Impact of porcine reproductive and respiratory syndrome virus on muscle metabolism of growing pigs¹

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ABSTRACT: Porcine reproductive and respiratory syndrome (PRRS) virus is one of the most economically significant pig pathogens worldwide. However, the metabolic explanation for reductions in tissue accretion observed in growing pigs remains poorly defined. Additionally, PRRS virus challenge is often accompanied by reduced feed intake, making it difficult to discern which effects are virus vs. feed intake driven. To account for this, a pair-fed model was employed to examine the effects of PRRS challenge and nutrient restriction on skeletal muscle and liver metabolism. Forty-eight pigs were randomly selected (13.1 ± 1.97 kg BW) and allotted to 1 of 3 treatments ($n = 16$ pigs/treatment): 1) PRRS naïve, ad libitum fed (Ad), 2) PRRS-inoculated, ad libitum fed (PRRS+), and 3) PRRS naïve, pair-fed to the PRRS-inoculated pigs' daily feed intake (PF). At days postinoculation (dpi) 10 and 17, 8 pigs per treatment were euthanized and tissues collected. Tissues were assayed for markers of proteolysis (LM only), protein synthesis (LM only), oxidative stress (LM only), gluconeogenesis (liver), and glycogen concentrations (LM and liver). Growth performance, feed intake, and feed efficiency were all reduced in both PRRS+ and PF pigs compared with Ad pigs ($P < 0.001$). Furthermore,

growth performance and feed efficiency were additionally reduced in PRRS+ pigs compared with PF pigs ($P < 0.05$). Activity of most markers of LM proteolysis (μ -calpain, 20S proteasome, and caspase 3/7) was not increased ($P > 0.10$) in PRRS+ pigs compared with Ad pigs, although activity of m-calpain was increased in PRRS+ pigs compared with Ad pigs ($P = 0.025$) at dpi 17. Muscle reactive oxygen species production was not increased ($P > 0.10$) in PRRS+ pigs compared with Ad pigs. However, phosphorylation of protein synthesis markers was decreased in PRRS+ pigs compared with both Ad ($P < 0.05$) and PF ($P < 0.05$) pigs. Liver gluconeogenesis was not increased as a result of PRRS; however, liver glycogen was decreased ($P < 0.01$) in PRRS+ pigs compared with Ad and PF pigs at both time points. Taken together, this work demonstrates the differential impact a viral challenge and nutrient restriction have on metabolism of growing pigs. Although markers of skeletal muscle proteolysis showed limited evidence of increase, markers of skeletal muscle synthesis were reduced during PRRS viral challenge. Furthermore, liver glycogenolysis seems to provide PRRS+ pigs with glucose needed to fuel the immune response during viral challenge.

Key words: liver, metabolism, muscle, pig, porcine reproductive and respiratory syndrome

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INTRODUCTION

It is nearly inevitable that at some point in production, growing pigs will experience immune system activation from either vaccination, bacterial challenge, or viral challenge. The latter is often accompanied by reductions in both feed intake and lean tissue accretion (Escobar et al., 2004; Curry et al., 2017; Schweer et al., 2017). It is widely accepted that reductions in lean tissue accretion observed during an event such as viral challenge result from both indirect effects of reduced nutrient intake and direct effects of nutrient and energy reallocation toward the immune response (Scrimshaw, 1977; Reeds et al., 1994; Elsasser et al., 2000). However, individual contributions of these indirect and direct effects during a pathogen challenge towards the total reduction in lean tissue accretion observed remain poorly defined.

Appetite reduction during health challenges, or disease anorexia, is highly conserved across species and appears to be a mechanism necessary for survival and recovery from disease (Plata-Salaman, 1996). In mice, interfering with disease anorexia via force feeding during a bacterial *Listeria monocytogenes* challenge led to mortality of 93% and shorter survival times compared with ad libitum fed mice (Murray and Murray, 1979), suggesting caloric restriction is a crucial host defense mechanism. However, there is evidence that caloric supplementation during viral challenges may not have the same effect, as providing enteral nutrition to mice challenged with the influenza virus protected them from mortality (Wang et al., 2016). In meat animal production, a consequence of reduced appetite during disease is reduced nutrient availability to tissues such as skeletal muscle, decreasing capacity for lean tissue accretion. Thus, if the primary cause of reduced lean tissue accretion during viral challenges is reduced appetite, strategies to increase caloric intake may be best to mitigate performance losses.

In addition to indirect effects of reduced feed intake, immunological stimulation in pigs may directly antagonize skeletal muscle protein synthesis (Orellana et al., 2002) and enhance protein degradation, although this has not been well characterized in pigs challenged with live bacterial or viral pathogens. Skeletal muscle is the largest protein store in the pig, thus enhanced skeletal muscle protein degradation may release amino acids that could be utilized for protein synthesis or energy generation during immune activation (Reeds et al., 1994). During mild enteric viral (Curry et al., 2018) and

bacterial (Helm et al., 2018) pathogen challenges, upregulation of skeletal muscle protein degradation does not appear necessary; however, it is unclear if these changes would be necessary during a more severe pathogen challenge.

One of the most economically significant pig pathogens worldwide is Porcine Reproductive and Respiratory Syndrome (PRRS) virus, as the resulting disease costs the U.S. pork industry over US\$660 million annually (Holtkamp et al., 2013). In growing pigs, PRRS presents clinically with fever, respiratory distress, lethargy, reduced feed intake, and ultimately reductions in lean tissue accretion (Greiner et al., 2000; Murtaugh et al., 2002; Zimmerman et al., 2012). In growing pigs, PRRS virus infection consistently attenuates ADG and ADFI, and these performance indicators can be reduced by 50% and 30%, respectively, during the first 2 wk of infection (Rochell et al., 2015). Despite a growing body of literature regarding PRRS virus etiology, its impact on pigs, and its known cost to the industry, individual contributions of indirect and direct effects of PRRS virus challenge to reductions in skeletal muscle protein accretion remain undefined.

The objectives of this study were to examine the indirect and direct effects of PRRS virus challenge on liver and skeletal muscle metabolism. We hypothesized that PRRS virus challenge would increase skeletal muscle proteolysis and reduce synthesis in order to provide amino acids for acute phase protein synthesis, a cost that would be partially, but not fully, described by reduced feed intake. To investigate these objectives, we completed a 17-d PRRS challenge study and employed a pair-feeding model, in which a group of PRRS naïve pigs were fed daily to match the voluntary feed intake of PRRS challenged pigs.

MATERIALS AND METHODS

All animal procedures in this study were approved by the Iowa State University Institutional Animal Care and Use Committee (protocol number 8-15-8074-S) and adhered to the ethical and humane use of animals for research.

