

The effect of porcine reproductive and respiratory syndrome virus and porcine epidemic diarrhea virus challenge on growing pigs I: Growth performance and digestibility¹

W. P. Schweer,* K Schwartz,† E. R. Burrough,† K. J. Yoon,† J. C. Sparks,‡ and N. K. Gabler*²

*Department of Animal Science, Iowa State University, Ames 50011; †Department of Veterinary Diagnostic and Production Animal Medicine, Iowa State University, Ames 50011; and ‡Huvepharma, Peachtree City, GA 30269

ABSTRACT: Porcine reproductive and respiratory syndrome (PRRS) and porcine epidemic diarrhea (PED) are two diseases costly to the U.S. swine industry. The objective of this study was to determine the impact of PRRS virus and PED virus, alone or in combination, on growth performance, feed efficiency, and digestibility in grower pigs. Forty-two gilts (16 ± 0.98 kg BW) naïve for PRRS and PED were selected and allocated to 1 of 4 treatments. Treatments included 1) a control, 2) PRRS virus infected, 3) PED virus infected, and 4) PRRS+PED coinfection (PRP). Pigs in treatments 2 and 4 were inoculated with a live field strain of PRRS virus via intramuscular and intranasal routes at 0 d after inoculation (dpi). Treatments 3 and 4 were orally inoculated with a cloned PED virus at 15 dpi. Infection with PRRS virus was confirmed by quantitative PCR and seroconversion. Infection with PED virus was confirmed with PCR. Control pigs remained PRRS and PED virus negative throughout the study. All pigs were offered, ad libitum, a standard diet with free access to water. During the test period, PRRS reduced ADG and ADFI by 30 and 26%, respectively ($P < 0.05$), compared with control pigs, whereas PRP decreased ADG, ADFI,

and G:F by 45, 30, and 23%, respectively ($P < 0.05$). Additional reductions in ADG and G:F were detected in PRP pigs compared with singular PED or PRRS treatments (33 and 16%, respectively). The impact of PED, alone or in combination, on performance (15–21 dpi) reduced ADG (0.66 vs. 0.35 vs. 0.20 kg/d; $P < 0.01$), ADFI (1.22 vs. 0.88 vs. 0.67 kg/d; $P = 0.003$), and G:F (0.54 vs. 0.39 vs. 0.31; $P = 0.001$) compared with control pigs. Compared with control pigs, PRRS infection did not reduce apparent total tract digestibility (ATTD) of nutrients and energy. However, PED infection, alone or in combination, decreased ATTD of DM and energy by 8 and 12%, respectively ($P < 0.05$). Compared with control pigs, PRP reduced N and OM ATTD by 13 and 3%, respectively ($P < 0.05$). No significant differences in apparent ileal digestibility (AID) were detected between virus challenges. However, Lys AID tended to be reduced in both PED treatments compared with the control (10 and 12%; $P = 0.095$). Altogether, PRRS reduced growth but did not alter digestibility. Pigs challenged with PED and, to a greater extent, the coinfection of PED and PRRS viruses had reduced ADG, ADFI, G:F, and ATTD of nutrients and energy.

Key words: digestibility, growth, pig, porcine epidemic diarrhea virus, porcine reproductive and respiratory syndrome virus

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INTRODUCTION

Porcine reproductive and respiratory syndrome (PRRS) and porcine epidemic diarrhea (PED) are arguably two of the most costly diseases in the U.S. swine industry today. Per year, PRRS alone has been estimated to cost the U.S. swine producers an estimated US\$664 million (Holtkamp et al., 2013), whereas PED has been attributed to the loss of over 8 million piglets since being discovered in the United

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²Corresponding author: ngabler@iastate.edu

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States (Meyer, 2014). In young pigs, PRRS has been shown to reduce growth performance and feed efficiency (Greiner et al., 2000; Escobar et al., 2004) and negatively affect intestinal morphology. These pigs also have an increased likelihood of secondary viral and bacterial infections due to the ability of PRRS to suppress the immune response (Van Reeth et al., 1996; Nakamine et al., 1998). Unlike PRRS virus, the PED virus localizes to the gastrointestinal tract of pigs and induces watery diarrhea along with severe villous atrophy, leading to malabsorption (Madson et al., 2014).

