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The effects of thermal environment and spray-dried plasma on the acute-phase response of pigs challenged with lipopolysaccharide1,2

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ABSTRACT: Forty barrows (TR4 × C22) were weaned at 17 d of age (BW = 6.27 ± 0.30 kg), housed (two pigs/pen) in a thermal-neutral environment (TN; constant 26.7°C), and fed diets with or without 7% (as-fed basis) spray-dried plasma (SDP). On d 7, one pig/pen was moved into a cold environment (CE; constant 15.6°C). Pigs were fitted with jugular catheters on d 11. On d 12, 16 pigs per environment (eight pigs per dietary treatment) were challenged i.v. with 75 μg of lipopolysaccharide (LPS)/kg of BW. Blood samples were collected over a 4.5-h period. Pigs were then killed and tissue samples were harvested for messenger RNA (mRNA) analysis. From d 0 to 7, pigs fed SDP diets had a lower gain:feed ratio (G/F) than pigs fed no SDP (533 ± 14 vs. 585 ± 17 g/kg; P < 0.03). Pigs housed in the CE consumed more feed and had a lower G/F than pigs housed in TN from d 7 to 11 (P < 0.001). There were no environment × diet interactions from d 7 to 11 (P > 0.78). Baseline concentrations of serum ACTH and cortisol were lower in the TN pigs than in the CE pigs (P < 0.001). Pigs fed diets without SDP had lower serum cortisol concentrations over the 4.5-h period than pigs fed SDP (time × diet, P < 0.001). Serum concentrations of tumor necrosis factor-α (TNF-α) were highest for pigs consuming SDP in the CE, whereas there were no differences among the other treatments (time × diet × environment, P < 0.02). Pigs housed in the CE had higher serum interleukin-1β (IL-1β) (P < 0.001) and interleukin-6 (IL-6; P < 0.001) than TN pigs. Pigs fed SDP also had slightly higher serum IL-1β concentrations (P < 0.10) and higher (P < 0.001) IL-6 concentrations than pigs fed no SDP. Pigs fed SDP had 9% lower liver and 13% lower thymus mRNA expression of tumor necrosis factor-α (TNF-α) than pigs that consumed no SDP (P < 0.06). Liver IL-1β, IL-6, and LPS-binding protein mRNA were higher in the CE than in the TN (P < 0.03, P < 0.001, and P < 0.05; respectively). In addition, spleen TNF-α (P < 0.03) and IL-6 (P < 0.01) mRNA levels were higher in the CE than in the TN. Pigs consuming SDP and challenged with LPS responded with elevated serum concentrations of cortisol and cytokines compared with pigs fed diets with no SDP. Housing pigs in a CE increased the baseline concentrations of ACTH and cortisol, and when coupled with an LPS challenge, resulted in elevated serum and tissue mRNA levels of cytokines. Cold stress and feeding SDP during a LPS challenge may result in increased stress and immune responses in young pigs.

Key Words: Pigs, Cold Stress, Blood Plasma, Cytokines, Cortisol, Lipopolysaccharides

Introduction

Diets for weaning pigs that contain spray-dried plasma (SDP) improve growth performance (Hansen et al., 1993; de Rodas et al., 1995). Interestingly, the use of SDP to improve pig performance in conventional, on-farm nurseries is more effective than in environments with a lower pathogen load (Coffey and Cromwell, 1995).

The enhanced performance of pigs consuming SDP may be the result of a lower level of immune stimulation compared with pigs consuming diets with no SDP (Carroll et al., 2002). However, pigs fed SDP exhibit greater immune stimulation when challenged with lipopolysaccharide (LPS), as demonstrated by increased serum tumor necrosis factor-α (TNF-α) and interferon-γ (Touche et al., 2002). Other research suggests that the inclusion of complex ingredients, such as SDP and fishmeal, in nursery diets has no effect on the growth
response of pigs when repeatedly challenged with LPS (Dritz et al., 1996). In addition to SDP, cold stress has been reported to influence serum TNF-α concentrations (Carroll et al., 2001) and the febrile response (Klir et al., 1997; Carroll et al., 2001) to an endotoxin challenge in young pigs.

Although the literature evaluating the effects of cold stress on young pigs is minimal, the prevailing theory relates to cold stress predisposing the pig to bacterial infection (Curtis et al., 1976). This effect may be related to the inability of the young pig to mount a febrile response (Klir et al., 1997) and/or the capacity to secrete adequate concentrations of TNF-α (Carroll et al., 2001) in a cold environment.

Since limited information is available regarding the influences of feeding SDP and housing pigs under cold stress on immune system activation, additional research is needed. This study was designed to evaluate the effects of feeding diets with or without SDP in a thermal-neutral or cold environment on the acute-phase response associated with an immune challenge in nursery pigs.