Animals, Housing, Experimental Design, and Sample Collection

Forty-eight gilts (13.1 ± 1.97 kg BW; Genetiporc 6.0 × Genetiporc F25, PIC, Inc., Hendersonville, TN) confirmed seronegative by ELISA for PRRS virus were randomly selected for this study. Pigs

were split across 3 identical rooms at the Iowa State Livestock Infectious Disease Isolation Facility, assigned to individual pens, and allowed to acclimate for 4 d prior to inoculation. Pigs were then allotted into blocks of 3 pigs/block (16 blocks total) based on initial BW. Within each block, pigs were assigned to 1 of 3 treatment groups: 1) PRRS naïve, Ad libitum fed (**Ad**, $n = 16$), 2) PRRS inoculated, ad libitum fed (**PRRS+**, $n = 16$), and 3) PRRS naïve, pair-fed (**PF**, $n = 16$) to mirror nutrient intake of PRRS+ pigs.

On days postinoculation (**dpi**) 0, PRRS+ pigs were inoculated with ORF5 RFLP_1-3-4 isolate of PRRS virus (1-mL intramuscular injection and 1-mL intranasal inoculation; 10^6 genomic copies per mL), whereas PF and Ad pigs received a saline sham inoculum. At and beyond dpi 0, each PF pig was fed daily to the previous day's voluntary feed intake of the PRRS+ pig in its respective block. To implement pair feeding, voluntary feed consumption (feeder weight determination of feed disappearance) of each PRRS+ pig was recorded every morning, and that amount of feed was given to the PF pig in its respective block the following morning. Any feed refusals were recorded for PF pigs. Regardless of treatment group, all pigs were fed the same corn-soybean meal based nursery diet formulated to meet or exceed [NRC \(2012\)](#) requirements for this size pig. Briefly, this diet was formulated to contain 3,332 kcal/kg metabolizable energy and a standardized ileal digestible lysine of 1.10%.

On dpi 10 and 17, 8 blocks (24 pigs/dpi) were randomly selected for euthanasia and necropsy. These 2 time points were chosen as dpi 10 would likely coincide with high viremia and dpi 17 would likely coincide with seroconversion, although there is variation with regard to the timing of these events ([Klinge et al., 2009](#)). Thus, necropsy time points were designed to capture 2 different stages of the immune response, whilst remaining within a period where peak growth performance impacts would also be observed. All pigs were fasted the night prior to euthanasia and were then fed 2 h prior to euthanasia to ensure fasting duration would not influence metabolic parameters to be assessed. Pigs were snared, bled, and euthanized via captive bolt followed by immediate exsanguination. At necropsy, sections of longissimus muscle (**LM**) and liver (left lobe) were excised, frozen in liquid nitrogen, and stored at -80°C until further analysis. Additionally, a section of LM was placed on ice and immediately transported back to the laboratory for fresh tissue analysis.

Blood Collection and Analysis

On dpi 10 and 17, blood samples (10 mL) were collected from pigs immediately prior to euthanasia. Blood samples were collected into BD Vacutainer tubes (Becton, Dickinson and Company, Franklin Lakes, NJ) via jugular venipuncture. Samples were allowed to clot at room temperature prior to centrifugation ($2,000 \times g$ for 10 min at 4°C), and serum was collected, aliquoted, and stored at -80°C until analysis.

To confirm PRRS virus infection, serum samples were submitted to the Iowa State University Diagnostic Laboratory to test PRRS viremia and antibody response. Viral presence of PRRS was assessed by real-time PCR using commercial reagents (VetMAX NA and EU PRRSV real-time RT-PCR, Thermo Fisher Scientific, Waltham, MA). The PRRS virus antibodies were detected via a commercially available indirect ELISA (PRRSX3 antibody test, IDEXX Laboratories, Inc., Westbrook, ME) according to the manufacturer's instructions.

In addition to diagnostic measures, serum samples were analyzed for glucagon, blood urea nitrogen (**BUN**), NEFA, glucose, insulin, haptoglobin, and C-reactive protein (**CRP**) concentrations. Commercially available ELISA kits were utilized to determine glucagon (R&D Systems, Minneapolis, MN), insulin (R&D Systems, Minneapolis, MN), haptoglobin (Immunotag, G-Biosciences, St. Louis, MO), and CRP (Alpco, Salem, NH) concentrations according to the manufacturer's instructions. Serum BUN was quantified with a QuantiChrom Urea Assay Kit (BioAssay Systems, Hayward, CA), and NEFA concentrations were measured with the Wako Diagnostics kit (Wako Chemical Inc., Richmond, VA) according to the manufacturer's instructions. Serum glucose concentrations were measured utilizing Glucose Oxidase/Peroxidase Reagent (**GO**, Sigma-Aldrich, St. Louis, MO) assay as described previously ([Helm et al., 2018](#)). All serum absorbance values were analyzed with a Cytation Hybrid Multi-Mode Reader using Gen 5 software (BioTek Instruments Inc., Winooski, VT) and intraassay CVs were under 12%.

Muscle Mitochondrial Isolation, Reactive Oxygen Species Production, and Oxidative Stress

Longissimus muscle reactive oxygen species (**ROS**) production and oxidative stress markers were assessed at dpi 10 and 17. Briefly, mitochondrial isolation was performed on ice from fresh LM samples using a procedure described previously

(Iqbal et al., 2004; Grubbs et al., 2013). Isolated mitochondrial protein concentrations were determined using a bicinchoninic acid (BCA) assay and samples were standardized to 2-mg mitochondrial protein/mL for use in the ROS production assay.

Mitochondrial ROS production was determined using a 2',7'-Dichlorofluorescein diacetate (DCFH) assay described previously (Iqbal et al., 2004; Grubbs et al., 2013). Fluorescence of DCFH was detected at an excitation/emission wavelength of 480/530 nm using a Cytation Hybrid Multi-Mode Reader using Gen 5 software (BioTek Instruments Inc., Winooski, VT). Mitochondrial hydrogen peroxide production was calculated from a hydrogen peroxide standard curve based on fluorescence values of DCFH. Samples were plated in triplicate using a black 96-well plate. Twenty units of superoxide dismutase (Sigma-Aldrich, St. Louis, MO) were added to each sample well to convert any superoxide produced into hydrogen peroxide. Either hydrogen peroxide standards or 90 μ g of mitochondrial protein were added to each well after which 45 μ L of an assay buffer (145 mM KCl, 30 mM HEPES, 5 mM KH_2PO_4 , 3 mM MgCl_2 , 0.1 mM EGTA, 51 μ M DCFH, 8 μ M glutamate) was added. Plates were incubated at 37 °C and read at 0, 5, 10, 15, and 20 min after adding the energy substrate. Readings were used to calculate the rate of hydrogen peroxide production per min, expressed as μ mol hydrogen peroxide produced/mg mitochondrial protein/min.

To assess oxidative damage to LM proteins, protein carbonyl concentrations were evaluated. Sarcoplasmic protein from frozen LM (0.5 g) was extracted in an EDTA/phosphate buffer (50 mM sodium phosphate, pH 6.7, 1 mM EDTA) and the resulting solubilized protein extracts were assayed for protein carbonyl concentrations using a commercially available kit (Cayman Chemical, Ann Arbor, MI). Carbonyl content was expressed as nmol carbonyls per mg protein, which was determined via a BCA assay performed on samples after carbonyl determination.