Health challenged pigs often exhibit reduced appetite and feed intake and have altered nutrient utilization in a tissue-specific manner (Johnson, 2002). Therefore, due to the significant prevalence of PRRS-positive pigs in the Midwest region of the United States, our objectives were 1) to develop a challenge model to study the effects of PED virus alone and coinfecting with PRRS virus on pig performance and feed efficiency and 2) to characterize the impact PED and PRRS virus challenges have on nutrient and energy digestibility.

MATERIALS AND METHODS

Animals and Experimental Design

All animal work was approved by the Iowa State University Institutional Animal Care and Use Committee (number 1-14-7710-S) and adhered to the ethical and humane use of animals for research. All animal work was conducted at the Livestock Infectious Disease Isolation Facility at the Iowa State University College of Veterinary Medicine (Ames, IA).

Forty-two Choice Genetics maternal line gilts (16 ± 0.98 kg BW), naïve for PRRS and PED, were selected and randomly assigned to 1 of 4 treatments for a 21-d growth study. Pigs were penned in pairs with each treatment in separate rooms to avoid viral cross-contamination. Treatments included 1) high health control gilts (**CON**; $n = 3$ pens), 2) PRRS virus inoculated gilts (PRRS; $n = 6$ pens), 3) PED-inoculated gilts (PED; $n = 6$ pens), and 4) gilts inoculated with PRRS and then PED (PRRS+PED coinfection [**PRP**]; $n = 6$ pens). After a 4-d acclimation period, treatments were administered as follows on d 0 after inoculation (**dpi**): pigs in treatment 2 and 4 were inoculated with a live field strain of PRRS virus (ORF5 RFLP 1-18-4 Wild-type), in which 500 genomic units were intramuscularly inoculated and 500 genomic units were nasally inoculated. Treatments 1 and 3 received a saline solution sham inoculation at dpi 0. On dpi 14, pigs in treatments 3 and 4 were intragastrically inoculated with 10^3 plaque-forming units of a plaque-cloned PED virus isolate (USA/Iowa/18984/2013; Iowa State University, Ames, IA) representing 6 cell culture

passages as previously described (Hoang et al., 2013; Madson et al., 2014), whereas treatments 1 and 2 received a saline sham inoculation.

Throughout the study, all pigs were fed a corn–soybean meal diet containing the digestibility marker titanium dioxide and formulated to meet or exceed NRC (2012) requirements for AA, minerals, and vitamins (Table 1). Pigs had ad libitum access to feed and water at all times. Individual BW was collected at 0, 7, 14, and 21 dpi and pen feed intake was recorded. Additionally, weekly pen feed efficiency was calculated for each treatment. All pigs were euthanized at 21 dpi by sodium pentobarbital overdose (Fatal-Plus Solution; Vortech Pharmaceuticals, Dearborn, MI) followed by immediate exsanguination.

Blood Collection and Analysis

Blood samples (10 mL) and fecal swabs were collected on all pigs at dpi 0, 14, and 21. All blood samples were collected via jugular venipuncture into BD Vacutainer serum tubes (Becton, Dickinson and Company, Franklin Lakes, NJ) while pigs were snare restrained. After clotting, serum was separated by centrifugation ($2,000 \times g$ for 15 min at 4°C) and stored at -80°C until analyzed or submitted to the Iowa State University Veterinary Diagnostic Laboratory (Ames, IA) for PRRS quantitative real-time qPCR) and serology analyses. Fecal swabs were submitted to the Iowa State University Veterinary Diagnostic Laboratory for PED S gene qPCR analysis (Madson et al., 2014). Testing for PRRS virus and PED virus was performed with commercial reagents (VetMAX NA and EU PRRS virus real time PCR [Thermo Fisher Scientific, Waltham, MA] and EZ-PED/TGE MPX [Tetracore Inc., Rockville, MD]). A commercial ELISA kit (HerdCheck PRRS X3; IDEXX Laboratories, Inc., Westbrook, ME) was used to detect anti-PRRS antibody according to manufacturer's instruction.

At dpi 21, serum insulin was measured using a Porcine Insulin ELISA kit (Mercodia AB, Uppsala, Sweden) according to manufacturer's instructions. Serum glucose and NEFA concentrations were measured using the Wako Diagnostics kits (Wako Chemical Inc., Richmond, VA). Blood urea nitrogen (**BUN**; BioAssay Systems, Hayward, CA) and glucagon (R&D Systems, Minneapolis, MN) was assessed using assay kits according to manufacturer's instructions. All assays were read with a Synergy 4 plate reader using Gen 5 software (BioTek Instruments Inc., Winooski, VT).

