established. It is reported that the dominance hierarchy in groups of sows is stable 3 d after mixing (Oldigs et al., 1992). According to Pritchard (1996), aggressive interaction of sows within a small group (6 sows) fell gradually over the following 2 d after mixing. Barnett et al. (1993) reported that in groups of 5 or 6 sows, the number of aggressive interactions decreased 2 d after mixing and had fallen to the average levels recorded over the following 15 d. Therefore, aggressive interactions in this study were recorded for a 4-d period after mixing on d 35 of gestation.

**Sow Reproductive Performance**

In this study, sows in HR had greater BW than sows in CON in early gestation, and greater than MR and LR through the whole gestation and lactation periods. This is consistent with previous studies (Mendl et al., 1992; Nicholson, 1994) which showed that dominant sows were heavier than intermediate and submissive sows. Results in this study indicated that sows with greater BW in a group may have physical advantage to win during aggressive interactions, thus they could stay in the top of the dominant hierarchy. This result also inspires us to think about the strategy to allot group housing sows. It might be helpful to establish the dominance hierarchy sooner and beneficial to the well-being of sows if the heavy and light sows are assigned into the same pens because their aggressive interactions could be short or slight due to the difference of their BW (Craig, 1986).

This study showed that there were no differences for the litter size and litter weight between sows in gestational crates (CON) and pens (HR + MR + LR). Hulbert and McGlone (2006) reported a similar result showing that sows in small gestational pens (5 or less sows per pen) had a similar litter size to sows in gestational crates.

| Table 6. Correlation of body weight of sows with plasma malondialdehyde (MDA) concentration on d 109 of gestation within different social ranks |
|-----------------|-----------------|-----------------|
| Item            | MDA in HR ¹     | MDA in MR ²     | MDA in LR ³   |
| BW on d 35 of gestation |                 |                 |               |
| \( r \)         | -0.447          | -0.579          | -0.685        |
| \( P \)         | 0.063           | 0.038           | 0.005         |
| BW on d 109 of gestation |                 |                 |               |
| \( r \)         | -0.347          | -0.704          | -0.722        |
| \( P \)         | 0.224           | 0.016           | 0.005         |
| BW on d 1 of lactation |                 |                 |               |
| \( r \)         | 0.146           | -0.717          | -0.586        |
| \( P \)         | 0.604           | 0.006           | 0.022         |
| BW on d 18 of lactation |                 |                 |               |
| \( r \)         | 0.034           | -0.636          | -0.657        |
| \( P \)         | 0.894           | 0.019           | 0.008         |

¹HR = sows in high social rank treatment.  
²MR = sows in middle social rank treatment.  
³LR = sows in low social rank treatment.

| Table 7. Correlation of sows’ reproductive performance with plasma protein carbonyl and 8-hydroxy-deoxyguanosine (8-OHdG) concentration in low social rank treatment (LR) |
|-----------------|-----------------|-----------------|
| Item            | Protein carbonyl ¹ | 8-OHdG ²        |
| Litter size, pig d 1, born alive |                 |                 |
| \( r \)         | -0.715           | -0.441          |
| \( P \)         | 0.006            | 0.114           |
| d 18 of lactation |                 |                 |
| \( r \)         | -0.774           | -0.542          |
| \( P \)         | 0.002            | 0.045           |
| Litter weight, kg d 1 of lactation |                 |                 |
| \( r \)         | -0.789           | -0.551          |
| \( P \)         | 0.001            | 0.041           |
| d 18 of lactation |                 |                 |
| \( r \)         | -0.898           | -0.223          |
| \( P \)         | < 0.001          | 0.464           |
| Gain from d 1 to 18 |                 |                 |
| \( r \)         | -0.844           | -0.033          |
| \( P \)         | 0.001            | 0.916           |

¹Plasma protein carbonyl concentration on d 60 of gestation.  
²Plasma 8-OHdG concentration on d 109 of gestation.
In addition, other studies reported no differences in total number of piglets born and born alive per litter for sows housed in gestational crates and pens (Johnson et al., 2001; Bates et al., 2003; Chapinal et al., 2010). The result in this study indicated that these two types of gestational housing systems did not have significant effect on reproductive performance of sows.

In this study, HR sows weaned the smallest number of piglets which was in agreement with a previous study (Nicholson et al., 1993). This was because HR sows had the lowest number of piglets born alive and the highest number of stillborn piglets compared with sows in the other treatments. Therefore, sows in HR had the smallest number of piglets and lower pressure to produce enough milk during lactation as indicated by smaller litter weight and litter weight gain compared with other treatments during lactation. The smallest litter size in HR also contributed to heavier individual pigs and greater ADG during lactation compared with other treatments. In summary, sows in HR showed decreased litter performance as indicated by their decreased litter size and litter weight at birth and weaning.

