Periparturient cortisol, acute phase cytokine, and acute phase protein profiles of gilts housed in groups or stalls during gestation

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ABSTRACT: Use of gestation stalls in pork production remains a controversial topic in animal welfare. Immune status and measures are frequently used to assess stress levels and thus well-being of confined animals. The important welfare issue of close confinement among gestating gilts was tested by quantifying cortisol, acute phase cytokine, and acute phase protein profiles before and after farrowing of gilts housed in 2 systems. Landrace × Yorkshire crossbred gilts housed in groups of 4 (group, n = 8) in pens (3.9 × 2.4 m with 4 individual feeding spaces, 9.36 m² total or 2.34 m²/gilt) were compared with gilts housed in standard industry stalls (stall, n = 16; 2.2 × 0.6 m, 1.32 m²/gilt). Floors were fully slatted, and a substrate was not provided for either system. Cortisol was determined from saliva on d 105 of gestation, 1 h after moving the gilts into farrowing stalls (d 111), and 24 h and 7 d after farrowing. Cortisol was greater (P = 0.04) for group gilts compared with stall gilts 1 h after moving them into farrowing stalls and 24 h after farrowing. Cortisol concentrations decreased (P = 0.001) over time. Leukocyte mRNA expression of IL-1, IL-1 receptor antagonist, and tumor necrosis factor-α was determined by quantitative, reverse transcription PCR on d 35, 63, and 91 of gestation and 72 h after farrowing. Cytokine mRNA expression of peripheral blood mononuclear cells did not differ between housing systems for IL-1, its receptor antagonist, or for tumor necrosis factor-α. Acute phase proteins, including fibrinogen, haptoglobin, and α1-acid glycoprotein were determined for plasma samples taken at d 35, 63, and 91 of gestation and 72 h and 14 d after farrowing. In contrast to cortisol, plasma fibrinogen concentrations increased (P < 0.005) over time. Haptoglobin did not differ between treatments (P > 0.10). Stall gilts tended to have greater (P = 0.07) plasma α1-acid glycoprotein concentrations than group animals at d 35 of gestation and d 14 after farrowing. These data showed a trend (P < 0.07) for α1-acid glycoprotein concentrations to return to baseline more quickly in group-housed gilts, which did not appear to be directly related to increased cortisol just before farrowing. In conclusion, few differences in the acute phase response were detected between housing systems, suggesting that the resting immunological responses are only mildly affected by gestation stalls.

Key words: acute phase response, cortisol, gestation stall, housing, pig, stress

INTRODUCTION

Use of gestation stalls in pork production continues to raise concern among the North American public (Rice, 2000). Currently, about two-thirds of all US sows are housed in gestation crates that restrict their movement and behavioral diversity (Barnett et al., 2001; USDA, 2001). Sow length in relation to stall length and sow height in relation to stall width have been related to injuries and therefore to animal well-being (Anil et al., 2002). The possible necessity for transformation of housing systems has arisen from concern of the restrictive and thus stressful nature of industry-size stalls for gestating sows (Anil et al., 2002). Stress, in the form of restraint stress during gestation, resulted in postpartum depressive-like behavior and elevated cytokines in rats (O’Mahony et al., 2006). Stress disturbs homeostasis by altering the equilibrium of hormones, which can impact immune response in general (Eicher and Burton, 2005). Interleukin-1 is one such mediator of disease and in production of the systemic acute phase protein responses (Dinarello, 1991).

The acute phase response in animals is a nonspecific reaction to disturbances in homeostasis due to infection,
inflammation, tissue injury, or immunological disorders. The response includes changes in concentrations of some plasma proteins known as acute phase proteins (Alsemgeest et al., 1994). Immune status and measures are frequently used to assess the stress level and thus the well-being of confined animals (Hessing et al., 1995). We addressed the welfare issue of close confinement among gestating gilts by quantifying the acute phase response of gilts housed in 2 systems.

The objective for this study was to determine whether gestating gilts housed in individual stalls experience more stress, physical or psychological, than gilts housed in groups, resulting in suppression or hyperactivity in immune measures during the time in the gestation stalls and as first parity sows in farrowing stalls.