Apparent Total Tract Digestibility and Apparent Ileal Digestibility

Feces were collected and pooled within pig at 18 to 20 dpi and ileal digesta from the last 100 cm of the

Table 1. Diet composition, as fed basis

Ingredient, %	Composition
Corn	60.93
Soybean meal, 48% CP	30.00
Corn DDGS ¹	5.00
Soybean oil	1.00
Limestone	0.94
L-Lysine HCl	0.50
Sodium chloride	0.35
Commercial VTM ²	0.30
Monocalcium phosphate, 21%	0.55
Heat Stable Optiphos 2000 ³	0.02
L-Threonine	0.22
DL-Methionine	0.19
Titanium dioxide	0.40
Calculated composition	
CP, %	21.13
ME, kcal/kg	3,388
NE, kcal/kg	2,433
Lys, SID ⁴ %	1.33
Lys, total %	1.48
Analysis	
DM, %	93.3
CP, %	21.0
GE, kcal/kg	3,895
Lys, total %	1.37

¹DDGS = distiller's dried grains with solubles.

²VTM = vitamin-trace mineral premix, which supplied, per kilogram of diet, 8,820 IU vitamin A, 1,653 IU vitamin D₃, 33.1 IU vitamin E, 4.4 mg vitamin K, 6.6 mg riboflavin, 38.9 mg niacin, 22.1 mg pantothenic acid, 0.04 mg vitamin B₁₂, 1.1 mg I as potassium iodide, 0.30 mg Se as sodium selenite, 60.6 mg Zn as zinc oxide, 36.4 mg Fe as ferrous sulfate, 12.1 mg Mn as manganous oxide, and 3.6 mg Cu as copper sulfate.

³Huvepharma Inc., Peachtree City, GA.

⁴SID = Standardized ileal digestibility.

distal ileum was also collected for apparent ileal digestibility (AID) immediately following euthanasia (21 dpi). Approximately 100 mL of ileal digesta was collected, frozen, and then freeze-dried for AID analysis. Pooled fecal and diet samples were homogenized and dried in a mechanical convection oven at 100°C to determine apparent total tract digestibility (ATTD) of N, DM, OM, and GE. Proximate analysis was performed on feed, ileal digesta, and fecal samples as previously described (Stein et al., 2007; Oresanya et al., 2008; Jacobs et al., 2011). Briefly, all samples were analyzed for DM (method 930.15; AOAC, 2005), titanium dioxide as described by Leone (1973), N using TruMac N (Leco Corporation, St. Joseph, MO), and GE using bomb calorimetry (Oxygen Bomb Calorimeter 6200; Parr Instruments, Moline, IL). Organic matter was determined using the ashing method and calculated as previously described (Faithfull, 2003). Amino acid analysis of feed and digesta samples was performed by the Agricultural Experiment Station Chemical Laboratories at the University of Missouri-

Columbia (Columbia, MO) using cation-exchange HPLC (L8900 Amino Acid Analyzer; Hitachi High-Technologies Corporation, Tokyo, Japan). For each of the 4 treatments, ATTD of DM, OM, N, and GE and AID of OM, DM, N, and AA were calculated using the index method (Oresanya et al., 2008).

Statistical Analysis

The PROC MIXED procedure of SAS version 9.4 (SAS Inst. Inc., Cary, NC) was used to compare the overall treatment effects. Pen was considered the experimental unit for performance, digestibility, and blood measures. For ATTD and AID analysis, ADFI was used as a covariate. Orthogonal contrast statements were used to determine the main effects of PRRS and PED infection as well as the interaction of the two infections. All data are reported as least squares means ± SEM and considered significant if $P \leq 0.05$ and a trend if $P \leq 0.10$.

RESULTS

Porcine Reproductive and Respiratory Syndrome Virus and Porcine Epidemic Diarrhea Virus Infection

All animals were naïve to PRRS and PED before inoculation based on serum and fecal swab qPCR analysis, respectively. By design, CON and PED-only gilts remained PRRS negative throughout the study. At 14 and 21 dpi, PRRS virus and antibodies were detected from serum of all pigs inoculated with PRRS (Table 2). Analysis of fecal swabs by qPCR revealed that PED virus was prevalent in the PED- and PRP-inoculated gilts, whereas the CON and PRRS treatments remained PED virus negative (Table 2).