**Materials and Methods**

**Experimental Design**

Forty barrows (TR4 × C22) were weaned at 17 d of age (initial BW = 6.27 ± 0.30 kg) and transported to the University of Missouri’s Brody Climatology Laboratory. All pigs were separated into groups of two per pen in a thermal-neutral environment (TN, constant at 26.7°C and 50% humidity) for 7 d and allotted to one of two experimental diets (0 or 7% spray-dried plasma protein; NSDP and SDP, respectively). Both diets (Table 1) were formulated to meet or exceed all nutrient recommendations of the NRC (1998). On d 7, one pig per pen was allotted to remain in the TN and the other pig was moved to the cold environment (CE, constant at 15.6°C and 50% humidity). On d 11, pigs were fitted nonsurgically with jugular catheters as described by Carroll et al. (1999). All pigs and feeders were weighed on d 0, 7, and 11 to calculate ADG and ADFI. The following day, eight pigs per treatment group were challenged i.v. with 75 μg of LPS/kg of BW (Escherichia coli serotype 0111:B4; Sigma L-2630, Sigma Chemical, St. Louis, MO) dissolved in 0.9% (wt/vol) NaCl solution. Blood samples were collected every 15 min via the jugular catheter from –0.5 to 4 h relative to challenge. Serum was harvested from blood samples and stored at –80°C until later analysis. Baseline concentrations of stress hormones, cytokines, and acute-phase proteins are reported as the mean serum concentration from –0.5 to 0 h. After the 4.5-h blood collection period, all pigs were killed by captive bolt followed by exsanguination for tissue sample collection. All tissues samples collected were immediately placed on dry ice and then stored at –80°C until they were extracted for messenger RNA (mRNA) analysis.

**Hormone Assays**

For each hormone, samples were analyzed in duplicate within a single assay as previously reported (Carroll et al., 2002). Serum concentration of ACTH was determined using a commercially available human ACTH double-antibody assay kit (Diagnostic Products, Los Angeles, CA), which were validated for use in swine serum in our laboratory (Daniel et al., 1999). Minimal detectability of this assay was 30 pg/mL, with an intraassay CV of 10.1%. Serum concentration of cortisol was determined using a Coat-a-Count assay kit (Diagnostic Products), which we previously validated for use in our laboratory (Daniel et al., 1999). Minimal detectability was 5 ng/mL, with an intraassay CV of 5.6%.

**Cytokine Analysis**

Serum concentration of tumor necrosis factor-α (TNF-α) was determined using a pig TNF-α ELISA kit per the instructions of the manufacturer (Pierce-Endogen, Inc., Woburn, MA). The dynamic range of the

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**Table 1. Experimental diets (as-fed basis)**

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>NSDP</th>
<th>SDP</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dried whey</td>
<td>30.0</td>
<td>30.0</td>
</tr>
<tr>
<td>Corn starch</td>
<td>17.9</td>
<td>12.1</td>
</tr>
<tr>
<td>Corn</td>
<td>17.2</td>
<td>26.5</td>
</tr>
<tr>
<td>Soybean meal (48% CP)</td>
<td>16.5</td>
<td>16.5</td>
</tr>
<tr>
<td>Soy protein concentrate</td>
<td>10.0</td>
<td>7.0</td>
</tr>
<tr>
<td>Spray-dried plasma*</td>
<td>—</td>
<td>7.0</td>
</tr>
<tr>
<td>Corn oil</td>
<td>3.0</td>
<td>3.0</td>
</tr>
<tr>
<td>Blood cells</td>
<td>1.75</td>
<td>1.75</td>
</tr>
<tr>
<td>Dicalcium phosphate</td>
<td>0.81</td>
<td>0.28</td>
</tr>
<tr>
<td>Limestone</td>
<td>0.75</td>
<td>1.14</td>
</tr>
<tr>
<td>Salt</td>
<td>0.65</td>
<td>0.14</td>
</tr>
<tr>
<td>Potassium chloride</td>
<td>—</td>
<td>0.37</td>
</tr>
<tr>
<td>Zinc oxide</td>
<td>0.30</td>
<td>0.30</td>
</tr>
<tr>
<td>t-Methionine</td>
<td>0.26</td>
<td>0.20</td>
</tr>
<tr>
<td>Mecadox premixb</td>
<td>0.25</td>
<td>0.25</td>
</tr>
<tr>
<td>Vitamin premixc</td>
<td>0.25</td>
<td>0.25</td>
</tr>
<tr>
<td>L-Lysine-HCl</td>
<td>0.15</td>
<td>0.05</td>
</tr>
<tr>
<td>Trace mineral premixd</td>
<td>0.15</td>
<td>0.15</td>
</tr>
<tr>
<td>L-Threonine</td>
<td>0.07</td>
<td>0.007</td>
</tr>
<tr>
<td>ME, Mcal/kg</td>
<td>3.27</td>
<td>3.27</td>
</tr>
<tr>
<td>Lysine, %</td>
<td>1.50</td>
<td>1.50</td>
</tr>
<tr>
<td>Methionine + cystine, %</td>
<td>0.90</td>
<td>0.90</td>
</tr>
<tr>
<td>Threonine, %</td>
<td>0.98</td>
<td>0.98</td>
</tr>
<tr>
<td>Calcium, %</td>
<td>0.80</td>
<td>0.80</td>
</tr>
<tr>
<td>Available phosphorus, %</td>
<td>0.40</td>
<td>0.40</td>
</tr>
<tr>
<td>Sodium, %</td>
<td>0.56</td>
<td>0.56</td>
</tr>
<tr>
<td>Potassium, %</td>
<td>1.23</td>
<td>1.23</td>
</tr>
</tbody>
</table>

*Appetein (APC, Inc., Ames, Iowa 50036).
bSupplied 55 mg/kg.
*Provided the following per kilogram of complete diet: 11,023 IU of vitamin A, 1,102 IU of vitamin D₃, 22 IU of vitamin E, 4 mg of menadione sodium bisulfate, 0.03 mg of vitamin B₁₂, 8.3 mg riboflavin, 28.1 mg of α-pantothenic acid, and 33.1 mg of niacin.
*Provided the following per kilogram of complete diet: 165.3 mg of Fe, 165.3 mg of Zn, 33 of Mn, 16.5 mg of Cu, 0.30 mg of I, and 0.285 mg of Se.
*All amino acid values expressed on a total basis.
assay was 38.4 to 1,500 pg/mL, with a sensitivity of 6.1 pg/mL. The interassay CV was 5.2% and the intraassay CV was 7.4%.

