Using serology in combination with acute phase proteins and cortisol to determine stress and immune function of early-weaned pigs

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Summary:
Objectives: To evaluate serum concentrations of cortisol and acute phase proteins (APPs), specifically alpha 1-acid glycoprotein (AGP) and haptoglobin (HPT), in the same early-weaned pigs over time and to determine whether changes in these APPs or cortisol are associated with health status and performance in pigs.

Methods: Groups of pigs were evaluated in two management systems, wean-nursery-finish (WNF; 998 pigs) and wean-to-finish (WF; 999 pigs). Serum samples were obtained from the same 20 pigs in each group at weaning and at approximately 50, 85, 110, and 130 days of age. Porcine reproductive and respiratory syndrome virus (PRRSV) and Mycoplasma hyopneumoniae were endemic in this herd. Fisher's r to z analysis was used to determine relationships between serum APPs, serum cortisol, and serum titers for M hyopneumoniae and PRRSV.

Results: There were no production differences between WNF and WF groups. Serum AGP was negatively correlated with weight and PRRS S:P ratio in both groups, but there was no correlation between AGP and M hyopneumoniae titer. In WF pigs, HPT persisted increased, while a sharp increase occurred in WNF pigs on entry into the finisher. Serum HPT was highly correlated with M hyopneumoniae titer in WNF pigs but not WF pigs.

Implications: Under the conditions of this study, increased serum AGP was negatively correlated with body weight, suggesting that an activated cellular immune response is a detriment to growth rate. Further investigations are needed to determine whether these or other APPs are reliable predictors of disease status in swine.

Keywords: swine, nursery, wean-to-finish, acute phase proteins, cortisol

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a natural and an experimental challenge (intranasal, 5.4 \times 10^9 colony forming units) with *Actinobacillus pleuropneumoniae* supports the use of serum HPT as an indicator of subclinical illness and therefore health status and weight gains in pigs. Son et al.\(^\text{13}\) showed that the concentration of AGP in pigs during acute phase response to infection may be high enough to alter protein binding of some drugs, resulting in a decrease in the unbound fraction of drug, thereby decreasing its pharmacological effect. This may help to explain why, in some instances, little or no response to antibiotic therapy is observed. Obtaining additional information with regard to serum APP profiles in swine in a commercial setting will add to our knowledge base regarding the regulation of APPs and their potential use as indicators of an animal's health status. Therefore, the objectives of this study were to evaluate serum concentrations of AGP and HPT in the same pigs over time and to determine whether changes in these APPs were associated with changes in health status and performance.

### Materials and methods

#### Experimental design

Pigs weaned from one sow herd were divided into two groups evaluated in different management systems: a wean-nursery-finish (WNF) group and a wean-to-finish (WF) group.

Approximately 2000 barrows from a 2400-sow, farrow-to-wean breeding stock farm were weaned over a 4-week period beginning on April 1, 1999. Twice a week, 250 pigs were weaned at 11 to 14 days of age and assigned to study groups in accordance with the normal production flow of the system. Nine hundred and ninety eight pigs were weaned at 11 to 14 days of age (WF pigs at 112 days of age and in the WF pigs at 112 days of age) and at 34 ± 4, 44 ± 4, 59 ± 4, 81 ± 3, 103 ± 5, and 119 ± 5 days of age. Pigs in the WNF group were weighed at weaning (11 to 14 days of age) and at 35 ± 2, 46 ± 5, 50 ± 3, 74 ± 3, 94 ± 3, and 139 ± 3 days of age.

Average daily gain (ADG), average daily feed intake (ADFI), and feed efficiency (FE) were calculated for the 20 feeders (40 pigs) in each pen. The opportunity to weigh pigs by pen ended when the first pigs were individually weighed on an electronic digital scale (Tru-Test SR2000; Auckland, New Zealand). Weights were entered directly into a spreadsheet for analysis.

Pigs in the WF group were weighed at weaning (11 to 14 days of age) and at 34 ± 4, 44 ± 4, 59 ± 4, 81 ± 3, 103 ± 5, and 119 ± 5 days of age. Pigs in the WNF group were weighed at weaning (11 to 14 days of age) and at 35 ± 2, 46 ± 5, 50 ± 3, 74 ± 3, 94 ± 3, and 139 ± 3 days of age.