Growth Performance

The effects of PRRS, PED, and PRP on ADG, ADFI, and G:F are summarized in Table 3 and Fig. 1. During the 21-d test period, PRRS infection reduced ADG and ADFI by 30 and 26%, respectively ($P < 0.05$), but not G:F compared with CON pigs. Coinfection greatly affected overall performance, reducing ADG, ADFI, and G:F by 46, 30, and 23%, respectively ($P < 0.05$). Reduction in overall performance in the PRP pigs was more influenced by infection with PRRS virus than infection with PED virus. The overall 21-d pig performance for ADG, ADFI, and G:F was not significantly different among PED and CON pigs. However, the main effect of PED was significant for ADG and G:F ($P = 0.001$ and $P = 0.008$, respectively) and showed a tendency for ADFI ($P = 0.076$). This indicated that PED virus alone and in combination reduced pig performance compared with CON and PRRS pigs.

Table 2. Effect of porcine reproductive and respiratory syndrome virus (PRRS) and porcine epidemic diarrhea virus (PED) infection on viremia and antibody titers

Parameter	Control ¹	PRRS ²	PED ²	PRP ²	SEM	<i>P</i> -value
PRRS virus titer (quantitative PCR log+1) ³						
14 dpi ⁴	Neg.	5.2	Neg.	4.9	0.31	0.64
21 dpi	Neg.	3.9	Neg.	3.4	0.36	0.36
PRRSX3 antibody (S:P ratio)						
14 dpi	Neg.	1.2	Neg.	1.4	0.14	0.10
21 dpi	Neg.	1.1 ^a	Neg.	1.5 ^b	0.16	0.003
PED virus titer (qPCR) ⁵						
0 dpi	Neg.	Neg.	Neg.	Neg.	–	–
21 dpi	Neg.	Neg.	Pos.	Pos.	–	–

^{a,b}Treatments with different superscripts differ ($P < 0.05$).

¹ $n = 3$ pens per treatment; healthy, virus naïve.

² $n = 6$ pens per treatment. PRRS = PRRS virus infected; PED = PED virus infected; PRP = PRRS+PED co-infection.

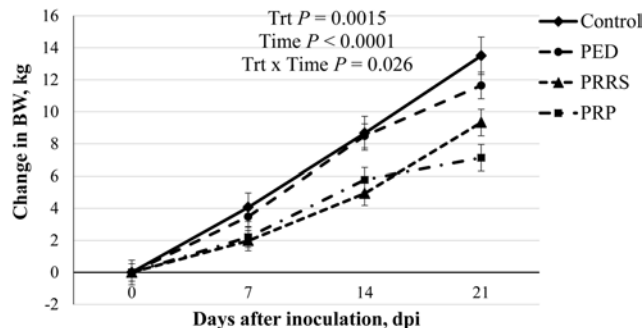
³Quantitative PCR (qPCR). Neg. = negative cycle threshold (Ct) > 37.

⁴dpi = days after inoculation.

⁵Neg.: Ct > 35; Pos. = positive: Ct < 35.

There was no interaction, suggesting no additive effects of coinfection (PRP) on overall growth performance.

When comparing the initial impact of PED on performance (15–21 dpi, 7 d of PED infection), CON and PRRS pigs showed no difference (Table 3). Control versus PED and PRP, however, resulted in sizable reductions in ADG (47 and 70%, respectively; $P < 0.001$), ADFI (29 and 45%, respectively; $P = 0.003$), and G:F (28 and 43%, respectively; $P = 0.001$), respectively. Performance parameters of ADG, ADFI, and G:F during the final week of performance were significantly

**Figure 1.** The effect of porcine reproductive and respiratory syndrome virus (PRRS), porcine epidemic diarrhea virus (PED), and PRRS+PED co-infection (PRP) health challenges on BW gains in nursery-grower gilts. Gilts were inoculated with PRRS virus (d after inoculation [dpi] 0) and PED virus (dpi 14) and BW were taken at 0, 7, 14 and 21 d. TRT = treatment.

decreased by PED ($P < 0.001$, all measures); however, PRRS tended ($P = 0.083$) to increase ADG and significantly ($P = 0.026$) influenced ADFI. There were no interactive effects during the final week of growth.

