# Porcine epidemic diarrhea virus reduces feed efficiency in nursery pigs

**S. M. Curry,[†] E. R. Burrough,[‡] K. J. Schwartz,[‡] K. J. Yoon,[‡] S. M. Lonergan,[†] and N. K. Gabler[†1]**

[†]Department of Animal Science, Iowa State University, Ames, IA 50011; [‡]Department of Veterinary Diagnostic and Production Animal Medicine, Iowa State University, Ames, IA 50011

**ABSTRACT:** Porcine epidemic diarrhea virus (**PEDV**) infects enterocytes and in nursery pigs, results in diarrhea, anorexia, and reduced performance. Therefore, the objective of this study was to determine how PEDV infection influenced growth performance and repartitioning of amino acids and energy in nursery pigs. A total of 32 barrows and gilts, approximately 1 wk post-wean (BW = 8.46 ± 0.50 kg), and naïve for PEDV were obtained, weighed, and allotted based on sex and BW to one of two treatments: 1) Control, PEDV naïve and 2) PEDV-inoculated (PEDV) with eight pens of two pigs each per treatment. On day post-inoculation (**dpi**) 0, PEDV pigs were inoculated via intragastric gavage with PEDV isolate (USA/Iowa/18984/2013). Pig and feeder weights were recorded at dpi −7, 0, 5, and 20 in order to calculate ADG, ADFI, and G:F. Eight pigs per treatment were euthanized on dpi 5 and 20, and tissues and blood were collected. At dpi 5, all PEDV pigs were PCR positive for PEDV in feces. Overall, PEDV pigs tended ($P < 0.10$) to increase ADFI, which resulted in reduced ($P < 0.05$) feed efficiency. At dpi 5, PEDV pigs had reduced ($P < 0.05$) villus height and increased ($P < 0.05$) stem cell proliferation in the jejunum compared with Control pigs. Pigs inoculated with PEDV had increased ($P < 0.05$) serum haptoglobin and increased insulin-to-glucose ratios compared with Control pigs at dpi 5. Markers of muscle proteolysis were not different ($P > 0.05$) between treatments within dpi; however, at dpi 5, 20S proteasome activity was increased ($P < 0.05$) in longissimus dorsi of PEDV pigs compared with Control pigs. Liver and jejunum gluconeogenic enzyme activities were not different ($P > 0.05$) between treatments within dpi. Overall, PEDV-inoculated pigs did recover the absorptive capacity that was reduced during PEDV infection by increasing proliferation of intestinal stem cells. However, the energy and nutrients needed to recover the epithelium may be originating from available luminal nutrients instead of muscle proteolysis and gluconeogenesis. This study provides insight into the effects of an enteric coronavirus on postabsorptive metabolism in nursery pigs.

**Key words:** feed efficiency, gluconeogenesis, metabolism, pig, porcine epidemic diarrhea virus, skeletal muscle



J. Anim. Sci. 2018.96:85–97
doi: 10.1093/jas/skx005

Pathogen challenges can antagonize animal health and performance, and are associated with allocation of nutrients and energy away from growth (Scrimshaw, 1977; Davies, 2012). Interestingly, improving feed intake during such challenges may not be advantageous as mice infected with *Listeria monocytogenes* that were force-fed to the energy intake of naïve counterpart's experienced 93% mortality (Murray and Murray, 1979). Although that study may not be directly translatable to pigs or other pathogen infections, there appears to be some advantage for the host to reduce feed intake. Unfortunately, for production animals, reduced feed intake results

Funding for this research was provided by the Agriculture and Food Research Initiative Competitive Grant no. 2016-67015-24574 from the USDA National Institute of Food and Agriculture.

[1]Corresponding author: ngabler@iastate.edu

Received July 6, 2017.
Accepted November 28, 2017.

in decreased gain and ultimately increases days to reach a final market BW.

Porcine epidemic diarrhea virus (**PEDV**), of the family Coronaviridae (Woo et al., 2012), infects enterocytes and in nursery pigs, infection results in clinical signs of diarrhea, dehydration, and anorexia (Stevenson et al., 2013). The pathology induced by this pathogen may result in altered nutrient and energy partitioning. Previous research in nursery pigs has demonstrated that PEDV challenge results in reduced growth performance (Kim and Lee, 2014; Schweer et al., 2015b; Curry et al., 2017a) and whole-body protein accretion rates compared with naïve counterparts (Curry et al., 2017a). However, the mechanism of action for this observation has not been determined. Muscle is the largest store of protein in the body; thus, changes in either protein degradation or protein synthesis during infection may result in reduced whole-body protein accretion. In addition, increased muscle protein turnover or degradation may provide AA that can be used for protein synthesis or energy production in other tissues. Rhoads et al. (2007) demonstrated that during peak rotavirus infection in pigs, protein translation initiation machinery were more abundant in the intestine compared with muscle of naïve counterparts. This demonstrates a reprioritization of energy and nutrients away from lean tissue growth to areas that are potentially damaged during infection and require resources to recover.