Skeletal Muscle Protein Degradation and Synthesis

Activities of calpain and calpastatin were fractionated and measured in fresh LM samples as described previously (Cruzen et al., 2013), using casein as a substrate (Koochmarai, 1990). Both assays occurred at 25 °C for 1 h and absorbance (278 nm) of TCA soluble casein peptides was measured. One unit of μ - or m-calpain activity was defined as the amount required to catalyze an increase of 1 absorbance unit. One unit of calpastatin activity was

defined as the amount required to inhibit 1 unit of purified porcine lung m-calpain.

Activity of the 20S proteasome was determined in triplicate in sarcoplasmic protein extracted in EDTA/phosphate buffer described above using a Chemicon 20S Proteasome Activity Assay Kit (MilliporeSigma, Billerica, MA) according to manufacturer's instructions. Activity was determined via fluorescence of fluorophore 7-amino-4-methylcoumarin after cleavage from LLVY-AMC and was expressed as units of activity per mg of extracted skeletal muscle protein, which was determined via BCA assay.

Caspase 3/7 activity was determined in frozen LM tissues in triplicate. A commercially available kit for caspase 3 activity (Cell Signaling, Danvers, MA) was used according to manufacturer's instructions, after proteins were extracted in EDTA/phosphate buffer described above and protein concentration was determined via BCA assay. Kit specificity does not distinguish between caspase 3 and 7; therefore, values determined include activity of both caspase 3 and 7 reported as relative fluorescent units per min per mg protein.

Easily releasable myofilaments (ERMs) were quantified from frozen LM tissue in duplicate using a procedure detailed by Neti et al. (2009). Protein was determined in both crude myofibrillar extracts and final ERM pellets via BCA assay. Quantity of ERM was reported as percent of ERM per mg crude myofibrillar protein.

Protein abundance of ribosomal protein S6 kinase (S6K1), eukaryotic translation factor 4E-binding protein (4E-BP1), adenosine 5' monophosphate kinase α (AMPK), and their phosphorylated forms were each determined via western blot analysis. Protein from frozen LM muscle (0.5 g) was extracted into HEPES cell lysis buffer containing SDS (50 mM HEPES, 150 mM NaCl, 50 mM NaF, 2 mM EDTA, 1% Triton X-100, 0.1% protease inhibitor cocktail, 5% glycerol, and 0.1% SDS). Equivalent protein concentrations (40 μ g per lane) were separated using SDS polyacrylamide gel electrophoresis (SDS-PAGE), with treatments evenly distributed amongst gels. Gels were run under reducing conditions, transferred to a nitrocellulose membrane, and then blocked for 1 h in 5% (wt/vol) bovine serum albumin (BSA) in Tris-buffered saline (TBS; 20 mM Tris-base and 150 mM NaCl, pH 7.4) containing 0.1% Tween-20 (TBST). The primary (phospho-) antibodies were diluted 1:1000 in TBST with 5% BSA and left overnight for incubation at 4 °C. Secondary antibody (Cell Signaling Technology, Danvers,

MA) was added at 1:1000 in TBST with 2.5% BSA and incubated for 1 h at room temperature. Membranes were incubated with Supersignal West Pico Chemiluminescent Substrate (ThermoFisher Scientific, Waltham, MA) for approximately 5 min to detect bands and then imaged using FluorChem M system (ProteinSimple, San Jose, CA). After imaging, membranes were stripped, reblocked, and reprobed for the total abundance of each respective protein. Bands were standardized to a reference control sample run on every blot, and then the ratio of phosphorylated to total protein was determined for each sample. Antibodies used were total S6K1 (Cell Signaling Technology #9202), S6K1 Thr⁴²⁴/Ser⁴²⁴ (Cell Signaling Technology #9204), total 4E-BP1 (Cell Signaling Technology #9452), 4E-BP1 Thr⁴⁶ (Invitrogen #44-1170g), total AMPK α (Cell Signaling Technology #2532), and AMPK α Thr¹⁷² (Cell Signaling Technology #2535).

Skeletal Muscle and Liver Glycogen

Stores of glycogen were determined from frozen LM and liver tissues in duplicate. Tissues were weighed (0.5 g) and homogenized in 4-mL phosphate buffered saline (PBS; 137 mM NaCl, 10 mM NaHPO₄, 2.7 mM KCl, 1.8 mM KH₂PO₄, pH 7.3). An equivalent volume of 1 M perchloric acid was added to each sample and then vortexed well. Two 300- μ L aliquots of this raw homogenate were taken for glycogen determination. Twenty-five microliters of 5.4 M KOH were added to each aliquot to re-balance the sample pH. A glucose assay was performed on this sample as described previously (Helm et al., 2018). After determining basal glucose concentrations, 500 μ L of 0.3 mg/mL amyloglucosidase were added to each sample. These samples were left to incubate on a rocker overnight at room temperature. After incubation, 50 μ L of 3 M perchloric acid were added to each sample, samples were incubated for 10 min on ice, and then were centrifuged at 1,700 $\times$ g for 15 min at 4 °C. The supernatant was collected to determine released glucose utilizing GO assay as described previously. Baseline concentrations were then subtracted from final glucose concentrations to determine tissue glycogen concentrations. Both tissue glucose and glycogen concentrations were reported as mM glucose per g starting tissue weight.

Liver Gluconeogenesis

Activity of 3 key gluconeogenic enzymes [phosphoenolpyruvate carboxykinase (PEPCK),

fructose 1,6-bisphosphatase (F1,6BP), and glucose 6-phosphatase (G6P)] was determined in triplicate from liver protein extracted in HEPES cell lysis buffer (50 mM HEPES, 150 mM NaCl, 50 mM NaF, 2 mM EDTA, 1% Triton X-100, 0.1% protease inhibitor cocktail, and 5% glycerol). Enzyme activities were normalized to sample protein concentration as determined by BCA assay. All assays were read on a Cytation Hybrid Multi-Mode Reader using Gen 5 software (BioTek Instruments Inc., Winooski, VT) and expressed on a per protein basis.

Briefly, PEPCK activity was determined by a modified method as described previously (Wimmer, 1988; Jin et al., 2004; Curry et al., 2018) via measuring the 2-step transformation of oxaloacetate to phosphoenolpyruvate and then to ATP. Luminometric production of ATP was determined using the luciferase ATP Determination Kit (ThermoFisher Scientific, Waltham, MA). Blank reactions were performed using samples incubated without oxaloacetate in reaction buffer I and without inosine-5'-triphosphate. All samples were corrected for their respective blanks, and assay results were expressed as mM of ATP produced per min per mg protein.