Serum concentration of interleukin-1β (IL-1β) was analyzed using a commercially available ELISA kit specific for porcine IL-1β (Quantikine P-PLB00, R & D Systems, Minneapolis, MN). Analyses were conducted as outlined by the manufacturer. The dynamic range of the assay was 39 to 2,500 pg/mL. The minimal detectable dose of porcine IL-1β for this kit is typically less than 10 pg/mL.

Serum concentration of interleukin-6 (IL-6) was analyzed using a commercially available ELISA kit specific for porcine IL-6 (Quantikine P–P6000, R & D Systems). Analyses were conducted as outlined by the manufacturer. The dynamic range if the assay was 39.1 to 2,500 pg/mL. The minimal detectable dose of porcine IL-6 for this kit is typically 10 pg/mL.

Acute-Phase Protein Analysis
The acute-phase proteins (APP), C-reactive protein (CRP), and haptoglobin, were analyzed using commercially available assay kits (Porcine C-Reactive Protein Assay and Haptoglobin Assay, Tri-Delta Diagnostics, Inc., Morris Plains, NJ). Analyses were conducted as outlined by the manufacturer. For each APP, reagents were pooled across assay kits and all serum samples were analyzed within one assay.

Quantification of Messenger Ribonucleic Acid
Total RNA was extracted from the pituitary gland, thymus, spleen, liver, and adrenal glands according to manufacturer’s instructions (Tri-reagent, Molecular Research Center, Inc., Cincinnati, OH) and transferred to a nylon membrane with a slot-blot apparatus (Bio-Dot SF, Bio-Rad Laboratories, Hercules, CA). Hybridization and detection were carried out with a commercially available kit according to the manufacturer’s instructions (BrightStar System, Ambion Inc., Austin, TX). Hybridization signal intensities were quantified by densitometry, with target mRNA values expressed relative to 28s ribosomal RNA for each sample. The probes and procedures for detection of mRNA specific for corticotropin-releasing hormone (CRH) receptor, proopiomelanocortin (POMC), ACTH receptor, IL-1β, IL-6, and TNF-α have been described previously (Daniel et al., 1999; Carroll et al., 2002; Touchette et al., 2002).

Polymerase chain reaction was used to amplify a complementary DNA (cDNA) for lipopolysaccharide-binding protein (LPSBP; RNA-PCR kit, Perkin-Elmer Corp., Foster City, CA). The up- and downstream oligonucleotide primers for PCR amplification were 5′-AGG TGA TGT TTA AGG GTG AA-3′ and 5′-CCA CAC TGA GCC GGA AGA CA-3′ for LPSBP (420-bp product; GenBank accession AF268388). The PCR product was subsequently cloned into a T-cloning vector (PCR-II, Invitrogen Corp., San Diego, CA). The identity of the cDNA clone was confirmed by dideoxy termination sequencing, which shared 81% homology with the human LPSBP sequence. A biotinylated riboprobe was synthesized for use in chemiluminescence-based detection (BrightStar System, Ambion, Inc., Austin, TX). All mRNA values are reported in relative units (ru).

Statistical Analysis
For the growth performance data from d 0 to 7, the pen mean performance data (n = 20) were analyzed as a completely random design using the GLM procedure of SAS (SAS Inst., Inc., Cary, NC). The experimental design from d 7 to 11 was a 2 × 2 factorial arrangement. The performance data from d 7 to 11 was analyzed as a randomized complete block design using the GLM procedure of SAS (1999). The statistical model included the main effects and interaction of dietary treatment and thermal environment. Individual pig was the experimental unit. Only those animals from which blood and tissue samples were collected were used for this portion of the analysis (n = 32). Statistical analyses for serum hormones and cytokines and tissue mRNA values were performed using Statview software (SAS Inst. Inc.) and the theory and rationale of Gardiner and Gettinby (1998). For all mRNA data, statistical analyses were performed using ANOVA and mean comparisons using Fisher’s protected LSD. The statistical model included the effects of diet (NSDP vs. SDP), temperature (TN vs. CE), and their interactions. Serum hormones and cytokines were analyzed by ANOVA specific for repeated measures. The statistical model included the effects of diet (NSDP vs. SDP), temperature (TN vs. CE), time, and their interactions. The within-pig error term was used to test for differences among diet, temperature, and diet × temperature. The within-pig-by-time error term was used to test for differences among time, time × diet, time × temperature, and time × diet × temperature. The variances for all data sets were proven to be homogeneous before analysis.