Therefore, the objective of this study was to determine how PEDV infection influenced growth performance and repartitioning of amino acids and energy in nursery pigs. We hypothesized that pigs inoculated with PEDV would exhibit decreased feed intake and growth, resulting in altered feed efficiency. This would drive energy and nutrients away from lean tissue growth toward immune activation and recovery of damaged intestinal tissue, such that muscle protein degradation and gluconeogenic enzyme activities in the liver and jejunum during peak infection and after recovery would differ between naïve and PEDV-infected nursery pigs.

## MATERIALS AND METHODS

All experimental protocols were reviewed and approved by the Institutional Animal Care and Use Committee at Iowa State University in Ames, IA (IACUC# 12-15-8147-S).

### *Animal Housing, Experimental Design, and Diets*

A total of 32 (Genetiporc 6.0 × Genetiporc F25 genetics; PIC, Inc., Hendersonville, TN) barrows and gilts 4 wks of age, approximately 1 wk post-wean (BW = 8.46 ± 0.50 kg), were obtained from a PEDV negative herd, weighed, and allotted based on sex and BW to one of two treatments: 1) Control, PEDV naïve and 2) PEDV-inoculated (PEDV). Each treatment was represented by eight pens with two pigs each. Pigs were housed in two rooms at the Livestock Infectious Disease Isolation Facility (biosecurity level 2) at Iowa State University (Ames, IA) with identical thermal environmental control, penning, and feeders, and appropriate biocontainment to prevent cross-infection.

All pigs had ad libitum access to a corn–soybean meal–based diet (Table 1) that met or exceeded the NRC (2012) requirements for nutrients and energy for this size pig, and all pigs had free access to water. Pig and feeder weights were recorded at day post-inoculation (**dpi**) −7, 0, 5, and 20 in order to calculate ADG, ADFI, and G:F.

### *Inoculation and Sample Collection*

On dpi 0, PEDV pigs were inoculated via intragastric gavage with 5 mL of $10^3$ tissue culture infectious dose $(TCID)_{50}$/mL of PEDV isolate (USA/Iowa/18984/2013) as previously described (Hoang et al., 2013; Madson et al., 2014). Fecal swabs were

**Table 1.** Diet composition, as-fed basis

| Ingredient | %, as-fed |
|---|---|
| Corn | 50.76 |
| Soybean meal, 48% CP | 19.00 |
| Soybean oil | 1.73 |
| Fishmeal, menhaden | 4.50 |
| Limestone | 0.35 |
| Whey, dried | 20.00 |
| Meat and bone meal | 2.04 |
| L-Lysine HCL | 0.43 |
| DL-Methionine | 0.16 |
| L-Threonine | 0.13 |
| L-Tryptophan | 0.03 |
| L-Valine | 0.07 |
| Salt | 0.40 |
| Vitamin–mineral premix[1] | |
| Calculated composition | |
| ME, kcal/kg | 3,408 |
| SID Lys, % | 1.35 |
| STTD P, % | 0.40 |

SID, standardized ileal digestibility; STTD, standardized total tract digestibility.

[1]Premix supplied (per kg 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.

taken on dpi 0, 2, 5, 10, 15, and 20 and submitted to Iowa State University Veterinary Diagnostic lab for quantitative real-time PCR (**RT-PCR)** of PEDV present in feces (Table 2) as described by Curry et al. (2017a). A cycle threshold (Ct) of <35 was considered positive for PEDV and ≥35 was considered negative. Blood was collected by jugular venipuncture from one randomly selected pig per pen in serum tubes (10 mL BD, Franklin, NJ) at dpi 5 and 20. The pig selected for blood collection was the pig that was euthanized at dpi 20. Serum was obtained by centrifugation at 2,000 × *g* for 10 min, aliquoted, and stored at −80 °C for later analysis.

On dpi 5 and 20, one pig per pen was euthanized by sodium pentobarbital overdose immediately followed by exsanguinated. Thereafter, tissues were harvested from each pig and processed immediately as described. Longissimus dorsi (**LD**) and liver samples were collected, trimmed, diced, snap-frozen in liquid nitrogen, and stored at −80 °C until later analysis. Whole jejunum sections were collected ~2 m prior to the ileal–cecal junction and flushed of luminal contents with Krebs–Henseleit buffer (**KB**; containing 25 mM $NaHCO_3$, 120 mM NaCl, 1 mM $MgSO_4$, 6.3 mM KCl, 2 mM $CaCl_2$, and 0.32 mM $NaH_2PO_4$, pH 7.4). Flushed jejunum sections were placed into 10% neutral buffered formalin (**NBF**) for 24 h and then into 70% ethanol for short-term storage until further processing. In addition, jejunum sections were cut longitudinally to expose the mucosa, which was harvested by scraping the length of the jejunum with a microscope slide. Mucosal scrapings were snap-frozen in liquid nitrogen and stored at −80 °C for later analysis.