Fructose-1,6-bisphosphatase activity was determined as described previously (Curry et al., 2018). All samples were blank corrected, and activity was expressed as μ mol NADPH produced per min per mg hepatic protein. Activity of G6P was quantified by measuring the release of phosphate from glucose-6-phosphate as described previously (Curry et al., 2018). Activity was expressed as mM Pi released per min per mg protein.

Statistical Analysis

The SAS program was used for the statistical analysis of all data (SAS Institute Inc., Cary, NC). The following mixed model was fitted to all parameters:

$$Y_{ijkl} = \mu + \text{PRRS}_i + \text{dpi}_j + (\text{PRRS} \times \text{dpi})_{ij} + i\text{BW}_k + e_{ijkl},$$

wherein Y_{ijkl} is the phenotype measured on animal l ; PRRS_i is the effect of treatment (fixed effect; Ad, PF, PRRS+); dpi_j is the effect of necropsy day (fixed effect; dpi 10, dpi 17); $(\text{PRRS} \times \text{dpi})_{ij}$ is the interaction effect between treatment i and dpi j ; $i\text{BW}_k$ is the blocking factor of initial body weight (random effect); and e_{ijkl} is the error term of animal l subjected to treatment i and dpi j in block k , $e_{ijkl} \sim N(0, \sigma_e^2)$. Least square means of treatment by dpi were

determined using the LS means statement and differences in LS means were produced using the pdiff option. All data are reported as LS means with a pooled SEM. Differences were considered significant when $P < 0.05$ and a tendency when $0.05 \leq P \leq 0.10$.

RESULTS

Confirmation of Challenge Model and Serum Parameters

At necropsy, PRRS serology diagnostics by serum PCR and ELISA confirmed that all PRRS+ pigs had been successfully inoculated and that Ad and PF pigs remained PRRS naïve (Table 1). For serum PRRS viral PCR Ct values, there was a treatment by dpi interaction ($P = 0.005$) in which PRRS+ pigs had lower Ct values than Ad and PF pigs at dpi 10 (19.8, 37.0, and 37.0, respectively; $P < 0.001$) and at dpi 17 (22.8, 37.0, and 37.0, respectively; $P < 0.001$). PRRS viral PCR Ct values did not differ between Ad and PF pigs at either time point ($P > 0.10$), but Ct values were lower in serum from PRRS+ pigs necropsied at dpi 10 compared with PRRS+ pigs necropsied at dpi 17 ($P < 0.001$; Table 1). Similarly, there was a treatment by dpi interaction ($P = 0.010$) for serum PRRS antibody titers. Antibody titers were increased in PRRS+ pigs compared with Ad and PF pigs at dpi 10 ($P < 0.001$) and dpi 17 ($P < 0.001$). Furthermore, PRRS antibody titers were lower PRRS+ pigs at dpi 10 than at dpi 17 (1.01 vs 1.41; $P < 0.001$). Taken together, this indicates that the 2 necropsy

dates corresponded to the predicted viremic and seroconversion time points in the PRRS immune response.

Serum glucose concentrations were significantly altered by treatment ($P = 0.041$; Table 1). Regardless of dpi, serum glucose concentrations were greater in PF pigs compared with PRRS+ pigs ($P = 0.017$) and tended to be greater in PF pigs compared with Ad pigs ($P = 0.059$); however, serum glucose concentrations did not differ between Ad and PRRS+ pigs ($P > 0.10$). There was a significant treatment by dpi interaction ($P = 0.025$; Table 1) for serum NEFA concentrations. Serum NEFA concentrations were greater in PRRS+ pigs compared with Ad ($P < 0.001$) and PF ($P < 0.001$) at dpi 10 but did not differ amongst treatment groups ($P > 0.10$) at dpi 17. There was an overall treatment effect for serum BUN concentrations ($P < 0.012$; Table 1) in which BUN concentrations were reduced in PRRS+ ($P < 0.006$) and Ad ($P < 0.017$) pigs compared with PF pigs regardless of dpi; serum BUN did not differ ($P > 0.10$) between PRRS+ and Ad pigs. Serum insulin concentrations reported a significant treatment by dpi interaction ($P < 0.001$), as insulin concentrations did not differ ($P > 0.10$) at dpi 10, but at dpi 17 insulin concentrations were greater in PRRS+ pigs compared with both PF ($P < 0.001$) and Ad ($P < 0.023$) pigs and greater in PF pigs compared with Ad pigs ($P = 0.001$). Glucagon concentrations were greater at dpi 17 than at dpi 10 ($P = 0.002$) but did not differ amongst treatments. C-reactive protein was significantly affected by treatment ($P < 0.001$), and by dpi ($P = 0.024$), but the interaction was not significant.

Table 1. Viremia and antibody serology¹

Item	dpi 10			dpi 17			SEM	P-value		
	Ad	PF	PRRS+	Ad	PF	PRRS+		Trt	dpi	Trt*dpi
PRRS viremia, Ct ²	37.0 ^a	37.0 ^a	19.8 ^b	37.0 ^a	37.0 ^a	22.8 ^c	0.531	<0.001	0.017	0.005
PRRS antibody, S:P ³	-0.04 ^a	-0.05	1.01 ^b	-0.06 ^a	-0.06 ^a	1.41 ^c	0.079	<0.001	0.047	0.010
Glucose, mg/dL	134 ^{ab}	188 ^a	129 ^b	146 ^{ab}	168 ^a	130 ^b	22.02	0.041	0.891	0.711
NEFA, mmol/dL	0.03 ^b	0.05 ^b	0.15 ^a	0.02 ^b	0.00 ^b	0.04 ^b	0.021	<0.001	0.005	0.025
BUN, mg/dL ⁴	12.2 ^b	14.1 ^a	11.7 ^b	10.9 ^b	14.3 ^a	10.23 ^b	1.207	0.012	0.339	0.674
Insulin, pg/mL	292 ^{cd}	272 ^d	267 ^d	793 ^b	470 ^c	1030 ^a	77.85	0.001	<0.001	0.001
Glucagon, pg/mL	197 ^a	174 ^a	217 ^a	610 ^b	418 ^b	590 ^b	97.60	0.232	0.002	0.177
CRP, µg/mL ⁵	139 ^c	28 ^c	472 ^a	318 ^b	99 ^c	535 ^a	48.40	<0.001	0.024	0.365
Haptoglobin, mg/mL	1.07 ^b	2.79 ^a	3.03 ^a	1.35 ^b	2.03 ^a	1.71 ^a	0.600	0.016	0.262	0.220

^{a-d}Means with differing superscripts indicate a significant ($P < 0.05$) difference.

¹Pigs were either challenged with porcine respiratory and reproductive syndrome virus (PRRS+), PRRS naïve and fed ad libitum (Ad), or PRRS naïve and pair-fed to PRRS+ pigs intake (PF). Pigs were euthanized at either days postinoculation (dpi) 10 or dpi 17.

²PRRS qPCR viremia Ct (cycle threshold) ≥ 37 denotes negative PRRS outcome.

