ABSTRACT: Ninety-six pigs from a herd naïve for porcine reproductive and respiratory syndrome (PRRS) virus were weaned (10 ± 3 d of age), penned individually in isolation rooms, and, at 29 ± 4 d of age, oronasally inoculated with a 2-mL dose of 10^{4.3} JA142 PRRS virus/mL. Body weight; feed intake; and serum concentrations of PRRS virus, interferon, and α₁-acylglycoprotein were determined for each pig every 4 d on d –8 to 24 postinoculation to quantify the effect of PRRS exposure on the immune response and growth of pigs. Another objective was to determine whether a quantitative relationship between a measure of systemic (serum) virus concentration and pig growth exists. Serum PRRS virus and interferon peaked at 10^5 virus/mL and 69% protection, respectively, at 4 d postinoculation and then declined steadily. Serum α₁-acylglycoprotein concentration peaked at 12 d postinoculation. Pig weight gains and feed intake were reduced sharply in the initial 8 d postinoculation and to a lesser degree for 24 d postinoculation. The serum concentration of virus and to a lesser degree serum concentrations of interferon and α₁-acylglycoprotein were quantitatively related to body weight gain and feed intake. The magnitude of the relationship was dependent on the stage of recovery from PRRS infection. Specifically, each log increase in serum virus concentration was associated with a reduction of 4-d pig gain and feed intake of .047 kg and .189 kg, respectively, in 5.5-kg pigs 4 d postinoculation and .085 kg and .036 kg, respectively, in 12.5-kg pigs at 20 d postinoculation. Based on these data, factors that minimize the systemic presence of a virus in pigs result in improvements in pig growth that are quantitatively related to the degree of systemic virus elimination or minimization.

Key Words: Growth Rate, Pathogen Elimination, Swine Diseases


Introduction

Following a pathogen exposure, the immune system of pigs becomes activated. One of the initial immune responses that occurs is the release of proinflammatory cytokines. These cytokines stimulate the proliferation of immune cells and the production of antibodies to aid the containment and elimination of the pathogen (Kuby, 1997). Chronic, subclinical pathogen exposure results in the reduction of body growth, particularly muscle growth, voluntary feed intake, and efficiency of feed utilization (Williams et al., 1997). However, the quantitative relationship of systemic viral concentration following an acute viral exposure and pig growth and immune response has not been defined.

Porcine reproductive and respiratory syndrome (PRRS) virus is a widespread viral disease in the pork industry. The PRRS virus has been shown to infect porcine macrophages, which become the host cells for the virus while it replicates. The virus eventually lyzes the cell and is released systemically into the body. In young pigs, symptoms of an infection include fever, anorexia, vomiting, and coughing (Benfield et al., 1992). Therefore, the objective of this study was to determine whether the systemic concentration of the virus present in the pig is related to growth and feed intake of pigs.

Materials and Methods

Animals

Ninety-six pigs from a high-lean strain were obtained from a herd naïve (noninfected, nonvaccinated) for
PRRS. The pigs were weaned at 10 ± 3 d of age, penned individually in 61- × 122-cm slatted-floor pens. Pigs were reared in the disease isolation rooms at the National Animal Disease Center, Ames, IA, to minimize exposure to other antigens. For each of the first 3 d after weaning, each pig was administered intramuscularly with 4.4 mg of Naxcel (Pharmacia and Upjohn Animal Health, Kalamazoo, MI) per kilogram BW/d. Subsequently, no therapeutic or subtherapeutic treatments were administered. A thermal climate of 29° to 24°C was maintained. Pigs were fed one common diet that provided nutrient concentrations that met or exceeded the estimated nutrient requirements of high-lean pigs (NRC, 1998). Pigs were allowed to consume feed and water ad libitum.

Blood from each pig was collected at weaning and immediately before PRRS inoculation to verify that the pigs did not have passive or active serological antibody titers for the PRRS virus nor have the PRRS virus present in serum. Eighteen days following weaning, titers for the PRRS virus nor have the PRRS virus per pigs did not have passive or active serological antibody immediately before PRRS inoculation to verify that the

Blood from each pig was collected via the jugular vein every 4 d from d 0 (preinoculation) to 24 postinoculation to determine serum concentrations of PRRS virus, interferon (IFN), and α1-acylglycoprotein (AGP). Feed intake, feed wastage, and BW also were measured (weighed to nearest gram) every 4 d from d -8 to 24 postinoculation to determine net feed intakes and BW gains for each 4-d period.