Apparent Total Tract and Ileal Digestibility

Dry matter, N, GE, and OM digestibility was assessed from 18 to 20 dpi by fecal grab samples (Table 4). Over this period, ATTD coefficients of DM, N, GE, and OM were unaffected by PRRS ($P > 0.10$). However, PED decreased DM and GE digestibility by 7.8 and 11.4%, respectively ($P < 0.01$), whereas N and OM remained unaffected ($P > 0.10$). Coinfection reduced DM, N, GE, and OM digestibility by 9.0, 12.8, 11.9, and 3.5%, respectively ($P < 0.05$), from CON. Reductions in all ATTD coefficients of PRP pigs were

Table 3. Growth performance of pigs infected with porcine reproductive and respiratory syndrome (PRRS) virus, porcine epidemic diarrhea (PED) virus, or co-infection

Parameter	Control ¹	PRRS ²	PED ²	PRP ²	SEM	<i>P</i> -value			
						Overall	PRRS ³	PED ⁴	Interaction ⁵
0–14 d performance									
ADG, kg	0.62 ^a	0.35 ^b	0.61 ^a	0.41 ^b	0.03	<0.001	<0.001	0.506	0.322
ADFI, kg	0.95 ^a	0.67 ^b	0.94 ^a	0.76 ^b	0.05	0.001	<0.001	0.441	0.354
G:F, kg/kg	0.65 ^a	0.53 ^b	0.65 ^a	0.55 ^{ab}	0.03	0.020	0.003	0.861	0.746
15–21 d performance									
ADG, kg	0.66 ^a	0.63 ^a	0.35 ^b	0.20 ^b	0.04	<0.001	0.083	<0.001	0.229
ADFI, kg	1.22 ^a	1.02 ^a	0.88 ^{ab}	0.67 ^b	0.07	0.003	0.026	<0.001	0.969
G:F, kg/kg	0.54 ^{ab}	0.62 ^a	0.39 ^{bc}	0.31 ^c	0.05	0.001	1.000	<0.001	0.133
0–21 d performance									
ADG, kg	0.63 ^a	0.44 ^b	0.51 ^{ab}	0.34 ^c	0.04	<0.001	<0.001	0.001	0.797
ADFI, kg	1.04 ^a	0.78 ^b	0.92 ^{ab}	0.73 ^b	0.04	0.002	<0.001	0.076	0.508
G:F, kg/kg	0.61 ^a	0.56 ^a	0.56 ^a	0.47 ^b	0.02	0.005	0.006	0.008	0.456

^{a-c}Treatments with different superscripts differ ($P < 0.05$).

¹ $n = 3$ pens per treatment; healthy, virus naïve.

² $n = 6$ pens per treatment. PRRS = PRRS virus infected; PED = PED virus infected; PRP = PRRS+PED coinfection.

³Main effects of PRRS effect (control and PED vs. PRRS and PRP).

⁴Main effects of PED effect (control and PRRS vs. PED and PRP).

⁵Interaction of PRRS and PED (control and PRP vs. PRRS and PED).

Table 4. Apparent total tract digestibility coefficients (%) of pigs infected with porcine reproductive and respiratory syndrome (PRRS) virus, porcine epidemic diarrhea (PED) virus, or combined infection

Parameter	Control ¹	PRRS ²	PED ²	PRP ²	SEM	P-value			
						Overall	PRRS ³	PED ⁴	Interaction ⁵
DM	88.7 ^a	87.5 ^a	81.8 ^b	80.7 ^b	0.90	<0.001	0.234	<0.001	0.956
OM	92.4 ^{ab}	92.7 ^a	90.3 ^{ab}	89.2 ^b	0.78	0.033	0.643	0.010	0.334
GE	87.2 ^a	86.4 ^a	77.3 ^b	76.8 ^b	0.90	<0.001	0.462	<0.001	0.909
N	85.9 ^a	84.7 ^a	80.1 ^{ab}	74.9 ^b	1.90	0.014	0.100	0.004	0.236

^{a,b}Treatments with different superscripts differ ($P < 0.05$).

¹ $n = 3$ pens per treatment; healthy, virus naïve.

² $n = 6$ pens per treatment. PRRS = PRRS virus infected; PED = PED virus infected; PRP = PRRS+PED coinfection.

³Main effects of PRRS effect (control and PED vs. PRRS and PRP).

⁴Main effects of PED effect (control and PRRS vs. PED and PRP).