Results
Growth Performance
All growth performance data are presented in Table 2. From d 0 to 7 all pigs were housed in the TN. While under TN conditions, pigs fed the diet containing 7% SDP had a 52 g/kg reduction in gain:feed ratio (G/F) compared to the NSDP group (P < 0.03). Feed intakes were not different; however, this did not translate into heavier body weights for the NSDP pigs at the end of the period. After pigs were moved into their respective environments for the remainder of the trial, the pigs consuming SDP tended to have lower ADG compared with the NSDP fed pigs in either environment (P < 0.10). Pigs housed in the CE had a 24% increase in ADFI and a 20% decrease in G/F compared to the pigs
Environmental temperature, spray-dried plasma, and LPS

Table 2. The effect of thermal environment and spray-dried plasma (SDP) or no SDP (NSDP) on pig growth performance

<table>
<thead>
<tr>
<th>Item</th>
<th>TN (26.7°C)</th>
<th>CE (15.6°C)</th>
<th>P-valuesb</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>NSDP</td>
<td>SDP</td>
<td>NSDP</td>
</tr>
<tr>
<td>Pre-test period</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>d 0 BW, kg</td>
<td>6.26</td>
<td>6.27</td>
<td>—</td>
</tr>
<tr>
<td>d 0 to 7 ADG, g</td>
<td>299</td>
<td>270</td>
<td>—</td>
</tr>
<tr>
<td>ADFI, g</td>
<td>511</td>
<td>507</td>
<td>—</td>
</tr>
<tr>
<td>G/F, g/kgc</td>
<td>555</td>
<td>533</td>
<td>—</td>
</tr>
<tr>
<td>d 7 BW, kg</td>
<td>8.36</td>
<td>8.16</td>
<td>—</td>
</tr>
<tr>
<td>Test period</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>d 7 BW, kg</td>
<td>8.53</td>
<td>8.51</td>
<td>8.65</td>
</tr>
<tr>
<td>d 7 to 11 ADG, g</td>
<td>426</td>
<td>378</td>
<td>437</td>
</tr>
<tr>
<td>ADFI, g</td>
<td>498</td>
<td>456</td>
<td>633</td>
</tr>
<tr>
<td>G/F, g/kgc</td>
<td>845</td>
<td>797</td>
<td>690</td>
</tr>
<tr>
<td>d 11 BW, kg</td>
<td>10.24</td>
<td>10.02</td>
<td>10.40</td>
</tr>
</tbody>
</table>

aAll pigs were housed two pigs/pen in the thermal-neutral (TN) environment from d 0 to 7 postweaning (pretest period). For the test period (d 7 to 11), pigs were allotted to either the TN or cold environment (CE) within their dietary treatment of NSDP or SDP and housed individually.

bMain effects of environment (E), dietary treatment (D), and their interaction (E × D). All measures of feed intake are on an as-fed basis.

Serum Hormone and Cytokine Profiles

Baseline concentrations of serum ACTH and cortisol were significantly elevated in pigs maintained in the CE compared to pigs maintained in the TN (Table 3). The SDP treatment group had 22% lower baseline concentrations of serum CRP compared with the NSDP group (P < 0.03); however, there was no diet effect for baseline concentrations of serum haptoglobin. Again, environment × diet interactions were not significant for baseline ACTH, cortisol, or APP.

As shown in Figure 1, no treatment differences in serum ACTH were detected over the sampling period. After the LPS challenge, a time effect (P < 0.001) was observed with peak ACTH concentrations of 600 pg/mL occurring at 1.5 h. In contrast, serum cortisol concentrations following the LPS challenge were lower in pigs maintained in the TN compared with pigs maintained in the CE (P < 0.05, Figure 2). Pigs consuming diets with SDP had greater serum cortisol concentrations than pigs fed NSDP diets after LPS challenge (time × diet, P < 0.001).

Serum TNF-α reached peak concentrations 1 h after LPS administration (Figure 3). From 1 h to approximately 3 h after the challenge, pigs housed in the CE and fed SDP had greater TNF-α concentrations compared with NSDP fed pigs. In the TN, there were no differences in serum TNF-α between the two dietary

Table 3. Effects of thermal environment and spray-dried plasma (SDP) or no SDP (NSDP) on baseline concentrations of serum ACTH, cortisol, and acute-phase proteins

<table>
<thead>
<tr>
<th>Item</th>
<th>TN (26.7°C)</th>
<th>CE (15.6°C)</th>
<th>P-valuesb</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>NSDP</td>
<td>SDP</td>
<td>NSDP</td>
</tr>
<tr>
<td>ACTH, pg/mL</td>
<td>61.3</td>
<td>57.7</td>
<td>85.1</td>
</tr>
<tr>
<td>Cortisol, ng/mL</td>
<td>24.1</td>
<td>27.6</td>
<td>39.1</td>
</tr>
<tr>
<td>APPc</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CRP, μg/mL</td>
<td>83</td>
<td>68</td>
<td>94</td>
</tr>
<tr>
<td>Haptoglobin, μg/mL</td>
<td>511</td>
<td>474</td>
<td>685</td>
</tr>
</tbody>
</table>

aAll pigs were housed two pigs/pen in the thermal-neutral (TN) environment from d 0 to 7 postweaning (pretest period). For the test period (d 7 to 11), pigs were allotted to either the TN or cold environment (CE) within their dietary treatment of NSDP or SDP and were housed individually. Baseline concentrations of ACTH, cortisol, and the APP are reported as the mean serum concentration from −0.5 to 0 h.

bMain effects of environment (E), dietary treatment (D), and their interaction (E × D).