In 16 pigs, randomly selected prior to inoculation, rectal body temperatures (to the nearest .1°C) were determined daily from d 0, before inoculation, to d 24 after inoculation. In 24 pigs, randomly selected at weaning, the presence of serological titers for five prevalent pathogens were determined at weaning as outlined by Williams et al. (1997), immediately before PRRS inoculation and at the removal from test (15 kg BW). The five pathogens evaluated were PRRS virus, transmissible gastroenteritis, swine influenza virus (H1N1), Mycoplasma hyopneumoniae, and Actinobacillus pleuropneumoniae. These data were used to validate that the pigs had not been exposed to other prevalent pathogens. The animal care procedures employed were approved by the Committee on Animal Care at the National Animal Disease Center, Ames, IA.

**PRRS Virus**

The serum PRRS virus concentration was determined through the use of a cytopathologic effect assay courtesy of William Mengeling, USDA/ARS/NADC. The assay consisted of growing MARC (Meat Animal Research Center, Clay Center, NE) 145 cells in culture medium (Eagle’s Minimum Essential Medium [MEM; JRH Biosciences, Lenexa, KS] supplemented with fetal bovine serum [FBS, Summit Laboratory, Ft. Collins, CO]) for 7 d, and then removing the medium and rinsing the flask with 10 mL of trypsin. A second 10 mL of trypsin was then added to the confluent cell monolayer and incubated at 37°C on a rocker until the cells were digested off the flask (5 min). The cell-trypsin solution was then pipetted into a centrifuge tube and spun at 1,000 × g for 5 min; the supernate was poured off of the pellet. The cells were resuspended in 5 mL of growth medium (MEM supplemented with 10% FBS and 50 mg/L of gentamycin) per flask used. The cell suspension was diluted into 150 mL of medium per flask. A 150-μL sample of each cell suspension was placed in each well of a 96-well plate. Cells were then allowed 3 d for multiplication in a 37°C incubator. Eight 1:10 serial dilutions of each serum sample were made. Fifty microliters of each serial dilution was then added to the plates. Each dilution for each sample was replicated 11 times. Each suspension was then incubated for 5 d to allow for viral replication.

The wells were evaluated based on the cytopathologic effect of the virus. Each dilution sample was evaluated for a cytopathologic effect defined as a clearing of the monolayer with associated morphological changes in the cells. A virus concentration was equated according to Reed and Muench (1938). The dilutions were replicated 11 times to increase the accuracy of the equation used. Serum taken from the pigs preceding inoculation was used as the negative control.

**Interferon**

Serum IFN concentrations were assayed using a modified bioassay (Rubinstein et al., 1981). Briefly, 50 μL of Madin Darby bovine kidney cells per well (American Type Culture Collection #CCL22, Manassas, VA; 1 × 10^6 cells/mL) were incubated in a 96-well plate for 24 h at 37°C in a 5% CO2 incubator. Four serial dilutions (1:8, 1:16, 1:32, and 1:64) of serum were prepared in triplicate with MEM supplemented with 10% FBS in a 96-well plate. Diluted sample (100 μL/well) and 50 μL of properly diluted vesicular stomatitis virus (Indiana strain, American Type Culture Collection) was added to each well. Plates were incubated another 24 h before addition of 100 μL of 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide (1 mg/mL of phosphate buffer saline [PBS]) per well. Plates were then incubated for 2 h, centrifuged, emptied by inversion, and washed two times with PBS before the addition of 150 μL of isopropanol to solubilize formazan crystals. The amount of formazan produced by the bovine kidney cells was directly proportional to viable cell number. Because IFN inhibits vesicular stomatitis virus replication, absorbance at 570 nm due to formazan production was used to measure the relative amount of IFN in each serum sample. Concentrations of IFN are reported as a percentage of cell protection. In this experiment, the 1:32 dilution was used so that the highest protection level was less than 100%. The intra- and interassay
Figure 1. Mean serum concentrations of porcine reproductive and respiratory syndrome (PRRS) virus (10^6/mL) from d 0 to 24 postinoculation in pigs (n = 96) infected with PRRS virus.

variations were 5.9 and 12.6, respectively. The percentage of hIFN recovery in spiked serum samples was 91.3. Human IFN was used as the standard in this assay. The hIFN was received from the National Institutes of Health (catalog number Ga23-902-530) at a concentration of 5 × 10^3 IU/mL of medium. Human IFN has been shown to have biological activity similar to porcine IFN (Cerretti et al., 1986; Townsend et al., 1988), and to serve as an acceptable standard for porcine IFN.