In the current study, sows in LR weaned the highest number of piglets per litter, and had greater pressure to produce milk during lactation than sows in HR as indicated by the greater litter weight gain during lactation compared with sows in HR. The higher lactation pressure pushed sows in LR to use their body reserve for milk production, therefore, resulted in the lowest backfat thickness at the end of lactation. This agrees with a previous study from Kranendonk et al. (2007), which showed that sows in the low social rank lost more backfat during lactation. However, although sows in LR harvested the highest number of piglets per litter at the end of lactation, it could not conclude that sows in LR had better reproductive performance compared with other treatments. Instead, the farrowing rate (72%) of sows in LR was lower than sows in MR (95.7%). Considering the litter size in each treatment, it was calculated that the total number of piglets weaned from all of the sows in CON or MR were greater than sows in LR and HR at the end of lactation, and results from this study indicated that there was no difference on the reproductive performance of sows between CON and MR. Thus, it was concluded that sows in CON and MR did not show inferior reproductive performance during gestation and lactation, whereas sow in LR had lower reproductive efficiency as they had lower farrowing rate which could increase their replacement rate and nonproductive days.

**Oxidative Stress Parameters and Correlation with Reproductive Performance**

Studies have focused on the effect of gestational housing systems and social ranks on the social stress of sows housed in gestational crates and pens (Johnson et al., 2001; Bates et al., 2003; Chapinal et al., 2010). The result in this study indicated that these two types of gestational housing systems did not have significant effect on reproductive performance of sows.

Table 8. Correlation of sows’ reproductive performance with plasma protein carbonyl and malondialdehyde (MDA) concentration in middle social rank treatment (MR)

<table>
<thead>
<tr>
<th>Item</th>
<th>Protein carbonyl</th>
<th>MDA</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>d 60 G</td>
<td>d 90 G</td>
</tr>
<tr>
<td>Stillborn</td>
<td>r</td>
<td>P</td>
</tr>
<tr>
<td></td>
<td>0.372</td>
<td>0.673</td>
</tr>
<tr>
<td>Piglet weight</td>
<td>r</td>
<td>P</td>
</tr>
<tr>
<td>d 1 of lactation</td>
<td>-0.772</td>
<td>0.003</td>
</tr>
<tr>
<td>d 18 of lactation</td>
<td>-0.023</td>
<td>0.943</td>
</tr>
</tbody>
</table>

1Plasma protein carbonyl concentration on d 60, 90, 109 of gestation, and d 18 of lactation.

2Plasma MDA concentration on d 109 of gestation.

In addition, other studies reported no differences in total number of piglets born and born alive per litter for sows housed in gestational crates and pens (Johnson et al., 2001; Bates et al., 2003; Chapinal et al., 2010). The result in this study indicated that these two types of gestational housing systems did not have significant effect on reproductive performance of sows.

In this study, HR sows weaned the smallest number of piglets which was in agreement with a previous study (Nicholson et al., 1993). This was because HR sows had the lowest number of piglets born alive and the highest number of stillborn piglets compared with sows in the other treatments. Therefore, sows in HR had the smallest number of piglets and lower pressure to produce enough milk during lactation as indicated by smaller litter weight and litter weight gain compared with other treatments during lactation. The smallest litter size in HR also contributed to heavier individual pigs and greater ADG during lactation compared with other treatments. In summary, sows in HR showed decreased litter performance as indicated by their decreased litter size and litter weight at birth and weaning.

In the current study, sows in LR weaned the highest number of piglets per litter, and had greater pressure to produce milk during lactation than sows in HR as indicated by the greater litter weight gain during lactation compared with sows in HR. The higher lactation pressure pushed sows in LR to use their body reserve for milk production, therefore, resulted in the lowest backfat thickness at the end of lactation. This agrees with a previous study from Kranendonk et al. (2007), which showed that sows in the low social rank lost more backfat during lactation. However, although sows in LR harvested the highest number of piglets per litter at the end of lactation, it could not conclude that sows in LR had better reproductive performance compared with other treatments. Instead, the farrowing rate (72%) of sows in LR was lower than sows in MR (95.7%). Considering the litter size in each treatment, it was calculated that the total number of piglets weaned from all of the sows in CON or MR were greater than sows in LR and HR at the end of lactation, and results from this study indicated that there was no difference on the reproductive performance of sows between CON and MR. Thus, it was concluded that sows in CON and MR did not show inferior reproductive performance during gestation and lactation, whereas sow in LR had lower reproductive efficiency as they had lower farrowing rate which could increase their replacement rate and nonproductive days.

**Oxidative Stress Parameters and Correlation with Reproductive Performance**

Studies have focused on the effect of gestational housing systems and social ranks on the social stress of
sows (Mendl et al., 1992; Nicholson et al., 1993). Mendl et al. (1992) found that primiparous pigs in a lower rank had the highest basal levels of salivary cortisol and the highest peak cortisol levels compared with pigs in a higher social rank in response to an ACTH hormone challenge during the first month after been mixed in a large indoor pen. However, only few studies have investigated oxidative stress status of sows during gestation and lactation (Berchieri-Ronchi et al., 2011), and the effects of gestational housing systems and social ranks on oxidative stress status of sows have not yet been studied. Malondialdehyde is one of the most frequently used indicators of lipid peroxidation and was determined in the current study. Protein carbonyl derivative is the most common product of protein oxidation (Simm and Brömme, 2005), which was used as an indicator for protein oxidation in this study. The major marker for oxidative damage to nucleic acids, 8-OHdG, was chosen to determine the DNA damage in the current study (Ravanat et al., 2000; Bowen, 2010).