### *Jejunum Histology*

To confirm the presence of PEDV in jejunum epithelium of pigs inoculated with PEDV, jejunum sections were collected in 10% NBF at 5 dpi, were paraffin-embedded into blocks, sectioned, and immunohistochemistry (**IHC**) was performed with PEDV antibody specific for the nucleoprotein of PEDV as described by Madson et al. (2014). In situ hybridization (**ISH**) was also performed using an oligonucleotide probe targeting the nucleocapsid (**N**) gene of PEDV (5′-TGTTGCCATTACCACGACTCCTGC-3′) obtained from a commercial vendor (Invitrogen Custom Oligos, Life Technologies, Carlsbad, CA) with a 5′ fluorescein label. The probe was reconstituted in a commercial hybridization buffer (BondTM Hybridization Solution, Leica Biosystems, Newcastle Upon Tyne, United Kingdom) at 5 ng/µL and the procedure was performed using a commercially available system (Leica Bond-III, Leica Biosystems, Melbourne, Australia). Tissue sections were dewaxed using a commercial dewaxing solution and then treated with a commercially available enzymatic pretreatment (BondTM Enzyme Pretreatment Kit, Leica Biosystems, Newcastle Upon Tyne, United Kingdom) for 5 min followed by five rinses with a commercial wash solution (BondTM Wash Solution, Leica Biosystems, Newcastle Upon Tyne, United Kingdom). The diluted probe was applied and allowed to hybridize at 45 °C for 12 h and then rinsed prior to incubation with an antifluorescein isothiocyanate (**FITC**) antibody for 30 min followed by application of a commercial chromagen system (Bond DAB Refine Kit, Leica Biosystems, Newcastle Upon Tyne, United Kingdom). A score for PEDV presence was assigned by a trained, blinded individual to each jejunum section with the following scoring system: 0 = no positive staining, 1 = <10% immunopositive enterocytes, 2 = 11% to 50% immunopositive enterocytes, and 3 = >50% immunopositive enterocytes, which has been used previously (Madson et al., 2013).

Jejunum sections were stained with hematoxylin and eosin, and images were obtained and analyzed

**Table 2.** PEDV detection in feces of pig inoculation with PEDV at dpi 2, 5, 10, 15, and 20[1]

| | Dpi | | | | | | |
|---|---|---|---|---|---|---|---|
| Parameter | 2 | 5 | 10 | 15 | 20 | SEM | *P*-value |
| Ct[2] | 29.15[a] | 21.01[b] | 31.52[a] | 32.69[a] | 33.50[a] | 1.826 | <0.001 |
| Positive/Total | | | | | | | |
| PCR[3] | 5/8 | 8/8 | 6/8 | 2/8 | 1/8 | – | – |
| ISH[4] | – | 7/8 | – | – | 0/8 | – | – |
| IHC[5] | – | 7/8 | – | – | 0/8 | – | – |

[1]Control pigs remained negative for PEDV throughout the duration of the study.

[2]Ct, cycle threshold. Ct value ≥35 is considered negative for PEDV.

[3]Positive pigs out of total pigs swabbed at that dpi. Pigs were considered positive for PEDV presence in feces when Ct <35.

[4]Positive pigs were those that PEDV nucleic acid was detected in the jejunum.

[5]Positive pigs were those that PEDV protein was detected in the jejunum.

[a,b]Means without a common superscript differ ($P \leq 0.05$).

as previously described (Curry et al., 2017b) in order to evaluate jejunum morphology. On the same sections, lesion scores were assigned based on villus atrophy and fusion and immune cell infiltrates. The scoring system was as follows: 0 = normal, 1 = mild villus blunting and rare fusion, 2 = moderate villus blunting and fusion with lymphoid infiltration, and 3 = severe blunting and fusion with lymphoid infiltration.

An anti-Ki67 antibody (Dako, Glostrup, Denmark) was used to detect proliferating stem cells in the jejunal crypts (Jung et al., 2015). Primary antibody was added at 1:500 (v/v) and mouse-on-Farma horseradish peroxidase (**HRP**)-Polymer (Biocare Medical, Concord, CA) was used as secondary. Staining conditions and analysis are as described elsewhere (Curry et al., 2017b).