α1-Acylglycoprotein

Serum AGP was analyzed by using radial immunodiffusion kits (Cardiotech Services Inc., Louisville, KY). The intra- and interassay variation was 4%, and the sensitivity range was 50 to 1,500 μg/mL.

Serological Antibodies to Common Swine Pathogens

The presence of serological titers was determined as follows: PRRS virus by ELISA (IDEXX Laboratories, Inc., Westbrook, ME); transmissible gastroenteritis via a serum neutralization (Foley and Patterson, 1998); swine influenza H1N1 via a hemagglutination assay (Dunne et al., 1967; Conrath, 1972; Mengeling, 1972); Mycoplasma hyopneumoniae via an ELISA (Bereiter et al., 1990); and Actinobacillus pleuropneumoniae via a complement fixation assay (Nicolet et al., 1971; Slavic and Switzer, 1972; Diagnostic Bacteriology Laboratory, National Veterinary Services Laboratories, 1986). The assays were conducted by the Iowa State University Veterinary Diagnostic Laboratory, Ames. These samples were taken to verify that the animals did not have passive or active titers for PRRS virus and to establish if the pigs had been exposed to other prevalent pig pathogens.

Data Analysis

The relationship of serum virus concentrations, pig performance traits, and immune response was analyzed by multiple regression techniques using the backward-stepwise regression model procedures of SAS (1996). Variables that were not significant (P > .10) were deleted from the regression model.

The independent variables included in the analysis were the linear and quadratic effects of serum concentrations of virus, IFN, AGP, and BW for d −4 to 24. The dependent variables were pig BW gain and feed intake for each 4-d period immediately before the measurement of the independent variable from −4 to 24 d. Pig BW; feed intake; serum virus concentration of PRRS, IFN, AGP; and rectal body temperature were reported as least squares means.

Results and Discussion

Determination of PRRS Virus Exposure

Based on serum virus analysis, all animals prior to inoculation were naive to PRRS. Postinoculation, all

Figure 2. Mean percentage of pigs (n = 96) possessing serum porcine reproductive and respiratory syndrome (PRRS) virus from d 0 to 24 postinoculation infected with PRRS virus.

Figure 3. Mean daily rectal temperature (°C) from d 0 to 24 postinoculation of pigs (n = 16) infected with porcine reproductive and respiratory syndrome virus.
animals possessed systemic (serum) PRRS virus. All pigs were free of passively or actively acquired titers for PRRS at weaning and before inoculation, but all pigs exhibited serum antibodies for PRRS at 24 d after inoculation.

Serological Antibody Titers for Other Pathogens

Pigs were free of passively or actively acquired antibody titers for *M. hyopneumoniae* at weaning, preinoculation, and at d 24 postinoculation. Sixty percent of the pigs possessed passively acquired titers for transmissible gastroenteritis and swine influenza, and 25% had passively acquired titers for *A. pleuropneumoniae* at weaning. The percentage of pigs exhibiting passively acquired titers had decreased to 50% for gastroenteritis and influenza and 25% for *A. pleuropneumoniae* by inoculation; and to 8% for gastroenteritis and influenza and 3% for *A. pleuropneumoniae* by termination of the study. Prior to infection, two pigs were killed due to food refusal. Postinoculation, two pigs died from a twisted gut, and one pig was euthanatized due to a severe joint infection.

Serum Virus Concentration and Immune Responses to PRRS Inoculation

Mean serum virus concentration increased sharply to $10^7$ viruses/mL at 4 d postinoculation, and then declined to a mean concentration of $10^{3.7}$ within 24 d (Figure 1). The percentage of pigs that possessed serum virus declined from 100% at d 4 and 8 to 50% at d 24 (Figure 2). Rectal body temperatures also increased sharply to 103.9°C at d 3 and then declined (Figure 3).