APP = acute-phase proteins; CRP = C-reactive protein.
Figure 1. Serum concentrations of ACTH following an i.v. challenge with 75 μg of lipopolysaccharide (LPS)/kg of BW at 0 h. Pigs were housed in either a cold environment (CE, 15.6°C) or thermal-neutral environment (TN, 26.7°C) and fed a diet with spray-dried plasma (SDP) or without SDP (NSDP). There was a time effect \((P < 0.001)\) such that serum ACTH increased over the 4.5-h sampling period. No significant treatment effects were observed. Error bars are omitted for presentation purposes. Pooled SEM = 7.5 pg/mL.

Figure 2. Serum concentrations of cortisol following an i.v. challenge with 75 μg of lipopolysaccharide (LPS)/kg of BW at 0 h. Pigs were housed in either a cold environment (CE, 15.6°C) or thermal-neutral environment (TN, 26.7°C) and fed a diet with spray-dried plasma (SDP) or without SDP (NSDP). Overall, pigs reared in the TN environment had lower serum cortisol levels compared to the CE pigs following the LPS challenge \((P < 0.05)\). There was also a time × diet interaction \((P < 0.001)\), such that mean serum cortisol concentrations following the LPS challenge were lower for pigs consuming diets with NSDP compared to pigs consuming diets with SDP. Error bars are omitted for presentation purposes. Pooled SEM = 3.0 ng/mL.

Figure 3. Serum concentrations of tumor necrosis factor-α (TNF-α) following an i.v. challenge with 75 μg of lipopolysaccharide (LPS)/kg of BW at 0 h. Pigs were housed in either a cold environment (CE, 15.6°C) or thermal-neutral environment (TN, 26.7°C) and fed a diet with spray-dried plasma (SDP) or without SDP (NSDP). After challenging the pigs with LPS, the CE-SDP group had a greater increase in serum TNF-α than the TN-SDP group, whereas there were no differences between the two environments for the pigs consuming NSDP. This resulted in a time × environment × diet interaction \((P < 0.02)\). Error bars are omitted for presentation purposes. Pooled SEM = 383 pg/mL.

At 2.5 h after LPS challenge, IL-6 peaked between 16,000 and 23,000 pg/mL depending on environment and diet (Figure 4). Pigs in the CE had greater increases in serum IL-6 than pigs in the TN in response to the LPS \((time \times environment, P < 0.001)\). Other differences included a time × diet interaction where SDP-fed pigs had elevated IL-6 concentrations compared with NSDP-fed pigs following challenge \((P < 0.001)\). Although this was more evident in the CE, there was no time × environment × diet interaction. The 4.5-h sampling period did not allow the determination of peak serum IL-1β concentrations.

Serum IL-1β concentrations began to increase between 1 and 2 h following LPS challenge (Figure 5). The concentrations of IL-1β were greater for pigs maintained in the CE compared with pigs maintained in the TN during the sampling period \((P < 0.001)\). Furthermore, SDP-fed pigs tended to have greater serum concentrations of IL-1β than those that were NSDP fed \((P < 0.10)\). However, no environment × diet interactions were observed for IL-1β. The 4.5-h sampling period did not allow the determination of peak serum IL-1β concentrations.

The APP analysis of CRP and haptoglobin are presented in Figures 6 and 7, respectively. Interestingly, there was a time × environment interaction for CRP treatments. This resulted in a time × environment × diet interaction \((P < 0.02)\). Serum TNF-α returned to near baseline levels approximately 3.5 h after challenge for all treatment groups.
Environmental temperature, spray-dried plasma, and LPS

Figure 4. Serum concentrations of interleukin-6 (IL-6) following an i.v. challenge with 75 µg of lipopolysaccharide (LPS)/kg of BW at 0 h. Pigs were housed in either a cold environment (CE, 15.6 °C) or thermal-neutral environment (TN, 26.7 °C) and fed a diet with spray-dried plasma (SDP) or without SDP (NSDP). Following the LPS challenge, pigs reared in the CE had a greater increase in serum IL-6 than pigs in the TN (time × environment, P < 0.001). In addition, pigs fed SDP showed a greater increase in serum IL-6 than pigs fed NSDP after the LPS challenge (time × diet, P < 0.001). Error bars are omitted for presentation purposes. Pooled SEM = 521 pg/mL.

Figure 5. Serum concentrations of interleukin-1β (IL-1β) following an i.v. challenge with 75 µg of lipopolysaccharide (LPS)/kg of BW at 0 h. Pigs were housed in either a cold environment (CE, 15.6 °C) or thermal-neutral environment (TN, 26.7 °C) and fed a diet with spray-dried plasma (SDP) or without SDP (NSDP). Pigs reared in the CE had a greater increase in serum IL-1β than pigs in the TN following the LPS challenge (time × environment, P < 0.001). Furthermore, pigs fed SDP showed a more moderate increase in serum IL-1β than pigs fed NSDP during the challenge period (time × diet, P < 0.10). Error bars are omitted for presentation purposes. Pooled SEM = 35 pg/mL.

Figure 6. Serum concentrations of C-reactive protein (CRP) following an i.v. challenge with 75 µg of lipopolysaccharide (LPS)/kg of BW at 0 h. Pigs were housed in either a cold environment (CE, 15.6 °C) or thermal-neutral environment (TN, 26.7 °C) and fed a diet with spray-dried plasma (SDP) or without SDP (NSDP). Pigs reared in the CE had a greater increase in serum CRP than pigs in the TN during the LPS challenge period (time × environment, P < 0.001). Error bars are omitted for presentation purposes. Pooled SEM = 3.0 µg/mL.