Average daily gain (ADG), average daily feed intake (ADFI), and feed efficiency (FE) were calculated for the 20 feeders (40 pens) in each barn. The opportunity to weigh pigs by pen ended when the first group of pigs reached a marketable weight of 118 kg and pen integrity was altered.

#### Feed

The WF and WNF pigs received the same rations throughout this study. A segregated early weaning diet and a transition diet were replaced by a succession of seven different rations.

All pigs were treated with neomycin (Neomix 325; Pharmacia and Upjohn Inc, Kalamazoo, Michigan) in the drinking water for 3 days at weaning. However, the WF pigs were treated with an additional dose of 5 mg per kg BW of neomycin (150 g per proportioner) when they were 33 days old because of an increasing incidence of *Escherichia coli* diarrhea.

Both groups received medicated feed containing tylosin (Tylan 40; Elanco Animal Health, Indianapolis, Indiana), 100 g per ton (110 mg per kg), for the 3 weeks after proliferative ileitis was diagnosed in the WF pigs at 112 days of age and in the WNF pigs at 126 days of age.

#### Diagnostic testing

Pathogen exposure at each site was monitored by obtaining repeated serum samples from 20 WNF pigs and 20 WF pigs to observe titer changes for *A. pleuropneumoniae* (types 1, 5, and 7), *Mycoplasma hyopneumoniae*, swine influenza virus (SIV), and porcine reproductive and respiratory syndrome virus (PRRSV), and to monitor changes in concentrations of serum HPT, AGP, and cortisol.

Serum samples were analyzed by Biovet Laboratories (St Anthony, Minnesota) using the Exposure Serum Antibody Profiles (ESAP; Biovet Laboratories, St Hyacinthe, Quebec) for all titer changes. The ESAP profile is usually reported as the percent of pigs seropositive for each of the pathogens. However, for the purpose of this study, the results of the serological tests for individual pigs were reported to facilitate statistical evaluation. The tests used in the ESAP were Tween 20 ELISA for SIV, with a sample:positive (S:P) ratio >0.099 considered positive; PRRSV ELISA, S:P ratio ≥0.4 considered positive; serum inhibition test for *M. hyopneumoniae*, inhibition >50% considered positive; and Tween 20 ELISA for *A. pleuropneumoniae* types 1, 5, and 7, S:P ratio >0.099 considered positive.

Assays for serum AGP and HPT (ELISA) were performed at the Veterinary Diagnostic Laboratory at the University of Illinois (Urbana, Illinois). These assays have been validated in swine (Jeff Sarno, Cardiotec Services, oral communication, 2001). Serum concentrations of cortisol were determined by the University of Missouri Animal Research Service (Columbia, Missouri) using a single commercially available kit (Diagnostic Products Corporation, Los Angeles, California). This technique has been validated in swine.\(^\text{15}\) The minimum detectable cortisol level was 2 ng per mL, with a within-assay coefficient of variation of 4.2%.

Tissue samples from pigs that died were submitted to the University of Missouri Veterinary Medical Diagnostic Laboratory (Columbia, Missouri) to determine the causes of death and identify pathogens.

#### Statistical analysis

All data were analyzed with the pig as the experimental unit utilizing Statview software.\(^\text{16}\) All between-group comparisons between WNF and WF groups at specific

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time points were made using an unpaired \( t \) test. All within-group comparisons between two data collection points were made using a paired \( t \) test. Correlation coefficients (r) are reported on the overall means for each variable for the WF and WNF groups using Fisher's r to z analysis to describe linear relationships between two variables within the same group (two sets of observations from the same pig).

Variables used for correlation comparisons were body weight, \( M \) hyopneumoniae \( \text{titers} \), PRRS ELISA S:P ratios, and serum concentrations of cortisol, AGP, and HPT. Correlation comparisons were made between the parameters of interest, and the positive or negative change in the linear relationship of the parameters compared over the same time period is reported. In all comparisons, \( P \) values < .05 were considered significant.