⁵Interaction of PRRS and PED (control and PRP vs. PRRS and PED).

influenced by the main effect of PED ($P < 0.001$, $P = 0.004$, $P = 0.010$, and $P < 0.001$, respectively) and not PRRS ($P > 0.05$). No significant differences between PED and PRP were detected.

Dry matter, N, OM, and specific AA digestibility were assessed by collection of ileal digesta at 21 dpi (Table 5). No AID differences were detected among treatments for DM, N, or OM. There were numerical differences, however, with PED and PRP having reduced DM (14 and 17%, respectively), N (9 and 4%, respectively), and OM (8 and 11%, respectively) compared with CON. Interestingly, OM AID was effected by PED ($P = 0.042$). Similar to ATTD, PRRS had a minimal effect on AID measures compared with the CON pigs ($P > 0.10$). There were no statistical differences reported for any specific AA AID measured (Table 5). However, Lys AID tended ($P = 0.095$) to be reduced in PED pigs from PRRS and CON pigs, attributed to PED ($P = 0.029$).

Blood Metabolites

Blood glucose, insulin, glucagon, and NEFA concentration and BUN were assessed at 21 dpi (Table 6). Blood urea nitrogen was increased ($P < 0.001$) 2-fold in PED and PRP pigs but was unaffected by PRRS ($P > 0.10$). Additionally, glucagon concentrations were increased ($P < 0.001$) 3-fold in PED and PRP treatments over CON, whereas the PRRS treatment did not affect blood glucagon concentrations. A main effect of PED was detected for both BUN and glucagon ($P < 0.001$ for both measures) whereas the main effect of PRRS had no effect ($P > 0.10$). Blood NEFA concentration was increased 23% by the PRP treatment compared with the CON, PRRS, and PED treatments. This resulted in an interaction ($P = 0.044$), suggesting an additive effect of PRRS and PED in combination. Blood glucose and insulin were unaffected by among treatments ($P = 0.61$ and $P = 0.36$, respectively).

DISCUSSION

The antibody response at 21 dpi was increased in PRP pigs versus PRRS-only pigs. This could be due to a later peak antibody response as a result of concurrent PED virus infection, which has been seen when PRRS-positive pigs challenged with other viruses (Zhang et al., 2012). Significant reductions in ADG and ADFI, but not G:F, in PRRS virus infected pigs throughout the duration of the study agrees with previous studies our group and others have reported (Escobar et al., 2004, 2006). This is the first reported characterization of growth performance and feed efficiency in PED- and PRRS+PED-infected pigs. Reduced performance was expected, as pigs with increased immune activity show reductions in ADG, ADFI, and G:F, as reported by Williams et al. (1997a,b,c). Similarly, coinfection with PRRS has reduced growth performance (Van Reeth et al., 1996; Brockmeier et al., 2001).

Recent work from our group reported significant differences in ATTD with regards to PRRS virus infection (Gabler et al., 2013); however, the same results were not seen in these younger pigs in the current study, as PRRS virus infection showed no difference in ATTD. However, PED, an enteric virus that replicates in villi enterocytes (Neumann et al., 2012), exhibited reductions in DM and GE ATTD. Unexpectedly, PED did not reduce N or OM ATTD. Similarly, PRP pigs had reduced DM, N, and GE ATTD whereas OM was not different from CON. Also, there were no differences between PED and PRP treatments, which was somewhat unexpected. Differences in ATTD may be explained by rate of passage. When livestock suffer from diarrhea, the rate of passage can be reduced by as much as 50% (Bush et al., 1963) and rate of passage is negatively correlated with total tract digestibility coefficients (Entringer et al., 1975). A shortened time of passage does not allow as much time for sufficient enzymatic breakdown of feedstuffs, which leads to poor absorption and diarrhea (Blaxter and Wood, 1953;

Table 5. Apparent ileal digestibility coefficients (%) of pigs infected with porcine reproductive and respiratory syndrome (PRRS) virus, porcine epidemic diarrhea (PED) virus, or combined infection