MATERIALS AND METHODS

Animals and Housing

The animals were cared for by the Purdue Swine Unit Animal Care Staff according to the research protocol approved by the Purdue Animal Care and Use Committee and in accordance with FASS (1999) guidelines. For this experiment, 48 Landrace × Yorkshire crossbred gilts were bred and housed at the Purdue University Animal Sciences Research and Education Center. Seven days after breeding, at approximately 8 mo of age, the gilts were randomly allocated to an individual stall (stall; 2.2 × 0.6 m, 1.32 m²/gilt) or to a group of 4 (group; 3.9 × 2.4 m with 4 individual feeding spaces, 9.36 m² total or 2.34 m²/gilt). The group housing system utilized gestation stalls as feeding spaces, with omission of the rear gate (Figure 1). Floors were fully slatted, and a substrate was not provided for either system. For experimental control, both housing systems were kept in a single room and treatments were maintained identically. Gilts in both systems had access to an individual waterer and feeding space. At 111 d of gestation, the gilts were moved into the farrowing barn into standard farrowing stalls (0.6 × 2.1 m).

Sampling Procedure

Blood was obtained by jugular venipuncture on d 35, 63, and 91 of gestation, and 72 h and 14 d after farrowing. Twenty milliliters of blood were drawn into 2 (10-mL) heparinized vacuum tubes. Samples were taken between 0900 and 1100 on each collection day. Blood samples were kept on ice until centrifuged at 700 × g for 15 min. Plasma was removed and frozen (−20°C) for later analysis, and theuffy coat (Peripheral blood mononuclear cells, PBMC) was collected for RNA extraction.

Salivary Cortisol Concentrations

Saliva samples were taken for cortisol analysis on d 105 of gestation, 1 h after moving the gilts into the farrowing barn on d 111, and 24 h and 7 d after farrowing. Cotton rolls (TIDI, Neenah, WI; 1 × 3.7 cm) tied to fishing line were used to collect saliva (Schonreiter et al., 1999). Gilts were allowed to chew on the cotton for approximately 45 s. The cotton rolls were put into 3-mL syringe sleeves and were stored inside 15-mL conical tubes. This allowed the saliva from the cotton to be easily extracted into the bottom of the conical tube upon centrifugation (600 × g for 20 min). After centrifugation, the saliva samples were submerged in ice and returned to a −80°C freezer until assayed.

Salivary concentrations of cortisol were quantified by using a Coat-A-Count cortisol kit (Diagnostic Products Corp., Los Angeles, CA). This RIA was designed for the quantitative measurement of cortisol in serum, urine, heparinized plasma, and saliva. All salivary samples were quantified in duplicate 200-μL aliquots in 1 of 5 assays. The use of the cortisol kit for porcine samples has been validated (Daniel et al., 1999). The interassay CV was 5.6%, the intraassay CV was 5.7%, the minimum detection limit was 2 ng/mL, and the assay sensitivity was 1.1 ng/mL.

Acute Phase Cytokine Response

Porcine peripheral blood mononuclear cells, collected on d 35, 63, and 91 of gestation and 72 h after farrowing, were used as a source of porcine cytokine mRNA to quantify IL-1β, IL-1 receptor antagonist (RA), and tumor necrosis factor (TNF)α using quantitative reverse transcription (RT) PCR. Porcine PBMC were isolated from heparin-treated blood samples by density gradient centrifugation (Histopaque 1077, Sigma, St. Louis, MO). The mononuclear cell fraction was washed 3 times in Hank’s balanced salt solution (Gibco, Carlsbad, CA) and resuspended in lymphocyte growth medium (Rose Park Memorial Institute 1640 medium, Gibco BRL, Life Technologies, Grand Island, NY). Ribonucleic acid was...
extracted from the pellet using an RNasea mini kit (Qiagen, Valencia, CA). Quantification of RNA was performed on a GeneQuant pro spectrophotometer (Amersham Pharmacia Biotech, Cambridge, UK) using a 10-mm cell path length at 260 and 280 nm wavelengths and a dilution factor of 20.

Genomic porcine sequences for II-1/β, II-1RA, and TNF-α were obtained from GenBank. Primers and probes (Table 1) were designed using Primer Express software (Applied Biosystems, Foster City, CA). Quantitation of primers was performed on the GeneQuant pro spectrophotometer (Amersham Pharmacia Biotech) using a 10-mm cell path length. The absorbance of each oligonucleotide was measured after a 1:100 dilution in Tris-EDTA buffer at 260 nm. Primers and probes were optimized for each gene of interest, and the genetic reference was hypoxanthine guanine phosphoribosyl transferase.

Random hexamers from the TaqMan Reverse Transcription Reagents were used for RNA RT using MultiScribe reverse transcription (Applied Biosystems). To improve accuracy, RT was performed in a 100-μL final volume. A 100-μL RT reaction will efficiently convert a maximum of 2 μg of total RNA to cDNA.