³PRRS antibody S:P (sample: positive ratio) < 0.40 denotes negative PRRS outcome.

⁴BUN = blood urea nitrogen.

⁵CRP = C-reactive protein.

At dpi 10, CRP concentrations were greater in PRRS+ pigs compared with Ad ($P < 0.001$) and PF ($P < 0.001$) pigs, which did not differ from each other ($P = 0.10$). At dpi 17 CRP concentrations were greater in PRRS+ pigs compared with Ad ($P = 0.020$) and PF ($P < 0.001$) pigs, and CRP concentrations were greater in Ad pigs compared with PF pigs ($P = 0.001$). Haptoglobin was affected by treatment ($P = 0.016$) in which haptoglobin concentrations were lesser in Ad pigs compared with PF ($P < 0.010$) and PRRS+ ($P = 0.018$) pigs, which did not differ from each other, regardless of dpi.

Growth Performance

Initial body weights did not differ (13.3, 13.0, and 12.8 kg for Ad, PF, and PRRS+ pigs, respectively; $P > 0.10$) among treatments, but end body weights were reduced in PRRS+ and PF pigs compared with Ad pigs at both dpi 10 (18.9, 14.4, and 12.5 kg for Ad, PF, and PRRS+ pigs, respectively; $P < 0.001$) and dpi 17 (21.0, 15.0, and 13.0 kg for Ad, PF, and PRRS+ pigs, respectively; $P < 0.001$).

Growth performance parameters are described in Figure 1. There were no treatment by dpi interactions, nor significant effect of dpi, in any of the growth performance parameters ($P > 0.10$). Average daily gain was significantly affected by treatment ($P < 0.001$) in which ADG was reduced in PF (54%; $P = 0.001$) and PRRS+ (135%; $P < 0.001$) pigs compared with Ad pigs and was reduced in PRRS+ pigs (176%; $P < 0.001$) compared with PF pigs, regardless of dpi. Consistent with experimental design, ADFI was reduced ($P < 0.001$; Figure 1) in PF ($P < 0.001$) and PRRS+ ($P < 0.001$) pigs compared with Ad pigs at both time points but did not differ between PRRS+ and PF pigs ($P > 0.10$). Feed efficiency was significantly affected by treatment ($P < 0.001$), wherein G:F was reduced in PF (54%, $P = 0.001$) and PRRS+ (135%, $P < 0.001$) pigs compared with Ad pigs and was reduced in PRRS+ pigs (177%, $P < 0.001$) compared with PF pigs, regardless of dpi.

Skeletal Muscle Mitochondrial ROS and Oxidative Stress

Reactive oxygen species production was evaluated in mitochondria extracted from the LM as a marker for potential for oxidative stress and protein carbonyls were evaluated in the LM as a marker for oxidative damage. Neither mitochondrial ROS production nor protein carbonyl concentrations differed ($P > 0.10$; Table 2) as a result of treatment.

Figure 1. Pig performance. (A) Average daily gain (ADG), (B) average daily feed intake (ADFI), and (C) feed efficiency (G:F) in pigs challenged with porcine respiratory and reproductive syndrome virus (PRRS+), naïve and fed ad libitum (Ad), or naïve and paired to PRRS+ pigs intake (PF) selected for necropsy at either days postinoculation (dpi) 10 or dpi 17. Differing letters a, b, and c represent $P < 0.05$. $n = 8$ pigs per treatment per dpi.

Protein carbonyls concentrations did differ due to dpi, in which carbonyl concentrations were greater ($P < 0.001$; Table 2) at dpi 10 compared with dpi 17.

Skeletal Muscle Protein Degradation and Synthesis

Skeletal muscle protein degradation markers are presented in Table 2. Activity of μ -calpain did not differ ($P > 0.10$) as a result of treatment or dpi. There was a treatment by dpi interaction ($P = 0.003$) for m-calpain activity in which m-calpain activity did not differ ($P > 0.10$) amongst treatment groups at dpi 10. However, at dpi 17 m-calpain activity was greater in PRRS+ pigs compared with

Table 2. Longissimus muscle markers of proteolysis, oxidative stress, and energy balance¹

Item	dpi 10			dpi 17			SEM	P-value		
	Ad	PF	PRRS+	Ad	PF	PRRS+		Trt	dpi	Trt*dpi
Mitochondrial ROS ²	111	118	71	121	133	106	37.56	0.29	0.671	0.856
Protein carbonyls ³	4.18 ^a	4.75 ^a	4.26 ^a	1.77 ^b	1.67 ^b	1.70 ^b	0.477	0.775	<0.001	0.725
μ-calpain ⁴	0.69	0.70	0.77	0.69	0.53	0.82	0.121	0.256	0.656	0.539
m-calpain ⁴	1.92 ^{abc}	1.74 ^{bc}	1.77 ^{bc}	1.65 ^c	2.11 ^{abc}	2.19 ^a	0.160	0.368	0.129	0.033
Calpastatin I ⁴	0.63 ^b	0.47 ^b	0.53 ^b	0.67 ^b	0.54 ^b	1.08 ^a	0.130	0.041	0.025	0.059
Calpastatin II ⁴	0.62 ^{ab}	0.62 ^b	0.83 ^b	0.48 ^{ab}	0.61 ^b	0.90 ^a	0.175	0.045	0.414	0.564
Total calpastatin ⁴	1.25 ^a	1.09 ^a	1.65 ^b	1.16 ^a	0.89 ^a	1.99 ^b	0.241	0.001	0.928	0.413
20S proteasome ⁵	24.62 ^a	23.88 ^a	23.59 ^a	5.81 ^b	4.72 ^b	4.79 ^b	1.897	0.802	<0.001	0.993
Caspase 3/7 ⁶	60877 ^a	67425 ^a	63986 ^a	8683 ^b	10756 ^b	63986 ^b	3879	0.316	<0.001	0.542
ERMs ⁷	0.15	0.11	0.12	0.14	0.14	0.13	0.015	0.232	0.464	0.361
Glucose ⁸	0.34 ^{ab}	0.31 ^b	0.22 ^c	0.37 ^a	0.37 ^a	0.29 ^b	0.019	<0.001	0.001	0.474
Glycogen ⁹	3.76	2.57	3.37	3.88	3.72	2.72	0.565	0.298	0.641	0.257

^{a-c}Means with differing superscripts indicate a significant ($P < 0.05$) difference.

¹Pigs were either challenged with porcine respiratory and reproductive syndrome virus (PRRS+), PRRS naïve and fed ad libitum (Ad), or PRRS naïve and pair-fed to PRRS+ pigs intake (PF). Pigs were euthanized at either days post inoculation (dpi) 10 or dpi 17.

²Basal reactive oxygen species (ROS) production from isolated mitochondria as H2O2 produced, μmol/min/mg protein when glutamate is provided as a substrate.

³nmol protein carbonyls/mg protein.

⁴Units of activity/g tissue.