Parameter	Control ¹	PRRS ²	PED ²	PRP ²	SEM	P-value			
						Overall	PRRS ³	PED ⁴	Interaction ⁵
DM	59.3	57.4	50.9	48.8	3.63	0.305	0.596	0.073	0.976
OM	77.7	75.8	71.2	69.0	2.45	0.208	0.409	0.042	0.945
N	68.0	70.3	62.0	65.1	4.11	0.423	0.534	0.291	0.927
Total AA	68.7	69.9	64.5	66.8	3.74	0.651	0.641	0.430	0.865
Essential AA, %									
Arginine	82.6	81.5	78.2	78.6	2.50	0.639	0.892	0.251	0.738
Histidine	76.5	73.8	71.5	73.3	2.81	0.688	0.870	0.430	0.359
Isoleucine	71.4	72.3	72.6	72.7	3.04	0.996	0.869	0.833	0.885
Leucine	71.0	71.2	70.8	73.3	2.98	0.920	0.648	0.806	0.671
Lysine	86.3	83.6	75.8	77.5	2.82	0.095	0.848	0.029	0.377
Methionine	83.7	83.5	82.2	83.4	1.94	0.919	0.806	0.761	0.683
Phenylalanine	73.0	71.6	73.8	74.3	2.93	0.900	0.873	0.639	0.719
Threonine	65.6	69.5	66.3	68.5	3.83	0.823	0.430	0.977	0.797
Tryptophan	76.5	81.5	76.1	79.1	2.95	0.367	0.190	0.699	0.695
Valine	61.3	64.9	61.3	66.3	3.67	0.623	0.253	0.883	0.821
Nonessential AA, %									
Alanine	66.0	66.3	62.2	65.2	3.85	0.786	0.677	0.609	0.684
Aspartic acid	68.8	68.8	61.0	62.4	4.40	0.486	0.872	0.209	0.853
Cysteine	51.4	53.0	38.7	41.3	6.62	0.374	0.740	0.171	0.929
Glutamic acid	73.0	70.8	63.9	67.6	4.20	0.445	0.854	0.248	0.421
Proline	66.5	66.2	59.7	60.4	4.81	0.672	0.972	0.297	0.909
Serine	64.7	67.2	64.1	64.4	4.28	0.915	0.740	0.745	0.769
Tyrosine	71.8	73.3	73.2	73.7	2.89	0.984	0.730	0.801	0.840

¹n = 3 pens per treatment; healthy, virus naïve.

²n = 6 pens per treatment. PRRS = PRRS virus infected; PED = PED virus infected; PRP = PRRS+PED coinfection.

³Main effects of PRRS effect (control and PED vs. PRRS and PRP).

⁴Main effects of PED effect (control and PRRS vs. PED and PRP).

⁵Interaction of PRRS and PED (control and PRP vs. PRRS and PED).

Weijers et al., 1959). It also reduces the amount of time spent in the large intestine for water reabsorption and bacterial fermentation (Levitan, 1969; Clausen et al., 1998; Marchelletta et al., 2013). Although limited work has been done with industry applicable pathogens, lipopolysaccharide (LPS) has been used to mimic pathogen challenge in pigs. These studies have reported that LPS induced inflammatory challenges attenuate OM, energy, and N ATTD (Rakhshandeh and de Lange 2012; Rakhshandeh et al., 2012). This agrees with the present study in which PED and PRRS were the immunological agents. Williams et al. (1997a) also reported decreases in N digestibility using a rearing method with piglets exposed to environmental antigens.

There were apparent differences in ATTD between treatments, but interestingly, there were only numerical differences in AID of DM, N, and OM. Apparent ileal digestibility of specific AA was also unaffected by treatment, with only Lys digestibility tending to be reduced in PED challenged pigs. Similarly, in LPS-challenged pigs, AID was not different from nonchallenged pigs (Rakhshandeh et al., 2010); however, this differs from a

previous study where pigs infected with *Salmonella typhimurium* had reduced AID of several AA (Lee, 2012). A limitation of using AID is that endogenous losses cannot be accounted for. Endogenous losses have been previously determined in pigs inoculated with *S. typhimurium*. This study found that endogenous losses of all AA were increased 4- to 9-fold at 24 h after infection; however, by 72 h after infection, there were no differences in endogenous losses (Lee, 2012). This may suggest that time of collection (21 dpi for PRRS and 7 dpi for PED) did not capture peak infection differences, which may influence AID. Villus height and crypt depth were severely reduced throughout the small intestine in PED and PRP pigs but minimally affected in PRRS pigs (Schweer et al., 2016). This leads to reduced surface area of the intestine, which may influence AID. Another diarrheal disease described in pigs, cryptosporidiosis, causes reductions in villi height and crypt hyperplasia and has been reported to decrease villous surface area 3-fold (Argenzio et al., 1990). Although intestinal morphology was changed, there was no difference in aminopeptidase activity, suggesting that digestive enzymes were still functioning normally