A reaction mixture of 25 μL of Universal Master Mix (Applied Biosystems), 5 μL each of the optimum primer concentrations, 5 μL of the optimum probe concentration, 5 μL of the cDNA sample, and 5 μL of water were added to a 96-well cDNA Sample, for a total volume of 50 μL/well/sample in duplicate. The plates were placed in an ABI Prism 7700 Sequence Detection System (Applied Biosystems) for sample amplification. Thermal cycling conditions were 50°C for 2 min for activation, 95°C for 10 min., and 40 cycles of 15 s at 95°C for TaqGold (Applied Biosystems) activation, 40 cycles at 95°C for 15 s, and 60°C for 1 min for annealing and extending. The relative standard curve method was chosen for analysis of PCR data. [User Bulletin #2, ABI Prism 7700 Sequence Detection System; available at: http://docs.appliedbiosystems.com/search.taf (last accessed 11 April 2007)].

**Acute Phase Protein Response**

Fibrinogen was determined using the IDEXX VetAuto read Hematology Analyzer using heat activation of fibrinogen (IDEXX Laboratories Inc., Westbrook, ME). Haptoglobin and αα-1-acid glycoprotein concentrations were determined for the plasma samples by radial immunodiffusion using a kit (Saikin Kagaku Institute Co. Ltd., Seda, Japan) specific for porcine haptoglobin and αα-1-acid glycoprotein. The porcine haptoglobin assay and αα-1-acid glycoprotein radial immunodiffusion kits had detection ranges of 50 to 1,500 μg/mL, a sensitivity of 5 μg/mL, and interassay CV of less than 4 and 2% for haptoglobin and αα-1-acid glycoprotein, respectively. The intraassay CV was <20% for haptoglobin and <10% for αα-1-acid glycoprotein.

**Statistical Analysis**

Eight pens of 4 gilts were compared as distinct experimental units (pen was the experimental unit) to 16 individually stalled gilts (each stall was the experimental unit). All physiological measures were analyzed as a repeated measures design (randomized complete block) using PROC MIXED (SAS Inst. Inc., Cary, NC). Compound symmetry and auto-regressive structures were used as appropriate for each variable. Block was a random variable, and the model effects were treatment, sampling time, and the treatment × sampling time interaction. Differences were considered significant at $P<0.05$ and trends at $P<0.10$. Therefore, differences were considered nonsignificant at $P>0.10$.

**RESULTS AND DISCUSSION**

**Cortisol Concentrations**

Salivary cortisol of group housed gilts was greater ($P = 0.04$) than stalled gilts on d 105 of gestation. This effect was eliminated by 7 d after farrowing (Figure 2). An overall time effect was also observed for both treatments ($P < 0.001$). Concentrations gradually declined over farrowing for group gilts but slightly increased for stall gilts upon moving into farrowing stalls and returned to baseline by 7 d after farrowing. It is possible that group cortisol concentrations were exacerbated in the first sampling due to unavoidable differences in collection methods between treatments. Researchers that collected the samples needed to get in

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**Table 1. Sequence of PCR primers and TaqMan probes used for porcine cytokines**

<table>
<thead>
<tr>
<th>Cytokine</th>
<th>Primer</th>
<th>Sequence (5’ to 3’)</th>
<th>Probe</th>
<th>Probe sequence (5’ to 3’)</th>
<th>Accession number</th>
</tr>
</thead>
<tbody>
<tr>
<td>IL-1/β</td>
<td>IL1b.748f</td>
<td>TCTGCCCTGTACCCCAACTG</td>
<td>IL1b.769t</td>
<td>TACATCAGCACCTCTTC</td>
<td>M86725</td>
</tr>
<tr>
<td></td>
<td>IL1b.812r</td>
<td>CCAAGGAAGACGGGCTTTT</td>
<td>ILRA.98t</td>
<td>TGCCTGCCACCCC</td>
<td>L38849</td>
</tr>
<tr>
<td>II-1RA</td>
<td>II1RA.74f</td>
<td>CTTTTTCTGTCCACCTAGAGA</td>
<td>TNF.373.t</td>
<td>TCAGATCATCGTCTCAAC</td>
<td>X57321</td>
</tr>
<tr>
<td></td>
<td>II1RA.143r</td>
<td>GAAAGCTTGCATCCTGGCAA</td>
<td>HPRT.349r</td>
<td>GCTTGCAACCTTGACCATCTTT</td>
<td></td>
</tr>
<tr>
<td>TNF-α</td>
<td>TNF.354f</td>
<td>AACCTTCTGGCCCAAGGA</td>
<td>HPRT.328f</td>
<td>CACATCAGCACCTCTTT</td>
<td></td>
</tr>
<tr>
<td></td>
<td>TNF.410f</td>
<td>GGCAGGCGCTTATCTGA</td>
<td>HPRT.349r</td>
<td>GCTTGCAACCTTGACCATCTTT</td>
<td></td>
</tr>
</tbody>
</table>

<sup>1</sup>RA = receptor antagonist; TNF-α = tumor necrosis factor-α; HPRT = hypoxanthine guanine phosphoribosyl transferase.