### *Serum Analysis*

Serum was analyzed in duplicate for glucagon concentration using commercially available ELISA kit (DuoSet Glucagon, catalog number DY1249, R&D Systems, Minneapolis, MN). Serum glucose concentration was measured in duplicate using glucose oxidase/peroxidase reagent with O-dianisidine (Sigma-Aldrich, St. Louis, MO) and a glucose standard (Sigma-Aldrich, St. Louis, MO). Insulin concentration was measured in duplicate using commercially available Human/Canine/Porcine Insulin Quantikine ELISA Kit (catalog number DINS00, R&D Systems, Minneapolis, MN). Insulin-to-glucose ratio was calculated by total mol insulin to total mol of glucose per liter of serum. Nonesterified fatty acid (**NEFA**) concentration [NEFA-HR(2), Wako Chemical USA Inc., Richmond, VA] and blood urea nitrogen (**BUN**) concentration (BioAssay Systems, Hayward, CA) in serum were measured in duplicate per manufacturer's instructions. Serum haptoglobin concentrations were analyzed in duplicate using commercial ELISA kit (ALPCO diagnostics, Salem, NH). A Synergy 4 plate reader using Gen 5 software (BioTek Instruments Inc., Winooski, VT) was used to read all assays.

### *LD Proteolysis Markers*

Protein from snap-frozen LD was collected after homogenization in HEPES buffer (50 mM HEPES, 150 mM NaCl, 50 mM NaF, 20 mM EDTA, 5% glycerol, 1% Triton-X 100, and 0.1% SDS) and centrifugation at 2,000 × g for 10 min at 4 °C. Equivalent protein concentrations (10 µg) were separated by 10% SDS polyacrylamide gel electrophoresis (**SDS-PAGE**). Gels were run under reducing conditions, transferred to a nitrocellulose membrane and blocked for 1 h in 5% (w/v) non-fat dry milk (**NFDM**) prepared in Tris-buffered saline (**TBS**; 20 mM Tris-base and 150 mM NaCl, pH 7.4) with 0.1% Tween-20 (**TBST**). Primary antibody for calpastatin (catalog number 2G11D6, ThermoFisher Scientific, Waltham, MA) or µ-calpain (catalog number MA3-942, ThermoFisher Scientific, Waltham, MA) was diluted 1:1,000 or 1:250, respectively, in TBST with 5% NFDM and then applied to the membrane for an overnight incubation. Membranes were washed with TBST for three times for 10 min each. Secondary antibody was added at 1:1,000 or 1:2,500, respectively, in TBST with 2.5% NFDM and incubated for 90 min at 4 °C before washing as described previously. 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). Calpastatin abundance was expressed as arbitrary units relative to dpi 5 Control pigs. The proportions of the µ-calpain catalytic subunit present as the intact 80 kDa band and the autolyzed 78 and 76 kilodalton bands were calculated (Bee et al., 2007). Figure 1 illustrates the separation of µ-calpain bands.

Myofibrillar protein and easily releasable myofilaments (**ERMs**) were extracted from LD as previously described (Neti, 2009) and determined in triplicates. Protein concentration in crude myofibril and ERMs were determined using bicinochoninic acid (**BCA**) protein analysis. Percent of ERMs was calculated as a percent of crude myofibrillar protein.

Total RNA was isolated from LD muscle as previously described (Schweer et al., 2015a). Total protein was set relative to total RNA to assess protein synthesis efficiency, which has previously been published (Pringle et al., 1993). Both total protein and total RNA were corrected for differences in start tissue weight prior to efficiency calculation.

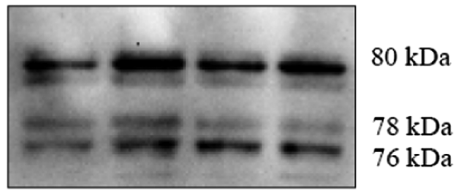


**Figure 1.** Western blot of intact µ-calpain illustrated by the 80-kilodalton band and autolyzed 78- and 76-kilodalton bands.

Protein extracted from LD in HEPES buffer as previously described was used to determine 20S proteasome activity (Cruzen et al., 2013). Activity of 20S proteasome was determined in duplicate using commercially available kit (catalog number APT280, EMD Millipore, Billerica, MA).