Our study showed no significant differences in MDA, protein carbonyl, and 8-OHdG between sows housed in gestational crates (CON) and pens (HR + MR + LR). Similar results were shown by Costantini and Lipp (2010) as there were no differences in oxidative stress markers between a restraint crate and a non-restraint environment in homing pigeons. On the other hand, Lee et al. (2009) found that mice exposed to a restraint environment had increased levels of lipid peroxidation. These varying results may relate to different species and housing environments.

This study showed that oxidative stress was closely related to reproductive performance of sows from all of the social ranks. Although plasma concentrations of MDA did not differ among treatments on different days of gestation and lactation, it was found that the MDA concentration on d 109 of gestation was negatively correlated with the BW of sows on d 109 of gestation, d 1 and 18 of lactation in both MR and LR, indicating that the level of lipid peroxidation at late gestation was related to the body weight of sows from middle and low social ranks during late gestation and lactation. Studies showed that the oxidative damage to DNA can cause DNA breakdown and mutation (Gutteridge and Halliwell, 1994). The elevated 8-OHdG can alter gene expression by inhibiting methylation and can cause mutation by pairing with adenosine rather than cytosine during DNA replication, leading to GC to AT conversion (Simm and Brömme, 2005). This study found that 8-OHdG concentration was higher in LR compared with that in HR and CON during late gestation and lactation, indicating that sows from LR had higher DNA damage during late gestation and lactation compared with other treatments. There was a negative correlation between 8-OHdG concentration and litter size and litter weight of sows in LR. The increased oxidative damage could contribute to the low reproductive efficiency in LR. This explanation could be supported by the previous study on pregnant women which showed that oxidative stress was associated with fetal growth retardation which cause a higher risk of prenatal mortality (Toy et al., 2009).

In this study, plasma protein carbonyl concentration during mid and late gestation showed negative correlations with litter size and litter weight of sows in MR and LR, indicating that the litter performance of sows in MR and LR could be affected by oxidative damage to protein during mid and late gestation. We also found that compared with sows in MR and CON, sows from HR had greater protein carbonyl concentration during late gestation and lactation, indicating that sows in HR received greater oxidative damage during late gestation and lactation. The increased oxidative damage to cellular proteins can cause chemical modifi-
cation of proteins, increased protein turnover, and cell death (Simm and Brömme, 2005), which could contribute to the decreased litter size in HR compared with other groups. It seems that sows from MR and CON did not show increased oxidative stress compared with sows in both HR and LR during gestation and lactation. This may indicate that sows in MR and CON suffer less oxidative damage compared with other two treatments during gestation and lactation.

Immunoglobulin Evaluation

Plasma concentrations of IgG and IgM were not different among treatments, which was similar with other studies (von Borell et al., 1992; Broom et al., 1995; McGlone et al., 2004). It was shown that all of the IgG and most of the IgM in the colostrum of sows came from the blood (Bourne and Curtis, 1973). In this study, we found that sows in HR tended to have higher IgG concentration in colostrums compared with sows in MR. The result was consistent with our finding that sows in HR had higher protein damage compared with sows in MR during late gestation and lactation. As reactive oxygen species can cause chemical modification of proteins, the generated protein carbonyl could then lead to protein aggregates which become resistant to proteolysis, thereby altering the structure of protein and affecting their functions (Simm and Brömme, 2005). The damaged proteins could be recognized as nonself proteins by the immune system, therefore, increasing circulating antibodies, which may explain the increased colostrums IgG concentration in HR from the current study (Grune et al., 1995, 1998; Ullrich et al., 1999; Simm and Brömme, 2005; Bender, 2009).

Collectively, the reproductive performance, oxidative stress status, and immune status did not differ between sows housed in gestational crates (CON) and pens (HR + MR + LR). Sows in CON and MR did not show inferior reproductive performance during gestation and lactation. Sows in HR showed decreased litter performance as indicated by their decreased litter size and litter weight at birth and weaning, and sows in LR had lower reproductive efficiency as indicated by their lower farrowing rate. The BW, litter size, and litter weight of sows from different social ranks were showed to be related to their oxidative stress status during gestation and lactation. Sows in HR had increased oxidative damage to protein during late gestation and lactation as indicated by increased plasma protein carbonyl concentration, whereas sows in LR showed increased oxidative damage to DNA during late gestation and lactation, and these oxidative damages could contribute to the reduced litter size and litter weight in HR, and lower farrowing rate in LR.

LITERATURE CITED


Simm, A., and H. J. Brömme. 2005. Reactive oxygen species (ROS) and aging: Do we need them, can we measure them, should we block them? Signal Transduction 5:115–125.