Table 6. Blood metabolites of pigs infected with porcine reproductive and respiratory syndrome virus (PRRS), porcine epidemic diarrhea virus (PED), or combined infection

Parameter	Control ¹	PRRS ²	PED ²	PRP ²	SEM	P-value			
						Overall	PRRS ³	PED ⁴	Interaction ⁵
Glucose, mg/dL	148.0	164.2	180.5	166.6	15.4	0.610	0.943	0.273	0.342
Insulin, ng/mL	0.118	0.157	0.110	0.078	0.021	0.363	0.923	0.227	0.320
BUN, ⁶ mg/dL	11.46 ^a	10.28 ^a	21.16 ^b	21.72 ^b	2.12	<0.001	0.885	<0.001	0.686
NEFA, mmol/L	0.134 ^a	0.134 ^a	0.131 ^a	0.174 ^b	0.010	0.010	0.044	0.077	0.044
Glucagon, ng/mL	308 ^a	487 ^a	1,159 ^b	1,398 ^b	162.6	<0.001	0.219	<0.001	0.857

^{a,b}Treatments with different superscripts differ ($P < 0.05$).

¹ $n = 3$ pens per treatment; healthy, virus naïve.

² $n = 6$ pens per treatment. PRRS = PRRS virus infected; PED = PED virus infected; PRP = PRRS+PED coinfection.

³Main effects of PRRS effect (control and PED vs. PRRS and PRP).

⁴Main effects of PED effect (control and PRRS vs. PED and PRP).

⁵Interaction of PRRS and PED (control and PRP vs. PRRS and PED).

⁶BUN = blood urea nitrogen.

(Schweer et al., 2016). That would leave only feed intake as a major difference between treatments, but feed intake does not greatly affect nutrient digestibility (Haydon et al., 1984; Albin et al., 2001).

Although there are limitations to single point estimates for metabolites and hormones, similarities in blood metabolites between CON and PRRS treatments were expected at 21 dpi (Roberts and Almond, 2003). Reductions in feed intake, leading to a catabolic state, presumably caused increased blood glucagon and BUN in PED and PRP treatments. This data conflicts with a previous report where pigs infected with rotavirus exhibited no difference in blood glucagon (Zijlstra et al., 1997). Although differences in BUN were reported here, pigs inoculated with *Brachyspira hyodysenteriae*, the causative agent of swine dysentery, reported no difference in serum total AA concentrations (Jonasson et al., 2007). Interestingly, blood NEFA in CON, PRRS, and PED treatments was not different, whereas it was increased in PRP pigs. This suggests an additive effect of the PRRS and PED and an increase in immune stimulation-induced changes in metabolism. This potentially results in an increase in lipolysis to provide substrates ATP production and fuel of immune cells (Doughty et al., 2006) as energy intake could be limiting. Fatty acid oxidation also plays an important in memory CD8+ cell generation (Pearce et al., 2009). Increased NEFA has been previously reported in pigs coinfecting with PRRS and *Mycoplasma hyopneumoniae* at 7 dpi compared with control and pair-fed pigs (Oliver, 2004). This suggests that feed restriction only partially explains the increase in NEFA. Blood glucose and insulin were unaffected by treatment. Similarly, pigs infected with rotavirus had no differences in circulating insulin levels (Zijlstra et al., 1997). Similar find-

ings for blood glucose have been reported in pigs during *B. hyodysenteriae* infection (Jonasson et al., 2007) whereas others have reported an increase in glucose during infection (Somchit et al., 2003). Utilization of glucose is increased in tissues and immune cells during immune response, which can cause hypoglycemia (Mizock, 1995); however, no differences in blood glucose in the present study suggests these pigs were likely meeting their glucose demands via gluconeogenesis.

In summary, PRRS and PED virus challenges, alone or in combination (PRP), antagonize pig growth rates and feed intakes. Pig ATTD coefficients were reduced by PED and a coinfection of PRRS and PED, but PRRS alone did not reduce ATTD. Similarly, PRRS, PED, or a combination of both did not significantly decrease AID coefficients of specific AA. Altogether, these results indicate that capability to digest nutrients (as determined by apparent digestibility) seems to be unaffected by systemic or enteric viral challenges. However, the reduction in feed intake resulting from PED and PRRS challenges is probably one of the main limitations for pigs not being able to maintain high growth rates during the 21-d test period.

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