Results

Health

Over the course of the study, 72 pigs died (7.2%) in the WF group and 44 (4.6%) in the WNF group. Ileitis was the greatest single cause of death in these pigs (4.8% of WF pigs, 2.7% of WNF pigs). Proliferative ileitis was first diagnosed in the WF pigs at 112 days of age and in the WNF pigs at 126 days of age, and \textit{Lawsonia intracellularis} was identified in intestinal mucosa and intestinal content samples collected from several pigs at necropsy over the course of the study period. Diarrhea caused by \textit{E coli} was first diagnosed at necropsy in the WNF pigs at 17 days of age, and in the WF pigs at 33 days of age. \textit{Escherichia coli} was isolated from intestinal content samples collected from several pigs at necropsy over the course of the study period.

Serological data

Due to technician error, serum collected from the pigs in both groups at 130 days of age was not tested for antibodies against PRRSV, \( M \) hyopneumoniae, SIV, or \textit{A pleuropneumoniae}.

PRRS ELISA S:P ratios. Mean PRRS ELISA S:P ratios and changes in each group during the study are illustrated in Figure 1. Between 12 and 85 days of age, the mean S:P ratios decreased (\( P < .001 \)) in the WNF group but did not change significantly in the WF group. Between 85 and 110 days of age, the mean S:P ratios decreased (\( P = .04 \)) in the WF group but increased (\( P = .01 \)) in the WNF group. Between 110 and 130 days of age, the mean S:P ratios decreased (\( P = .01 \)) in the WNF group but increased (\( P = .06 \)) in the WF group.

Figure 1: Results of assays for acute phase serum proteins\(^1\) and serum cortisol and serological tests\(^2\) in pigs weaned at 11 to 14 days and managed either in a wean-to-finish facility (WF; 999 pigs) or in a nursery for 5 or 7 weeks and then a grow-finish facility (WNF; 998 pigs). Serum samples were collected from the same 20 pigs in each group at each of the five data points.

\(^1\) Alpha 1-acid glycoprotein (AGP) and haptoglobin (HPT)

\(^2\) Serum inhibition test for \textit{Mycoplasma hyopneumoniae} and enzyme-linked immunosorbent assay for porcine reproductive and respiratory syndrome virus

\[\text{AGP} (\mu g/mL)\]

\[\text{HPT} (mg/dL)\]

\[\text{Cortisol} (\mu g/dL)\]

\[\text{PRRS ELISA S:P ratio}\]

\[\text{\textit{M} hyopneumoniae MI} (\%)\]
increased ($P < .02$) in both the WNF and WF groups, and mean S:P ratio at 110 days of age was approximately 3.3-fold greater ($P < .001$) in the WF group than in the WNF group.

**M. hyopneumoniae titers.** Mean minimum inhibition (MI) titers for *M. hyopneumoniae* and changes in titers for each group during the study are illustrated in Figure 1. Between 12 and 50 days of age, mean MI titer decreased ($P < .01$) in the WNF group, and increased ($P < .001$) in the WF group. Between 50 and 85 days of age, mean MI titer increased ($P < .001$) in the WNF group and decreased in the WF group ($P < .01$). Between 85 and 110 days of age, mean MI titer decreased by a factor of 1.6 in the WNF group ($P < .001$), while increasing by a factor of 8.5 in the WNF group ($P < .001$). As a result, the mean MI titer was approximately 5.6-fold greater ($P < .001$) in the WF group than in the WNF group at 110 days of age. The maximum number of *M. hyopneumoniae*-positive pigs was observed at 85 days of age for the WNF group and at 110 days of age for the WF group.

**SIV titers.** The percent of pigs seropositive for SIV at 110 days of age was higher ($P < .01$) in the WNF group, and remained relatively constant ($P = .61$) in the WF group. Maximum serum concentration of HPT was observed at 85 days of age for the WNF and at 130 days of age for the WF group.