Figure 7. Serum concentrations of haptoglobin following an i.v. challenge with 75 µg of lipopolysaccharide (LPS)/kg of BW at 0 h. Pigs were housed in either a cold environment (CE, 15.6 °C) or thermal-neutral environment (TN, 26.7 °C) and fed a diet with spray-dried plasma (SDP) or without SDP (NSDP). Pigs reared in the TN had lower serum haptoglobin levels than pigs in the CE following the LPS challenge (time × environment, P < 0.05). Error bars are omitted for presentation purposes. Pooled SEM = 20 µg/mL.
where concentrations in the CE increased at 1 h and subsequently decreased at 2 h relative to LPS challenge, whereas in the TN, the opposite phenomenon occurred (\(P < 0.001\)). The concentrations of CRP appeared to plateau between 3 and 4 h after LPS administration. As observed with CRP, at 1-h post challenge there was a decrease in serum haptoglobin in the TN environment and a slight increase in the CE; however, these differences were not as prominent (\(P < 0.05\)).

**Messengers Ribonucleic Acid Expression**

Analyses of mRNA expression are presented in Table 4. No significant environment or dietary treatment differences in pituitary mRNA levels of POMC and CRH-R or adrenal ACTH-R were found. Although no differences were detected in these tissues, environment and dietary treatment influenced cytokine mRNA expression in the liver, thymus, and spleen.

Feeding SDP resulted in an 18% decrease in liver TNF-\(\alpha\) mRNA expression at 4-h post challenge (\(P < 0.06\)). A similar effect was noted with thymus TNF-\(\alpha\) mRNA expression, where pigs consuming SDP diets had lower expression compared with NSDP-fed pigs (0.23 ± 0.01 vs. 0.26 ± 0.02 ru, respectively; \(P < 0.06\)). No other effect of diet was observed on the mRNA analysis. Environment had a much greater influence on tissue mRNA expression. Pigs housed in the TN had 20% lower IL-1\(\beta\) and 45% lower IL-6 mRNA expression in the liver compared with the pigs in the CE (\(P < 0.03\) and \(P < 0.001\), respectively). In addition, liver LPSBP mRNA expression was reduced in the TN pigs vs. the CE pigs (0.43 ± 0.02 vs. 0.49 ± 0.03 ru, respectively; \(P < 0.05\)). Spleen TNF-\(\alpha\) mRNA levels were 0.33 ± 0.02 ru for pigs in the TN and 0.38 ± 0.02 ru for pigs in the CE (\(P < 0.03\)). For spleen IL-6 mRNA, the pigs in the CE had 30% greater expression than pigs in the TN 4 h after challenge (\(P < 0.01\)). A tendency for an environment × diet interaction was observed for thymus mRNA of IL-1\(\beta\) (\(P < 0.09\)). In the TN, pigs fed NSDP had slightly increased IL-1\(\beta\) mRNA levels compared with pigs fed SDP (1.43 ± 0.03 vs. 1.39 ± 0.10 ru, respectively); however, in the CE, pigs consuming NSDP had lower expression of IL-1\(\beta\) mRNA than SDP-fed pigs (1.37 ± 0.07 vs. 1.68 ± 0.16 ru, respectively). No other environment × diet interactions approached significance.

**Discussion**

In the present study only one time × environment × diet interaction occurred. This was for serum concentrations of TNF-\(\alpha\) following the LPS challenge. Although the role of TNF-\(\alpha\) in relation to the acute-phase response and metabolism is highly significant (Johnson, 1997; Spurlock, 1997), the inconsistency of additional environment × diet interactions following the LPS challenge for IL-1\(\beta\) and IL-6 would indicate that feeding SDP is no more beneficial to piglet growth performance and immune system activation in the CE than in the TN. This lack of environment × diet interactions for the
stress hormones, IL-6, IL-1β, and the APP indicates the effects of feeding SDP and housing pigs in the CE were additive. Feeding SDP is associated with improved growth performance (Kats et al., 1994; Coffey and Cromwell, 1995; de Rodas et al., 1995), likely the result of a lower level of immune stimulation in SDP-fed pigs (Carroll et al., 2002; Touchette et al., 2002). Hyun et al. (1998a,b) also determined that various stresses, such as high temperature, high stocking density, and regrouping, affect pig growth performance in an additive manner. Considering these findings, the main effects of environment and diet are the primary focus of the discussion.

It is well known that the effects of an environmental temperature below the thermal-neutral zone results in an increase in feed intake and decreased feed conversion. Schenck et al. (1992) conducted a trial in which pigs were weaned into a constant thermal temperature of 20°C or 32°C. During d 0 to 21, a 22% increase in ADFI was observed in the cool environment accompanied by a 19% reduction in ADG and a 2% increase in feed:gain ratio. In the present study, pigs were exposed to a CE with a constant temperature of 15.6°C for a 4-d period. Our results are consistent with the findings of Schenck et al. (1992), in that a 24% increase in ADFI was observed. In slight contrast to those findings was the 20% reduction in G/F observed in our study. However, other data in growing pigs have shown a 16 and 13% decrease in G/F (Stahly and Cromwell, 1979; Stahly et al., 1979, respectively) when pigs were reared in a cold environment of 10°C vs. a thermal neutral environment of 22.5°C.