⁵Activity measured as μM of released fluorescent 7-Amino-4-methylcoumarin (AMC) from LLVY-AMC per mg protein.

⁶Change in RFU/min/mg protein.

⁷Easily releasable myofilaments (ERMs) as a % of crude myofibrillar protein.

⁸mM/g tissue.

⁹Liberated glucose, mM/g tissue.

Ad pigs ($P = 0.025$) and tended to be greater in PF pigs compared with Ad pigs ($P = 0.05$). Skeletal muscle m-calpain activity did not differ between PRRS+ and PF pigs ($P > 0.10$) at dpi 17. There was a tendency for a treatment by dpi interaction ($P = 0.059$) for the activity of calpastatin I, in which calpastatin I activity did not differ ($P > 0.10$) amongst treatments at dpi 10; however, calpastatin I activity was increased in PRRS+ pigs compared with Ad ($P = 0.031$) and PF ($P = 0.006$) pigs at dpi 17. Activity of calpastatin II was significantly affected by treatment ($P = 0.045$), driven by PRRS+ pigs having increased activity of calpastatin II compared with PF pigs (0.012) regardless of dpi. As changes to both calpastatin isoforms were observed as a result of treatment, total calpastatin activity was significantly affected by treatment ($P < 0.001$). Regardless of dpi, total calpastatin activity was greater in PRRS+ pigs compared with PF pigs ($P = 0.019$) and tended to be greater in PRRS+ pigs compared with Ad pigs ($P = 0.052$). Total calpastatin activity did not differ ($P > 0.10$) between Ad and PF pigs.

Activity of the 20S proteasome and caspase 3/7 did not differ ($P > 0.10$) as a result of treatment; however, activity of both enzymes was significantly affected by dpi. Activities of both the

20S proteasome and caspase 3/7 were reduced ($P < 0.001$) at dpi 17 compared with dpi 10, regardless of treatment. Concentrations of ERMs did not differ ($P > 0.10$) as a result of treatment or dpi.

Total protein abundance of S6K1, 4E-BP1, and AMPK did not differ among treatments at either timepoint (data not shown). There was a highly significant treatment by dpi interaction ($P = 0.006$; [Figure 2](#)) for the ratio of phosphorylated to total S6K1. At dpi 10, pS6K:S6K was greater in Ad pigs compared with PRRS+ (96%, $P < 0.001$) and PF (64%, $P = 0.002$) pigs, and pS6K:S6K tended to be reduced in PRRS+ pigs compared with PF pigs (19%, $P = 0.092$).

However, pS6K1:S6K1 did not differ ($P > 0.10$; [Figure 2](#)) among treatments at dpi 17. There was a tendency for a treatment by dpi interaction ($P = 0.057$) for the ratio of p4E-BP1:4E-BP1. At dpi 10, p4E-BP1:4E-BP1 was greater in PF pigs compared with PRRS+ (50%, $P = 0.014$) and Ad (33%, $P = 0.048$), whereas PRRS+ and Ad pigs did not differ from each other ($P > 0.10$). At dpi 17, p4E-BP1:4E-BP1 was greater in Ad pigs compared with PRRS+ pigs (51%, $P = 0.010$), with PF pigs intermediate and not differing from either of the treatments. The ratio of phosphorylated to total AMPK did not differ ($P > 0.10$; [Figure 2](#)) as a result of treatment or dpi.

Figure 2. Relative LM protein abundances of phosphorylated to total (A) S6K1, (B) 4E-BP1, and (C) AMPK in pigs challenged with porcine respiratory and reproductive syndrome virus (PRRS+), naïve and fed ad libitum (Ad), or naïve and pair-fed to PRRS+ pigs intake (PF) at days postinoculation (dpi) 10 and 17. Differing letters a, b, c, and d represent $P < 0.05$.

Table 3. Liver metabolic profile¹

Item	dpi 10			dpi 17			SEM	P-value		
	Ad	PF	PRRS+	Ad	PF	PRRS+		Trt	dpi	Trt*dpi
Gluconeogenesis										
G6P ²	0.46 ^a	0.35 ^b	0.37 ^b	0.42 ^a	0.35 ^b	0.26 ^b	0.032	0.001	0.128	0.172
F16BP ³	2.62 ^a	2.15 ^{ab}	1.36 ^{bc}	1.70 ^{bc}	2.89 ^a	0.86 ^c	0.298	<0.001	0.359	0.021
PEPCK ⁴	2.02	2.04	1.94	1.93	1.96	2.16	0.104	0.731	0.87	0.185
Glucose ⁵	3.30 ^a	3.39 ^a	2.28 ^b	1.05 ^c	1.23 ^c	0.81 ^c	0.261	0.012	<0.001	0.270
Glycogen ⁶	20.0 ^a	32.0 ^b	3.5 ^c	30.18 ^b	33.7 ^b	10.4 ^c	3.425	<0.001	0.067	0.355

^{a-c}Means with differing superscripts indicate a significant ($P < 0.05$) difference.

¹Pigs were either challenged with porcine respiratory and reproductive syndrome virus (PRRS+), PRRS naïve and fed ad libitum (Ad), or PRRS naïve and pair-fed to PRRS+ pigs intake (PF). Pigs were euthanized at either days postinoculation (dpi) 10 or dpi 17.

²Glucose-6-phosphatase activity; Pi, mM/min/mg protein.

³Fructose-1,6-bisphosphatase activity; NADPH, μ mol/min/mg protein.

⁴Phosphoenolpyruvate carboxykinase activity; ATP, μ M/min/mg protein.

⁵mM/g tissue.

⁶Liberated glucose, mM/g tissue.

LM Glycogen Balance

There were no treatment by dpi interactions ($P > 0.10$; Table 3) for either glucose or glycogen concentrations in the LM. Glucose concentrations were

decreased ($P < 0.05$) in PRRS+ pigs compared with Ad (27%, $P < 0.001$) and PF pigs (23%, $P < 0.001$), which did not differ from each other, regardless of dpi. There was also a dpi effect in which LM glucose concentrations were lesser ($P = 0.001$) at dpi

10 compared with dpi 17. Glycogen concentrations did not differ ($P > 0.10$) due to treatment or dpi.

Liver Gluconeogenesis and Glycogen Balance

Liver gluconeogenic enzyme activities and glycogen concentrations are presented in Table 3. The interaction of treatment by dpi was not significant for activity of G6P; however, activity of G6P was significantly different among treatments ($P = 0.001$). Regardless of dpi, activity of G6P was decreased in PRRS+ ($P < 0.001$) and PF ($P = 0.003$) pigs compared with Ad pigs. Activity of G6P did not differ ($P > 0.10$) between PRRS+ and PF pigs. There was a significant treatment by time interaction ($P = 0.021$) for activity of F16BP. At dpi 10, activity of F16BP was reduced in PRRS+ pigs ($P = 0.005$) compared with Ad pigs, tended to be reduced in PRRS+ pigs ($P = 0.005$) compared with PF pigs, and did not differ ($P > 0.10$) between PRRS+ and PF pigs. However, at dpi 17, F16BP activity tended to be reduced in PRRS+ pigs ($P = 0.055$) compared with Ad pigs, and was increased in PF pigs compared with Ad ($P < 0.001$) and PRRS+ ($P < 0.001$) pigs. Activity of PEPCK did not differ ($P > 0.10$) due to treatment or dpi.