**Serum cortisol.** Mean serum cortisol for the two groups, and changes in concentration during the study, are shown in Figure 1. Between 12 and 50 days of age, serum cortisol decreased ($P < .001$) in the WNF group and increased ($P < .001$) in the WF group. Between 50 and 85 days of age, serum cortisol increased ($P < .001$) in the WNF group but did not change in the WF group ($P = .05$). Serum cortisol did not change in the WNF group between 85 and 130 days of age, but tended to decline ($P = .06$) in the WF group between 85 and 110 days of age. Maximum serum concentration of cortisol was observed at 85 days of age for the WF group and at 110 days of age for the WNF group.

**Correlations.** Correlations of overall means for the five data points for the serum cortisol and APPs and body weight means, and for the four data points for the serological assays, are shown in Table 1.

**Discussion.** In a previously reported companion study, there were no differences detected in the ADG, ADFI, or FE between the WNF and WF groups. Although APPs as indicators of immune activation have proven to be challenging due to the variability of the results. Serum AGP in both the WNF and the WF groups tended to peak when the pigs were 7 weeks of age, then returned to previously reported values for young adult swine. An increase in serum AGP concentrations may be considered an indicator of intracellular communication, suggesting an increase in the cellular immune response. In both the WNF and WF groups, serum AGP concentration and PRRS ELISA S:P ratio were negatively correlated, suggesting that when the cellular immune response is active, there may be suppression or limitation of the humoral immune response associated with circulating PRRS virus. A negative correlation between AGP and weight, as observed in this study, suggests that an active cellular immune response adversely affects nutrient accretion and growth rate. Itoh et al noted that in pigs chronically exposed to a pathogen, both disease and stressors such as weaning may contribute to increases in AGP.

The gradual increases in serum HPT concentrations in the WF group suggest that these pigs were being chronically stressed throughout the testing period. In contrast, a dramatic increase in HPT was observed in the WNF group in the sample collected when they were 85 days of age. This sudden increase in HPT may reflect the stress associated with movement of the pigs from the nursery to the grow-to-finish facility, where they were exposed to chilling (room temperature 24°C initially) and a large pig space for their size, and the additional stress of finding feed and water in the new environment. In a study by Francisco et al., ADG was better in pigs with limited pen space, higher pig density, and warmer room temperature compared to pigs housed with a greater amount of pen space, lower pig density, and a cooler room temperature. In the present study, differ-
Serum HPT concentrations increased in both groups during the study, and were highly correlated with *M. hyopneumoniae* titers in the WNF group. Hall et al. reported a rapid response of HPT after either a natural or an experimental challenge with *A. pleuropneumoniae*, and concluded that serum HPT is an appropriate indicator of subclinical illness and health status in pigs.

Serum concentrations of AGP and HPT did not correlate to any change in serum cortisol concentrations, although there was a trend for serum concentrations of HPT to increase at the same time as cortisol concentrations in both the WNF and WF groups. Although serum concentrations of HPT for the WF and WNF pigs were notably different at weaning, cortisol levels were not. However, in the WNF pigs, the sharp increase in HPT between 50 and 85 days of age, when these pigs were adjusting to being moved from the nursery to the finisher, was associated with a similar increase in mean cortisol concentration. In the WF group, serum cortisol had increased between 12 and 50 days of age, before *M. hyopneumoniae* titers and PRRS S:P ratios increased in these pigs, and while serum cortisol levels were decreasing in the WNF pigs. The early increase in serum cortisol in the WF pigs might be attributed to stress. At this time, the WF pigs had been weaned and placed in a new wean-to-finish building, where they were allowed 0.70 m² per pig with black mats and heat lamps providing zone heating. In this type of building, there is a possibility for chilling of the newly weaned pigs. Cold stress may have been responsible for at least part of the observed increase in serum cortisol concentrations during this period. We speculate that the conventional nursery room, warmed to 29°C, minimized chilling in the WNF pigs. This implies that the environment in the conventional nursery was more suited to the age and size of the early-weaned pig than the WF facility in this study.

**Implications**
- Serum AGP concentrations of pigs in two management systems were negatively correlated with overall body weights, suggesting that an activated cellular immune response is a detriment to growth.
- Serum HPT may be of value as an indicator of stress in swine herds.
- A combination of serum HPT and serum cortisol concentrations may be a more reliable indicator of disease status or stress in pigs than either parameter alone.
- Further investigations are needed to determine whether serum cortisol, APPs, or a combination of both are reliable predictors of disease status in swine herds.