### *Gluconeogenic Enzyme Activities*

Protein from liver and jejunum mucosal scrapings was extracted in HEPES buffer as described above. Phosphoenolpyruvate carboxykinase (**PEPCK**) activity was measured in duplicate as described by Wimmer (1988) with modifications. The activity of PEPCK was calculated by subtracting endogenous ATP concentration in homogenates from ATP produced in the presence of oxaloacetate and thus a result of PEPCK. Luminometric determination of ATP was performed in a 96-well plate and fluorescence was measured at 538 nm. Assay mixture after the formation of ATP was diluted 10-fold and 100-fold for liver and jejunum mucosal scrapings, respectively, prior to luminometric determination of ATP. Fructose-1,6-bisphosphatase (**F1,6BP**) activity was measured in duplicate using protocol EC 3.1.3.11 (Sigma-Aldrich, St. Louis, MO) with modifications. In brief, test buffer contained 50 mM Tris–HCl (pH 8.5), 1.5 mM $MgCl_2$, 0.1 mM EDTA, 0.2 mM NADP, 0.1 mM fructose-1,6-phosphate, 0.83 U phosphoglucose isomerase, and 0.1 U glucose-6 phosphate dehydrogenase. Blank buffer contained test buffer without fructose-1,6-phosphate. Glucose-6 phosphatase (**G6P**) activity was measured in duplicate using protocol EC 3.1.3.9 (Sigma-Aldrich, St. Louis, MO) with modifications. After addition of 20% TCA, 96 well plates were centrifuged at 750 × *g* for 5 min at 25 °C. The resulting assay mixture was used for inorganic phosphorous determination using the molybdovanadate method (Ueda and Wada, 1970). These enzymes were chosen to be evaluated as they are enzymes necessary to overcome the irreversible steps of glycolysis and thus key enzymes of gluconeogenesis.

### *Statistical Analysis*

Pen was the experimental unit for growth performance, while pig was the experimental unit all other parameters, with fixed effects of dpi (5 or 20), treatment (Control or PEDV), and their interaction. For all data, significance was determined when $P \leq 0.05$ and tendency for significance at $0.05 < P \leq 0.10$. The effect of dpi on Ct in PEDV pigs and the effect of treatment on growth performance parameters were analyzed using the MIXED procedure of SAS (SAS Inst. Inc., Cary, NC) with pen as the experimental unit. Least square (**LS**) means of dpi or treatment were determined using the LS means statement and differences in LS means were produced using the pdiff option. Data are reported as LS means estimates with a pooled SEM.

Morphology, serum parameters, proteolysis markers in the LD, and gluconeogenic enzyme activities in the liver and jejunum mucosal scrapings were analyzed using the MIXED procedure of SAS with pig as the experimental unit. The model included treatment and dpi and the interaction of treatment and dpi as fixed effects. 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. Data are reported as LS means estimates with a pooled SEM.

Count data from Ki67 analyses were analyzed using the GLIMMIX procedure of SAS using a Poisson distribution. Data are reported as LS means and SEM. For lesion scores, Fisher's exact test in the FREQ procedure was used to assess if the interaction of treatment and dpi contributed to lesion score distribution.

## RESULTS

### *Confirmation of PEDV Challenge*

All Control pigs were RT-PCR negative for PEDV throughout the duration of the study (data not shown). As expected, all pigs inoculated with PEDV had lower ($P < 0.001$) Ct values at dpi 5 compared with all other time points (Table 2) and therefore greater presence of PEDV in feces. The Ct estimate at dpi 20 indicates that PEDV pigs were still positive for PEDV; however, only one out of eight pigs sampled had PEDV detectable in feces by RT-PCR. All sampled PEDV challenged pigs were PCR-positive for PEDV at least once during the sampling period. Nucleic acid and protein from PEDV was detected by ISH and IHC, respectively, in seven out of the eight PEDV-inoculated pigs euthanized on dpi 5 and 0 of the eight PEDV-inoculated pigs euthanized on dpi 20 (Table 2). During necropsy, lesions typical of bacterial infections were not observed by present veterinarians in any of the pigs.

### *Growth Performance*

There was no difference ($P > 0.05$) in start or end BW between Control and PEDV pigs (Table 3).

**Table 3.** Growth performance of naïve (Control) and pigs inoculated with PEDV over 20 d[1]

| | Treatment | | | |
|---|---|---|---|---|
| Parameter, kg | Control | PEDV | SEM | *P*-value |
| Start BW | 8.06 | 8.85 | 0.496 | 0.280 |
| End BW | 17.89 | 17.72 | 1.051 | 0.912 |
| Dpi 1–5 | | | | |
| ADG | 0.28 | 0.18 | 0.049 | 0.156 |
| ADFI | 0.39 | 0.35 | 0.047 | 0.493 |
| G:F | 0.70 | 0.45 | 0.077 | 0.039 |
| Dpi 6–20 | | | | |
| ADG | 0.55 | 0.52 | 0.034 | 0.433 |
| ADFI | 0.63 | 0.79 | 0.053 | 0.047 |
| G:F | 0.90 | 0.66 | 0.050 | 0.003 |
| Dpi 0–20 | | | | |
| ADG | 0.49 | 0.44 | 0.033 | 0.328 |
| ADFI | 0.57 | 0.68 | 0.043 | 0.088 |
| G:F | 0.88 | 0.66 | 0.046 | 0.004 |

[1]Estimates are represented by eight pens per treatment.