The design of our study was to challenge all pigs with LPS; therefore, baseline concentrations of serum ACTH and cortisol were measured to confirm that the CE was in fact eliciting a stress response. Indeed, pigs reared in the CE had greater baseline levels of both ACTH and cortisol regardless of dietary treatment. Other work from our laboratory has shown differences in serum cortisol due to a cold stress in neonatal pigs 24 h of age; however, no differences were observed with ACTH concentrations (Carroll et al., 2001). Likewise, Klir et al. (1997) found numerical differences in serum cortisol due to a cold stress in 28-d-old pigs. In contrast to the differences in baseline concentrations of ACTH and cortisol observed in our study, baseline levels of the APP were not affected by environmental temperature. This was not surprising because baseline levels of TNF-α were also not different between the two environments (data not shown). Baseline IL-6 and IL-1β concentrations were not measured because no differences were detected in serum TNF-α. Although IL-6 may be one of the most potent stimulators of APP synthesis from the liver, TNF-α has the potential to modulate hepatic APP responses as well (see reviews by Steel and Whitehead, 1994; Johnson, 1997).

After all pigs were challenged with LPS, the environmental effects on serum ACTH and cortisol were no longer detectable. However, after challenge, the concentrations of IL-6 and IL-1β were increased to a greater extent in the CE pigs compared with the TN pigs. To our knowledge, this is the first report evaluating the interaction between environmental temperature and LPS challenge on cytokines IL-6 and IL-1β in the neonatal pig. Carroll et al. (2001) and Klir et al. (1997) evaluated TNF-α in their studies. In the former, serum TNF-α was increased to a greater extent in the cold-stressed vs. thermal-neutral housed pigs 3-h after an LPS challenge. Klir et al. (1997), who observed numerical differences in serum cortisol due to a cold stress, also found numerical differences in TNF-α following the LPS challenge in the pigs housed in the cold vs. warm environment. The inability of Klir et al. (1997) to detect significant differences may have been due to the high amount of variation observed in the cold stress × LPS treatment group. Although in the current study, a time × environment × diet interaction was detected in serum TNF-α following the endotoxin challenge, the redundancy associated with the proinflammatory cytokines IL-6, IL-1β, and TNF-α would indicate our data are in general agreement with those of Carroll et al. (2001) and Klir et al. (1997).

Serum haptoglobin concentrations have been shown to be negatively correlated with growth in pigs (Eurell et al., 1992). In addition, Asai et al. (1999) reported an increase in serum haptoglobin in pigs infected with porcine reproductive and respiratory syndrome virus. In the study by Asai et al. (1999), serum TNF-α was not detected throughout the study, whereas increases in IL-6 were documented. Using a turpentine challenge model, Eckersall et al. (1994) reported peak concentrations of haptoglobin and CRP to occur 48 h following administration of the challenge. Both CRP and haptoglobin returned to baseline concentrations approximately 6 d later. Realizing APP synthesis and release are stimulated by the proinflammatory cytokines TNF-α, IL-6, and IL-1β (Baumann and Gauldie, 1994; Steel and Whitehead, 1994), an increase in serum concentrations of these proteins should not occur within the timeframe of our study. However, following the challenge with LPS, we observed a time × environment effect, where haptoglobin and CRP were elevated in the CE. Although these increases were not great, the elevated concentrations of the proinflammatory cytokines in the CE were likely the stimulus. The abrupt increase in CRP in the CE at 1 h post challenge warrants further investigation to determine if this is an artifact of the data or a true event. The biological significance of this elevation is unknown at this time.

The mRNA expression of IL-6 in the liver and spleen and the mRNA expression of IL-1β in the liver compare favorably with serum concentrations of these cytokines. As described above, serum concentrations of these cytokines were elevated in the CE, and the same is true for mRNA expression. We did not detect these differences in the thymus. The spleen is the major site of immune responses to blood-borne antigens, acting as a filter for the blood, while the thymus is the site of T
lymphocyte maturation (Abbas et al., 2000). In this study the LPS was administered i.v. and animals were sacrificed 4-h following the challenge. This sampling period would not allow for the events of adaptive immunity to be measured. Therefore, it is not surprising that no differences in mRNA expression of the proinflammatory cytokines were observed in the thymus.

A slight increase in LPSBP mRNA expression was also detected in the liver of pigs in the CE vs. pigs in the TN. Although LPSBP has been shown to enhance and neutralize the biological activities of LPS (reviewed by Fenton and Golenbock, 1998), the increases in liver mRNA expression observed in our study would correlate positively to the increased release of proinflammatory cytokines. Therefore, assuming the increased mRNA expression of LPSBP translated to increased LPSBP in the serum, our results would suggest LPSBP enhanced the immune response of pigs maintained in the CE.

As previously reported, feeding SDP to commercially housed weaned pigs increases feed intake and daily gain (Hansen et al., 1993; Kats et al., 1994); however, these effects are much less pronounced under experimental conditions (Coffey and Cromwell, 1995). The pigs used in our study were housed in environmental chambers that were cleaned and disinfected prior to arrival of the pigs. Considering this level of cleanliness, these pigs were likely not exposed to environmental immunogens. Indeed, SDP-fed pigs did not outperform the pigs fed diets without SDP. In fact, pigs fed SDP had slightly lower G/F than pigs that consumed NSDP. This effect was lost once the pigs were divided into their respective thermal environments on d 7.