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**Table 1**: Correlations among body weight, concentrations of two acute phase serum proteins (AGP and HPT) and serum cortisol, and serological titers against porcine reproductive and respiratory syndrome (PRRS) virus and *Mycoplasma hyopneumoniae* (MH), in two groups of pigs.

<table>
<thead>
<tr>
<th>Parameters compared</th>
<th>WNF pigs</th>
<th>WF pigs</th>
</tr>
</thead>
<tbody>
<tr>
<td>Weight (kg), AGP (µg/mL)</td>
<td>Correlation -0.540, P value &lt;.0001</td>
<td>Correlation -0.475, P value .0007</td>
</tr>
<tr>
<td>Weight (kg), HPT (mg/dL)</td>
<td>0.704, &lt;.0001</td>
<td>0.279, .0603</td>
</tr>
<tr>
<td>Weight (kg), PRRS ELISA S:P ratio</td>
<td>0.661, &lt;.0001</td>
<td>0.807, &lt;.0001</td>
</tr>
<tr>
<td>Weight (kg), MH titer</td>
<td>0.780, &lt;.0001</td>
<td>0.846, &lt;.0001</td>
</tr>
<tr>
<td>Weight (kg), cortisol (µg/dL)</td>
<td>-0.015, 0.9098</td>
<td>0.087, .5656</td>
</tr>
<tr>
<td>AGP (µg/mL), HPT (mg/dL)</td>
<td>-0.343, 0.0868</td>
<td>-0.223, .1377</td>
</tr>
<tr>
<td>AGP (µg/mL), PRRS ELISA S:P ratio</td>
<td>-0.514, &lt;.0001</td>
<td>-0.545, &lt;.0001</td>
</tr>
<tr>
<td>AGP (µg/mL), MH titer</td>
<td>-0.361, 0.0545</td>
<td>-0.322, .0285</td>
</tr>
<tr>
<td>AGP (µg/mL), cortisol</td>
<td>-0.268, 0.0432</td>
<td>0.104, .4944</td>
</tr>
<tr>
<td>HPT (mg/dL), PRRS ELISA S:P ratio</td>
<td>0.241, 0.0710</td>
<td>0.098, 0.5185</td>
</tr>
<tr>
<td>HPT (mg/dL), MH titer</td>
<td>0.806, &lt;.0001</td>
<td>0.042, .7832</td>
</tr>
<tr>
<td>HPT (mg/dL), cortisol</td>
<td>0.117, 0.3872</td>
<td>0.321, .0294</td>
</tr>
<tr>
<td>PRRS ELISA S:P ratio, MH titer</td>
<td>0.364, 0.0515</td>
<td>0.852, &lt;.0001</td>
</tr>
<tr>
<td>Cortisol (µg/dL), PRRS ELISA S:P ratio</td>
<td>-0.047, 0.7309</td>
<td>-0.144, .3413</td>
</tr>
<tr>
<td>Cortisol (µg/dL), MH titer</td>
<td>0.055, 0.6874</td>
<td>0.112, .4623</td>
</tr>
</tbody>
</table>

1 Correlations are reported on the overall means for five data points (weight, acute phase proteins and serum cortisol at 12, 50, 85, 110 and 130 days of age) or four data points (serological assays at 12, 50, 85, and 110 days of age) using Fisher’s r to z analysis. P values <.05 were considered significant.

2 Alpha 1-acid glycoprotein

3 Haptoglobin

4 Serum profiles were performed using the MH serum inhibition test (>50% inhibition considered positive) and PRRS ELISA (sample:positive (S:P) ratio ≥0.4 considered positive).

5 Pigs were weaned from sows in the same production unit and either placed in a wean-to-finish building (WF) or placed in a nursery and moved into a grow-finish facility 5 or 7 weeks later (WNF). Of the approximately 1000 pigs in each group, 20 were randomly selected and were tested for each parameter at each data point.
References – refereed

References – non refereed