Mean serum concentration of interferon responded in a pattern similar to that of PRRS virus; however, the AGP response was delayed by 8 to 12 d and then declined with time (Figures 4 and 5). In uninfected animals, serum AGP concentrations in pigs declines
Table 1. Quantitative relationship of serum porcine reproductive and respiratory syndrome (PRRS) virus concentration and immune parameters on individual 4-d pig gain and feed intake in 96 pigs from d −4 to 24 postinoculation. Days 0 to 4 pig gain and feed intake values were regressed to d −4 virus concentration, interferon, and α1-acylglycoprotein

<table>
<thead>
<tr>
<th>Factor</th>
<th>Gain, kg/4 d&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Feed intake, kg/4 d&lt;sup&gt;a&lt;/sup&gt;</th>
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<td></td>
<td>b</td>
<td>SE</td>
</tr>
<tr>
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<td>BW, kg</td>
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<td>Virus, 10&lt;sup&gt;9&lt;/sup&gt;/mL</td>
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</tr>
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</tr>
<tr>
<td>Interferon (IFN), %</td>
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</tr>
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<td>IFN × IFN</td>
<td>−.000066</td>
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<tr>
<td>α&lt;sub&gt;1&lt;/sub&gt;-acylglycoprotein (AGP), μg/mL</td>
<td>.000002</td>
<td>.001</td>
</tr>
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</table>

<sup>a</sup>R² for pig gain = .71; R² for feed intake = .82; b = coefficient for each factor.

steadily as the animals mature (Williams et al., 1997). However, infection will cause the release of the proinflammatory cytokines, which will cause a rise in AGP levels 8 to 12 d after infection. The increase in serum IFN concentration suggests that T cytotoxic and natural killer cells were actively releasing this cytokine to aid in stimulating macrophage activity and that the infection was significant enough to stimulate an immune response. The relationship of PRRS virus and AGP demonstrated the time necessary for an acute phase protein response to occur following a PRRS exposure. The activation of macrophages and B cells would cause increases in IL-1 and IL-6 release. These two cytokines are responsible for the rise in serum AGP levels during infection (Kuby, 1997).

Growth and Feed Intake Response to PRRS Inoculation

Gain and feed intake were depressed sharply for 8 d postinoculation and to a lesser degree for 24 d (Figures 6 and 7). The average 4-d pig gain decreased from .956 kg during the 4-d period preceding inoculation to .464 kg for d 0 to 4. Similarly, 4-d feed intake decreased from 1.00 kg during the 4-d period preceding inoculation and to .776 kg for d 4 to 8.

Quantitative Relationship of Serum Virus Concentration and Growth of Pigs

A multiple regression analysis was used to determine the relationship of serum concentrations of virus, AGP, IFN, and BW on the associated 4-d pig gains, feed intakes, and gain:feed ratios (Table 1). Data were analyzed using a backward-stepwise regression procedure, which eliminated all effects that had a probability greater than .10. The components that accounted for a significant proportion of the variation in pig gain and feed intake are outlined in Table 1. The regression equation accounted for 71% and 82% of the variation in pig gain and feed intake, respectively (Table 1).

The serum virus concentration was negatively related to pig growth and feed intake. The magnitude of the relationship was dependent on stage of virus infection. As serum virus concentration increased, pig gain and feed intake were reduced, with the greatest reduction occurring at heavier pig weights when systemic virus concentration was lower. Specifically, each additional log increase in serum PRRS virus concentration was associated with a mean reduction of .047 kg in 4-d pig gain and .189 kg in 4-d pig feed intake in 5.5-kg pigs at d 4 postinoculation. However, each log increase in serum PRRS virus was associated with a reduction of .085 kg and .036 kg in 4-d pig gain and feed intake, respectively, in 12.5-kg pigs at d 20. The specific magnitude of reduction in gain also was dependent on serum concentrations of IFN and AGP because both measures would increase during various periods and degrees of viremia. For instance, an increase in serum IFN concentrations from 60 to 70% protection was associated with a reduction of .046 kg in 4-d pig gain and increase in serum AGP from 440 to 460 μg/mL in a virally challenged pig was associated with a decrease in 4-d pig gain of .005 kg (Table 1). We conclude that the magnitude of biological responses that occurred in pigs infected with PRRS was directly related to the animal’s serum virus concentration.

Implications

The systemic concentration of a virus seems to be quantitatively related to the growth of young naive pigs. Thus, factors such as immune modulators that minimize serum virus concentrations in the body should result in improved growth of animals.

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