There were no treatment by time interactions for either liver glucose or liver glycogen. Liver glucose concentrations were decreased in PRRS+ pigs compared with Ad (31%, $P = 0.008$) and PF (33%, $P = 0.004$) pigs, which did not differ from each other, at dpi 10. However, glucose concentrations did not differ amongst treatments at dpi 17. Regardless of treatment, glucose concentrations were lesser ($P < 0.001$) at dpi 17 compared with dpi 10. Regardless of dpi, liver glycogen concentrations were decreased in PRRS+ pigs compared with Ad (72%, $P < 0.001$) and PF (79%, $P < 0.001$) pigs, which did not differ from another. Regardless of treatment, liver glycogen concentrations tended to be greater ($P = 0.067$) at dpi 17 compared with dpi 10.

DISCUSSION

Achieving optimum accretion of skeletal muscle is a major goal of swine producers, which is dependent on maximizing the rate of skeletal muscle protein synthesis while minimizing the rate of skeletal muscle proteolysis. However, pathogen challenges antagonize growth performance, threatening achievement of this goal, and lead to losses in lean tissue accretion and feed efficiency that can ultimately affect time to market body

weight (Schweer et al., 2017). Losses in growth performance during pathogen challenges such as PRRS virus are the combined impact of indirect effects of disease anorexia and direct effects of supporting an immune response, although individual contributions of each are not well defined (Klasing et al., 1987; Klasing and Johnstone, 1991). As skeletal muscle composes 60% to 70% of lean tissue mass, it is one of the largest sources of both amino acids and energy substrates that could be used by the body in times of need (Frost and Lang, 2008). It has been widely hypothesized that the rate of skeletal muscle myofibrillar protein degradation is enhanced during pathogen challenges in order to provide the body with amino acids (Lochmiller and Deerenberg, 2000). These amino acids can be used to produce immune system components such as acute phase proteins (Reeds et al., 1994) or can be converted into energy via gluconeogenesis to support immune system energy demands (Bruins et al., 2003). However, the extent to which this occurs in growing pigs during viral challenges has been poorly defined.

In previous studies utilizing live pathogen challenges in pigs, our group has been unable to find evidence of enhanced skeletal muscle proteolysis during enteric viral (Curry et al., 2018) or respiratory and enteric bacterial- (Helm et al., 2018) challenged pigs. However, it is important to note that both of these studies reported mild clinical or sub-clinical disease, leaving it unclear if skeletal muscle proteolysis mechanisms only need to be utilized in cases of severe pathogen challenge and/or nutrient restriction. To address this, a PRRS virus strain that consistently produces severe morbidity and mortality (Cornelison et al., 2018) was chosen and a pair-feeding model was employed to differentiate effects of nutrient restriction from viral challenge.

In growing pigs, PRRS virus challenge has been shown to reduce pig ADG by 30% to 50% over a 14–21 dpi period (Escobar et al., 2004; Rochell et al., 2015; Schweer et al., 2016). In the current study, both PRRS+ and PF pigs had severely reduced ADG compared with Ad pigs. Pair-fed pigs had a 50% to 60% reduction in ADG compared with Ad pigs, and PRRS+ pigs in both necropsy groups lost weight from dpi 0 to the point of necropsy. Further, PRRS+ pigs had reductions in ADG compared with PF pigs, indicating that losses in growth performance during this PRRS viral challenge were not fully driven by reductions in feed intake. This is also supported by the observation that G:F was reduced in PRRS+ pigs compared with PF pigs at both dpi 10 and 17. Thus, this viral challenge

resulted in severe reductions in growth performance parameters, making it an excellent model to examine what alterations in skeletal muscle metabolism are utilized to fuel the immune response and how those alterations contribute to losses in lean tissue accretion.

Lean tissue accretion is governed by the independent processes of protein synthesis and degradation. If degradation processes occur at a faster rate than synthesis processes, muscle atrophy occurs (Smith et al., 2008). Intracellular protein degradation is primarily controlled by the lysosomal-autophagy, caspase, calpain-calpastatin, and ubiquitin-proteasome systems. The lysosomal system is not readily present within skeletal muscle fibers; thus, it likely is not a large contributor to myofibrillar turnover (Wildenthal et al., 1980; Lowell et al., 1986). Caspase-3, involved in apoptosis of damaged cells (Goll et al., 2008) and destruction of muscle fibers for further degradation, is activated during acute lipopolysaccharide (LPS) inflammation challenge in neonatal pigs (Orellana et al., 2012). In the current experiment, we did not observe any increase in skeletal muscle Caspase-3 activity as a result of PRRS virus challenge. Caspase-3 is thought to be triggered by inflammation and tissue damage as a result of excess ROS production, and LPS has been demonstrated to cause an increase in murine myotubule mitochondrial ROS production (Hansen et al., 2015). Herein, we also reported no increase in mitochondrial ROS production, or protein damage as a result of oxidative stress in the LM of PRRS+ pigs at either time point. These data are in agreement with Seelenbinder et al., (2018) who reported no differences in mitochondrial ROS production due to PRRS virus infection. Therefore, PRRS viral challenge may not cause the same skeletal muscle inflammatory stress as LPS inflammation, and there is no trigger to activate caspase induced skeletal muscle proteolysis.

Although Caspase-3 plays a role in myofibrillar protein degradation, the primary mechanism by which myofibrillar protein degradation occurs is via the calpain and ubiquitin-proteasome systems, which work together to break muscle fibers down into amino acids that can be released into circulation (Goll et al., 2008). The abundance of ubiquitin proteasome components is increased in a mouse model of acute and chronic sepsis induced by *Escherichia coli* challenge when compared with pair-fed controls (Voisin et al., 1996). In the current experiment, no increase in skeletal muscle μ -calpain or 20S proteasome activities was observed as a

result of PRRS challenge, despite severe reductions in growth performance. Skeletal muscle m-calpain did show increased activity in PRRS+ pigs; however, this was only observed at dpi 17. Serum BUN concentrations were also not elevated in PRRS+ or PF pigs compared with Ad pigs, consistent with what others have observed during PRRS challenge (Seelenbinder et al., 2018), but contradicts data from LPS-challenged pigs (Webel et al., 1997). In addition to having limited increases in proteolytic activity, PRRS+ pigs had potential for greater calpain inhibition with increased calpastatin I activity compared with Ad pigs at dpi 17 and increased total calpastatin activity compared with Ad and PF pigs at both time points. This suggests that a skeletal muscle saving mechanism is occurring under PRRS challenge which does not occur during nutrient restriction alone, and likely more than compensates for the changes observed in m-calpain activity at dpi 17. Conservation of skeletal muscle protein has been observed previously (Curry et al., 2018; Helm et al., 2018). Skeletal muscle turnover is energetically costly, thus slowing protein turnover may be a mechanism to conserve energy expenditure and body resources (Herd and Arthur, 2009). Furthermore, as skeletal muscle conservation only occurred in PRRS+ pigs, this may be a form of disease tolerance designed to aid survivability from pathogen challenge (Medzhitov et al., 2012).