From dpi 0 to 5, PEDV pigs had a 36% reduction ($P = 0.039$) in feed efficiency compared with Control pigs; however, there was no difference ($P = 0.493$) in feed intake between treatments. Although not significant, there was a 35% reduction ($P = 0.156$) in ADG of PEDV pigs compared with Control pigs. From dpi 6 to 20, PEDV pigs increased ($P = 0.047$) their ADFI by 20% compared with Control pigs; however, there was no difference ($P = 0.433$) in ADG between treatments. Therefore, feed efficiency was reduced ($P = 0.003$) by 27% in PEDV pigs compared with Control pigs. Pigs inoculated with PEDV did not differ ($P = 0.328$) in overall (dpi 0 to 20) ADG compared with Control pigs; however, there was a tendency for overall ADFI to be greater ($P = 0.088$) in PEDV pigs compared with Control pigs. This resulted in a 25% overall (dpi 0 to 20) reduction ($P = 0.004$) in feed efficiency in PEDV pigs compared with Control pigs.

### *Jejunal Morphology, Stem Cell Proliferation, and Lesion Scores*

Villus height (**VH**) was reduced ($P = 0.002$) by 34% at 5 dpi for PEDV pigs compared with Control pigs, but did not differ at 20 dpi (Table 4). The interaction of treatment and dpi tended ($P = 0.054$) to be significant for VH-to-crypt depth ratio with the same pattern observed as for VH. This was driven by the lack of difference ($P = 0.662$) for crypt depth between treatment and dpi.

Crypt stem cell proliferation as assessed by counts of cells positive for Ki67 was significantly ($P = 0.001$) increased at 5 dpi for PEDV pigs compared with Control pigs; however, there was no difference between treatments at 20 dpi (Table 4).

Protein-to-RNA ratios were used as a crude assessment of protein synthesis translation efficiency in jejunum mucosal scrapings (Table 4). There was a tendency ($P = 0.053$) for an interaction between treatment and dpi for protein-to-RNA ratios, where Control pigs had greater protein-to-RNA ratios at dpi 5 compared with PEDV pigs; however, at dpi 20, there was no difference between treatments. These differences in efficiency of protein translation were driven by the reduction in total protein in PEDV pigs at dpi 5 compared with dpi 20, whereas total protein was no different for Control pigs at either time point. There was no difference ($P > 0.05$) between treatment and dpi for total RNA in jejunum mucosal scrapings.

Lesions score distribution as assessed by villus atrophy and fusion and immune cell infiltrates in the jejunum was significantly ($P < 0.001$, Table 4) influenced by treatment and dpi. This was driven by PEDV pigs having more scores above 1 at 5 dpi compared with Control pigs and all pigs scored a 0 or 1 at dpi 20 regardless of treatment.

### *Serum Parameters*

There was a treatment by dpi interaction ($P = 0.010$) for insulin concentration, where at dpi 5, there was no difference in concentration; however, at dpi 20, PEDV pigs had 67% greater insulin in serum compared with Control pigs (Table 5). There was an interaction of treatment and dpi ($P = 0.024$) for serum glucose concentrations where there was no difference between treatments at dpi 5; however, at dpi 20, PEDV pigs had 19% greater serum glucose compared with Control pigs. There was a tendency ($P = 0.100$) for an interaction

**Table 4.** Morphology, stem cell proliferation, and lesion scores in the jejunum of naïve (Control) and PEDV-inoculated pigs at dpi 5 and 20[1]

| | Dpi | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | 5 | | 20 | | | *P*-value | | |
| Parameter | Control | PEDV | Control | PEDV | SEM | trt | dpi | trt × dpi |
| Morphology | | | | | | | | |
| VH, µm | 288[b] | 190[c] | 348[a] | 355[a] | 15.310 | 0.006 | <0.001 | 0.002 |
| CD, µm | 255 | 219 | 232 | 240 | 15.260 | 0.958 | 0.373 | 0.662 |
| VH:CD | 1.31[x] | 0.88[y] | 1.53[x] | 1.57[x] | 0.119 | 0.110 | <0.001 | 0.054 |
| Ki67[2] | 43[c] | 57[a] | 49[b] | 52[b] | 1.446 | <0.001 | 0.493 | 0.001 |
| Protein[3], % | 6.96[ab] | 6.15[b] | 6.80[ab] | 7.68[a] | 0.331 | 0.904 | 0.048 | 0.017 |
| RNA[3], % | 0.13 | 0.14 | 0.14 | 0.14 | 0.017 | 0.581 | 0.536 | 0.877 |
| Protein:RNA | 57.81[x] | 45.94[y] | 42.28[y] | 51.87[xy] | 5.277 | 0.831 | 0.373 | 0.053 |
| Lesion scores[4] | | | | | | | | |
| 0 | 4/8 | 0/8 | 3/8 | 6/8 | – | – | – | <0.001 |
| 1 | 4/8 | 1/8 | 5/8 | 2/8 | – | – | – | – |
| 2 | 0/8 | 5/8 | 0/8 | 0/8 | – | – | – | – |
| 3 | 0/8 | 2/8 | 0/8 | 0/8 | – | – | – | – |

trt, treatment; CD, crypt depth.