<sup>2</sup>f = forward primer and r = reverse primer.
Figure 2. Mean salivary cortisol concentrations (ng/mL) ± SE of gilts housed in groups (group, n = 8) or in stalls (stall, n = 16) during gestation and in stalls during farrowing and lactation. *Means within a time lacking a common superscript differ (P < 0.05). A trend existed between treatments (P = 0.09) at 1 h after moving the gilts into farrowing stalls. Time effect (P < 0.05). Time × treatment was not significant (P = 0.14).

Acute Phase Cytokine Response

Neither treatment nor time effects were apparent for IL-1β (time effect P = 0.16) or IL-1RA (Figure 3). From d 35 to 91, IL-1β concentrations tended to decrease for group and stall gilts (P = 0.10 and 0.11, respectively). A time effect (P < 0.003) was observed in TNF-α concentrations. Concentrations of TNF-α remained stable throughout gestation at 0.30 to 0.80 and then increased to 1.2 and 1.6 relative to hypoxanthine guanine phosphoribosyl transferase 72 h after farrowing (Figure 4). A treatment × time effect was not apparent for any of the cytokines. This study investigated use of quantitative RT-PCR to assess gene transcription in PBMC of healthy gilts during gestation. Overall, the real-time TaqMan PCR offered a quantitative PCR system for high analytical sensitivity and specificity, and increased reliability and reproducibility. Changes over time but no treatment differences were detected using this method.

Concentrations of IL-1, IL-1RA, and TNF-α remained in relatively normal ranges throughout gestation. Tumor necrosis factor-α doubled at farrowing. Tissue damage and inflammation during the birthing process may explain the reason for the increase. Interleukin-1 is an important inflammatory mediator involved in destruction of cartilage and bone that is a feature of atrophic rhinitis, erysipelas, and arthritis. Interleukin-1 localizes to inflammatory cells of affected joints (Davies et al., 1992). However, IL-1β and IL-1RA might also be expected to change at this time. This is not apparent in our results. Without specific pathogen signaling (lipopolysaccharide, flagellin, or other bacterial or viral components), an acute phase response would not be activated because of the redundancy of controls for IL-1. Interleukin-6 is another acute phase cytokine that might have been useful in determining differences in housing systems because α1-acid glycoprotein tended to be reduced in group-housed gilts. Interleukin-6 MRNA expression (which triggers α1-acid glycoprotein production) might be expected to be elevated in the stall-housed gilts if they had chronic stress or inflammation (Gabay, 2006). However, IL-1 and IL-6 concentrations frequently have similar response profiles. Interleukin-1β concentrations at d 91 suggest the suppression of immunity preceding parturition.

Conceptually, status of the immune system (immunosuppression vs. immunocompetence) will depend on the net effect of these changes (Khansari et al., 1990). Sows
in gestating housing systems are at risk of physical (fighting) or psychological (unresolved aggression) stressors (Harris et al., 2006). Therefore, stressors associated with housing systems such as those listed above may affect immune status during gestation (Geverink et al., 2003) by glucocorticoid mechanisms. Interleukins are cell signals secreted by immune cells to prepare surrounding tissue, attract and activate lymphocytes, and communicate with other physiological systems. Foremost among these are IL-1, IL-6, and TNF-α (Maule and VanderKooi, 1999). Circulating concentrations of IL-1 are elevated in a variety of clinical situations and, together with similarly elevated levels of TNF and IL-6, correlate with the severity of some diseases (Dinarello, 1991). The function of these acute phase proteins includes scavenging hemoglobin, free radicals, and cell nests, binding bacterial components, activating complement and having a role in cholesterol distribution, whereas some are thought to promote immunoglobulin production (Sorensen et al., 2006). These acute phase proteins have previously been described in pigs as indications of inflammation and disease (Eurell et al., 1990; Lampreave et al., 1994; Eckersall et al., 1996) and appeared to be correlated with atrophic rhinitis lesions in swine. However, the profile of acute phase protein response to stimulation differs among breeds (Clapperton et al., 2005) and species.