Although markers of degradation were not significantly altered in PRRS+ pigs at dpi 10 and 17, a reduction in skeletal muscle protein synthesis would also contribute to a reduction in lean tissue accretion. The major pathway by which skeletal muscle protein synthesis occurs is via the mammalian target of rapamycin (mTOR) pathway, which acts as a primary mediator and activator of protein translation initiation. Phosphorylation of mTOR, which can be controlled via several mechanisms such as insulin and amino acid availability (Preedy and Garlick, 1986), activates a signaling cascade to increase translation initiation and protein synthesis. This signaling cascade includes phosphorylation and inactivation of 4E-BP1, an mRNA translation suppressor, and phosphorylation and activation of S6K1, an mRNA translation enhancer (Richter and Sonenberg, 2005). A decrease in phosphorylation of skeletal muscle protein translation initiation markers such as 4E-BP1 or S6K1 is indicative of a reduction in skeletal muscle protein synthesis rate. Reductions in phosphorylation of 4E-BP1 and S6K have been reported in LPS models of acute (8–12 h challenge) endotoxemia using neonatal pigs in both fasted and fed states (Kimball et al., 2003;

Orellana et al., 2004). However, these translation initiation factors did remain sensitive to activation by amino acids, suggesting nutrient supply does play a role in phosphorylation of these enzymes (Orellana et al., 2007). In support of this body of work, we observed phosphorylation of S6K1 to be decreased 96% in PRRS+ pigs compared with Ad pigs at dpi 10, and phosphorylation of 4E-BP1 to be decreased in PRRS+ pigs compared with Ad pigs at dpi 17. Furthermore, PRRS+ pigs had more severe decreases in phosphorylation of translation initiation markers than PF pigs. This suggests that changes in skeletal muscle protein synthesis were driven partially by reduced feed intake, but there is an additional reduction in skeletal muscle protein synthesis as a result of PRRS challenge. Reductions in protein synthesis, coupled with no or limited changes to the rate of protein degradation, would decrease lean tissue accretion and in the most severe cases even result in a net loss of skeletal muscle protein. Furthermore, it would allow dietary amino acids to be reallocated away from growth and towards fueling the immune response.

There is still much unknown about the response of the immune system to PRRS virus, but viral clearance requires eventual recruitment and activation of B and T cells for antibody secretion and removal of virally infected cells, although this is hindered in PRRS challenges due to the lack of an adequate interferon- α response (Loving et al., 2015). Synthesis of these immune cells and noncellular immune components such as acute phase proteins demands both free amino acids and energy. Additionally, once activated, immune cells have a high demand for glucose as an energy substrate (Calder et al., 2007; Kvidera et al., 2017), likely exceeding that which is consumed via the diet. Energy expenditure is also increased in the presence of fever, which is observed during PRRS challenge (Greiner et al., 2001a, b). High demands for glucose and other energy substrates comes at a time when pigs have a reduced nutrient supply, putting further metabolic strain on the body. Despite this, we observed no differences in serum glucose concentrations due to PRRS, indicating that PRRS+ pigs were able to maintain circulating glucose homeostasis despite reductions in nutrient intake.

One source of energy pigs may be using to maintain glucose homeostasis is glycogen reserves. Glycogen is stored primarily in skeletal muscle and liver; however, only liver glycogen can be released directly into the blood (Magnusson et al., 1992; Jensen et al., 2011). In the current experiment, we observed dramatic liver glycogen reductions in

PRRS+ pigs compared with both Ad and PF pigs. Pair-fed pigs had no reduction in liver glycogen concentrations, so it appears liver glycogen is degraded and mobilized to replenish circulating glucose that is being utilized in the immune response. Glycogenolysis is the preferred method of glucose generation following acute LPS challenge in dogs (Meinz et al., 1998); thus, liver glycogen stores may preferentially be used in PRRS+ pigs to fuel the immune system. After depletion of glycogen stores, other hepatic glucose production systems such as gluconeogenesis may be needed to produce additional glucose for the immune response. However, markers of hepatic gluconeogenesis did not show increased activity due to PRRS challenge. In fact, PRRS+ pigs had decreased activity of G6P and F16BP compared with Ad pigs at dpi 10. These data are supported by Seelenbinder et al. (2018), who observed no change in PEPCK mRNA abundance after a 14-d PRRS challenge. Activity of gluconeogenesis is determined by availability of gluconeogenic substrates to the liver as well as its ability to metabolize those substrates (McGuinness, 2005). It is possible that a lack of substrates such as amino acids, due to limited evidence of skeletal muscle proteolysis and mobilization of amino acids, being provided to liver eventually resulted in the observed reduction in gluconeogenic activity in PRRS+ pigs. It is also possible that glycogen provided pigs with sufficient glucose, and that additional energy may be provided via lipolysis and mobilization of adipose stores to release fatty acids. Although lipolysis was not directly measured, PRRS+ pigs had increased serum NEFA concentrations at dpi 10 compared with both Ad and PF pigs. This is consistent with what we have observed previously in PRRS and PED dual-challenged pigs (Schweer et al., 2016) and suggests that lipolysis may also be providing substrates for ATP production to support the immune system when energy demands exceed that which can be provided as glucose via hepatic glycogen reserves.

Taken together, this work demonstrates the impact of a severe PRRS challenge on the growth performance and skeletal muscle metabolism of growing pigs. Although a large portion of the reduction in growth performance can be attributed to reductions in feed intake, PRRS+ pigs had additional losses in growth and feed efficiency compared with that of feed restricted pigs, indicating the additional cost of immune activation. These changes reflect differences in post absorptive metabolism in PRRS+ pigs, such as increases in liver glycogenolysis, which can be attributed to immune

system energy demands and maintaining glucose homeostasis. Additionally, it appears that even during severe viral challenge (where pigs are in weight stasis or losing weight), skeletal muscle proteolysis shows limited upregulation, and reductions in whole body lean tissue accretion are primarily due to reduced activation of protein synthesis pathways, which may allow for reallocation of dietary amino acids towards producing and providing energy for immune system components. Although further investigation is needed to determine strategies to specifically provide for the immune response and mitigate the additional cost of immune activation, it does appear that calorie consumption explains a significant portion of lost lean tissue accretion and finding strategies to increase calorie consumption may prove beneficial in managing PRRS-challenged pigs.

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