[1]Represented by eight observations per treatment per time.

[2]Cells positive for Ki67 were counted in three intact villi of correct orientation and an average per villus was calculated. Ki67 is a marker for stem cell proliferation.

[3]Represented as a percent (g/g) of tissue.

[4]Lesion score: 0 = normal; 1 = mild villus blunting with rare fusion; 2 = moderate villus blunting and fusion with lymphoid infiltration; 3 = severe villus blunting and fusion with lymphoid infiltration. The interaction of treatment and dpi significantly influenced lesion score in the jejunum as determined by Fisher's exact test.

[a,b]Means without a common superscript differ ($P \leq 0.05$); [x,y]Means without a common superscript differ ($P < 0.10$).

**Table 5.** Serum parameters in naïve (Control) and PEDV-inoculated pigs at dpi 5 and 20[1]

| | Dpi | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | 5 | | 20 | | | *P*-value | | |
| Parameter | Control | PEDV | Control | PEDV | SEM | trt | dpi | trt × dpi |
| Glucagon, pg/mL | 132 | 135 | 205 | 148 | 25.517 | 0.304 | 0.104 | 0.252 |
| Glucose, mg/dL | 96[b] | 83[b] | 105[b] | 130[a] | 8.002 | 0.463 | 0.002 | 0.024 |
| Insulin, ng/mL | 0.09[b] | 0.18[b] | 0.17[b] | 0.51[a] | 0.044 | <0.001 | <0.001 | 0.010 |
| Insulin:glucose[2] | 2.60[c] | 7.29[b] | 4.57[bc] | 11.18[a] | 1.203 | <0.001 | 0.021 | 0.433 |
| NEFA, mEq/L | 0.16[a] | 0.20[a] | 0.09[b] | 0.09[b] | 0.023 | 0.534 | 0.005 | 0.473 |
| BUN, mg/dL | 6.78[a] | 5.68[ab] | 6.46[ab] | 4.81[b] | 0.599 | 0.030 | 0.328 | 0.643 |
| Haptoglobin, µg/mL | 280[y] | 460[x] | 240[y] | 224[y] | 57.350 | 0.162 | 0.023 | 0.100 |

trt, treatment.

[1]Estimates are represented by eight observations per treatment per time.

[2]Units in mol:mol/L × $10^{-9}$.

[a,b]Means without a common superscript differ ($P \leq 0.05$); [x,y]Means without a common superscript differ ($P < 0.10$).

between treatment and dpi for haptoglobin concentration, where PEDV pigs had greater concentrations compared with Control pigs at dpi 5, but no difference was observed between treatments at dpi 20. All other serum parameters did not have a significant interaction of treatment and dpi. There was no main effect of treatment ($P = 0.304$) or dpi ($P = 0.104$) for serum glucagon concentrations. Pigs inoculated with PEDV had significantly ($P < 0.001$) greater insulin-to-glucose ratios compared with Control pigs and pigs at dpi 20 had significantly ($P = 0.021$) greater insulin-to-glucose ratios compared with dpi 5 pigs. At dpi 5, pigs had greater ($P = 0.005$) serum NEFA concentrations compared with pigs at dpi 20; however, there was no effect ($P = 0.534$) of treatment. Pigs inoculated with PEDV had significantly ($P = 0.030$) less serum BUN compared with Control pigs.

### *Skeletal Muscle Proteolysis Markers*

There was no significant interaction of treatment and dpi on any of the measured markers of protein degradation in the LD, except for 20S proteasome