**Acute Phase Protein Response**

A time effect ($P < 0.005$) was present in fibrinogen concentrations, where both treatments increased steadily over the 5 sampling times (Figure 5). Concentrations of fibrinogen within 72 h of farrowing for group-housed...
gestation stalls or group housing

Figure 5. Mean plasma fibrinogen concentrations (mg/dL) ± SE of gilts housed in groups (group, n = 8) or in stalls (stall, n = 16) during gestation and in stalls during farrowing and lactation. Time effect (P < 0.001). No treatment effect (P > 0.10).

gilts were not different than stall gilts. A time effect (P < 0.001) was also detected in concentrations of plasma haptoglobin (Figure 6). Concentrations of haptoglobin increased steadily for both treatments until 72 h after farrowing, but treatment differences were not detected. A trend for a treatment effect (P = 0.10) was observed in plasma α1-acid glycoprotein (Figure 7). Stalled gilts tended to express greater concentrations of plasma α1-acid glycoprotein at d 35 of gestation (P = 0.07) and d 14 after farrowing (P = 0.07) than did group-housed gilts. In contrast to the other 2 acute phase proteins, a time effect was not detected for α1-acid glycoprotein concentrations; a time × treatment effect was also not evident.

At the site of injury, a number of responses of the tissue itself and of vascular system involving platelets, clot formation, leakage, and inflammatory cells are elicited. These responses in turn are associated with production of mediators or cytokines. The mediators activate receptors on different target cells leading to a systemic reaction characterized by fever, anorexia, leukocytosis, increased secretion of ACTH and glucocorticoids, activation of the complement system and clotting cascades, and changes in the concentration of some plasma proteins. Changes in these plasma proteins are observed within hours or days after onset of infection or inflammation, although many acute phase changes also indicate persistent disease (Dinarello, 1984).

Plasma concentrations of acute phase proteins are related to the severity of the underlying condition and provide a ready means of evaluating the presence and extent of disease. Slife (1994) reported that an elevated α1-acid glycoprotein level was a stress signal, whereas others suggest glucocorticoids play a role as inducers in the acute phase process (Neuhaus et al., 1966; Baumann et al., 1986) and act synergistically with cytokines to mediate changes in α1-acid glycoprotein gene expression (Stone and Maurer, 1987; Richards et al., 1992). Sows that had chronic exposure to lipopolysaccharide were more reactive during lactation (d 18) than unexposed sows (Sauber et al., 1999). In the current study, a trend for greater α1-acid glycoprotein concentrations was observed in stall-housed gilts on d 35 of gestation (P = 0.07) and d 14 after farrowing (P = 0.07). Gilts appear to be much less responsive during the lactation, never exceeding 550 μg/mL. The tendency for stalled gilts to have greater α1-acid glycoprotein concentrations during lactation suggests that their immune system had been activated (perhaps chronically). But the question remains whether this is a sign of a

Figure 6. Mean plasma haptoglobin concentrations (μg/mL) ± SE of gilts housed in groups (group, n = 8) or in stalls (stall, n = 16) during gestation and in stalls during farrowing and lactation. Time effect (P < 0.001). No treatment effect (P > 0.10).

Figure 7. Mean plasma α1-acid glycoprotein concentrations (μg/mL) ± SE of gilts housed in groups (group, n = 8) or in stalls (stall, n = 16) during gestation and in stalls during farrowing and lactation. A trend for treatment differences existed at d 35 of gestation (P = 0.07) and again on d 14 of after farrowing (P = 0.07). Time effect not significant (P > 0.10).
compromised immune system unable to rid the gilt of a chronic pathogen.

Because few differences were detected between treatments, we might conclude that gilts housed in groups and in stalls respond the same and therefore have equal welfare. However, we must consider that results are based on first parity sows and in this particular system in which gilts had access to individual feeding space and water. The time they were in the stall housing is short compared with older parity sows. Additionally, older parity sows are larger, with less room to move in the stalls, but that would hold true for group-housed sows in this system. Older parities and other systems with group feeding and water should be compared with gestation stall housing to better understand the full impact of housing conditions on the porcine adaptive and innate immunity.

This study is part of a larger controlled comparison of stall and small group effects in conjunction with a multidisciplinary approach including production, health, and behavior (Harris et al., 2006), piglet growth and behavioral response (Sorrells et al., 2006), and immunology presented here enabling a comprehensive evaluation of well-being. Few differences were found between housing systems for gilts. However, differences between the systems observed in this study accentuated the importance of habituation of the gilts to any system, greater cortisol concentrations associated with more social environments, and the role of immune activation on postpartum homeostatic responses. It is important to continue research in this area because these results can only apply to the particular housing system evaluated for first parity gilts. Results may vary for sows of higher parities, or smaller or larger stalls. Larger groups, more floor space, bedding, or different feeding methods may affect results and well-being differently.

**LITERATURE CITED**


Gestation stalls or group housing