Prior to endotoxin challenge, serum samples were collected from all pigs. Haptoglobin and CRP were analyzed in these samples to evaluate baseline immune stimulation in the pigs. Whereas no treatment differences were detected for haptoglobin, small decreases in CRP were associated with feeding SDP. It is possible that pigs fed SDP also had lower baseline IL-6 and IL-1β concentrations resulting in reduced levels of CRP, although no growth performance differences were found. Escobar et al. (2002) used a Mycoplasma hyopneumoniae model to induce immune activation in nursery pigs. Whereas increases in the inflammatory cytokines and the induction of disease were noted, there were no effects on growth performance or whole body composition.

Recently, our laboratory published research evaluating the use of SDP during a LPS challenge and evaluated the effects on the hypothalamic-pituitary-adrenal axis (HPA) and the immune system in weaned pigs (Carroll et al., 2002; Touchette et al., 2002). A biphasic increase in serum ACTH was detected in addition to a more linear increase in cortisol in the pigs fed SDP diets compared with those pigs not receiving SDP. Following the endotoxin challenge in the current study, we did not observe any differences in serum ACTH; however, the profiles of serum cortisol concentrations between the SDP and NSDP pigs were similar to those in the previous research. In addition, no differences were found in the expression of pituitary CRH-R and POMC mRNA or adrenal gland ACTH-R mRNA in our study. Daniel et al. (1999) has reported an association between serum ACTH concentration and adrenal gland ACTH receptor mRNA expression. This observation is further corroborated by the findings of Carroll et al. (2002), in which mRNA expression and serum concentrations of the hormones associated with stimulation of the HPA axis follow similar patterns. The increased serum cortisol concentrations in the SDP pigs following challenge were not associated with increased ACTH in our study. Carroll et al. (2002) speculated that the increased concentrations of serum cortisol might have been the result of direct adrenal stimulation by cytokines. Other researchers using a rat model have also demonstrated that cortisol can be released independent of CRH following LPS challenge (Suzuki et al., 1986; Elenkov et al., 1992). Our data would support these findings. Pigs fed diets containing SDP had elevated serum cortisol and IL-6 compared to the pigs fed diets without SDP. At 1 h post challenge, the dietary effects on serum cortisol concentrations were more evident, and simultaneously serum IL-6 began to increase.

Interestingly, tissue mRNA expression of TNF-α in the liver and thymus were increased in the pigs fed NSDP. Touchette et al. (2002) found an influence of SDP on liver and thymus expression of TNF-α mRNA in both saline-treated controls and LPS-challenged pigs. The higher level of TNF-α mRNA expression in the NSDP-fed pigs administered saline was suggested to be influenced by pathogen exposure of these pigs. In our study, the lower levels of TNF-α mRNA expression in the liver and thymus of SDP-fed pigs may be associated with feedback inhibition from the proinflammatory cytokines and cortisol.

These observations taken together with the results of Carroll et al. (2002) and Touchette et al. (2002) indicate that feeding SDP to pigs makes them more susceptible to an immunological challenge with LPS. This increased susceptibility may be related to immunological priming in pigs fed NSDP in combination with administration of the endotoxin intravenously circumventing mucosal immune functions. Feeding SDP is thought to have a protective effect in the pig, such that the immunoglobulins present in SDP are thought to bind to and prevent attachment of pathogens to the intestine, although other mechanisms may be involved (Nollet et al., 1999). Recent literature using E. coli challenge in cattle and pigs have demonstrated decreased mortality with the feeding of SDP (Quigley and Drew, 2000; Bosi et al., 2001). Others using an E. coli challenge reported that feeding SDP did not reduce piglet mortality or bacterial colonization in the intestine, although ADG, feed intake, and fecal scores were improved (Van Dijk et al., 2002).

Housing pigs in a cold environment may predispose the animal to bacterial infection based on cytokine and
APP production. Kliir et al. (1997) demonstrated that pigs reared in a cool environment were not able to mount a febrile response to endotoxin challenge. In addition, 24-h-old neonatal pigs entered a state of hypothermia when administered LPS (Carroll et al., 2001). Previous research has also shown that maintaining pigs in a cold environment inhibited pulmonary bacterial clearance, although this effect decreased as the age of the pig increased (Curtis et al., 1976). The cold stress treatment used in our study was much more effective at altering the HPA and immune response than the dietary treatments of SDP and NSDP. Furthermore, no consistent interactions of environment and dietary treatment were observed in our study, suggesting the effects were additive.

Implications

Rearing pigs in thermal-neutral environments not only improves growth performance, but it also affects the response to an immune system challenge. When housed under conditions of cold stress, feed intake increases and gain:feed ratio decreases, and results with a lipopolysaccharide challenge model suggest that the immune responsiveness of the animal might be affected as well. Pigs consuming spray-dried plasma and exposed to an acute endotoxin challenge responded with increasing serum concentrations of cortisol and cytokines. These effects may result in greater reductions in growth performance compared with pigs fed diets without spray-dried plasma. Research evaluating the physiological mechanisms of recovery following an immune challenge needs to be conducted on pigs fed diets with and without spray-dried plasma.

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