**Table 6.** Proteolysis markers in LD of naïve (Control) and PEDV-inoculated pigs at dpi 5 and 20[1]

| | Dpi | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | 5 | | 20 | | | *P*-value | | |
| Parameter | Control | PEDV | Control | PEDV | SEM | trt | dpi | trt × dpi |
| Calpastatin, AU[2] | 1.00 | 1.05 | 0.40 | 0.77 | 0.125 | 0.092 | <0.001 | 0.201 |
| µ-Calpain,[3] % | | | | | | | | |
| 80 kDa | 44.28 | 47.54 | 54.18 | 52.84 | 3.802 | 0.802 | 0.051 | 0.548 |
| 78 kDa | 18.74 | 16.03 | 26.42 | 28.03 | 3.259 | 0.865 | 0.004 | 0.511 |
| 76 kDa | 36.98 | 36.44 | 19.40 | 19.14 | 3.408 | 0.907 | <0.001 | 0.967 |
| Myofibrillar protein,[4] mg | 31.78 | 32.89 | 23.68 | 24.35 | 2.250 | 0.696 | 0.009 | 0.921 |
| ERMs, mg | 0.06 | 0.06 | 0.05 | 0.07 | 0.01 | 0.303 | 0.698 | 0.247 |
| ERMs,[5] % | 0.20 | 0.20 | 0.22 | 0.31 | 0.041 | 0.324 | 0.112 | 0.325 |
| Protein:RNA | 24.69 | 26.31 | 25.20 | 23.29 | 2.807 | 0.958 | 0.659 | 0.534 |
| 20S proteasome[6] | 20.97[b] | 28.59[a] | 17.24[b] | 9.81[c] | 2.477 | 0.970 | <0.001 | 0.005 |

trt, treatment.

[1]Estimates are represented by eight observations per treatment per time.

[2]AU = arbitrary units relative to Control at dpi 5.

[3]Protein density percentages of unautolyzed 80 kilodalton µ-calpain subunit and its 78 and 76 kilodalton subunit autolysis products.

[4]Obtained from 1.5 g frozen LD.

[5]Represented as a percent of crude myofibrillar protein extraction.

[6]20S proteasome activity measured as micromolar (µM) of released fluorescent 7-amino-4-methylcoumarin (AMC) from LLVY-AMC per mg protein.

[a,b]Means without a common superscript differ ($P \leq 0.05$).

activity (Table 6). Pigs inoculated with PEDV had 27% more ($P = 0.005$) 20S proteasome activity in the LD at dpi 5 compared with Control pigs; however, at 20 dpi, PEDV pigs had 43% less activity in LD compared with Control pigs. Pigs inoculated with PEDV tended ($P = 0.092$) to have greater protein abundance of calpastatin, an endogenous inhibitor of calpain and there was more ($P < 0.001$) calpastatin protein abundance in LD of pigs at dpi 5 compared with pigs at dpi 20. Protein abundance of intact µ-calpain (80 kilodalton) and autolyzed 78 kilodalton µ-calpain was greater ($P < 0.01$) in LD of pigs at dpi 20 compared with pigs at 5 dpi; however, protein abundance of autolyzed 76 kilodalton µ-calpain was greater ($P < 0.001$) in LD of pigs at dpi 5 compared with pigs at dpi 20.

To further assess skeletal muscle proteolysis, LD myofibrillar protein and ERMs were determined (Table 6). The amount of myofibrillar protein extracted from the LD was greater ($P = 0.009$) at dpi 5 compared with pigs at dpi 20, regardless of treatment. However, the amount and percent of ERMs were not different ($P > 0.05$) between treatments or dpi. Protein-to-RNA ratios were assessed as a crude estimate of protein synthesis translation efficiency, and there were no differences ($P > 0.05$) due to treatment or dpi.

### *Gluconeogenic Enzyme Activities*

There was greater ($P = 0.018$) activity of PEPCK in the liver of pigs at dpi 20 compared with pigs at 5 dpi (Table 7). However, there was less ($P < 0.001$) activity of F1,6BP in the liver of pigs at 20 dpi compared with pigs at 5 pi. There was an interaction of treatment and dpi ($P = 0.043$) for activity of G6P where there was a 50% increase in liver G6P activity in Control pigs compared with PEDV pigs at dpi 5, but no difference between treatments at dpi 20.

In jejunum mucosal scrapings, there were no significant ($P > 0.05$) interactions of treatment and dpi on gluconeogenic enzyme activities. There was a tendency ($P = 0.060$) for a treatment-by-dpi interaction for F1,6BP activity driven by the overall increased activity in mucosal scrapings of Control pigs compared with PEDV pigs.

## DISCUSSION

Pigs inoculated with PEDV had reduced ADFI and ADG during the first wk post-inoculation compared with naïve counterparts (Schweer et al., 2015b; Curry et al., 2017a). Over a 42-d PEDV challenge period in nursery pigs, PEDV reduced whole-body protein accretion and growth compared with naïve counterparts (Curry et al., 2017a). Thus, it was hypothesized that PEDV infection modulated growth performance and tissue accretion, in part by reducing feed intake and by augmenting skeletal muscle proteolysis and gluconeogenesis in order to provide energy and nutrients to support an activated immune response and recovery of the intestinal epithelium. The extent to which muscle and liver metabolism are altered by PEDV infection has not been investigated or defined. Therefore, the

**Table 7.** Gluconeogenic enzymatic activity in liver and jejunum mucosal scrapings of naïve (Control) and PEDV-inoculated pigs at dpi 5 and 20[1]

| | Dpi | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | 5 | | 20 | | | *P*-value | | |
| Parameter | Control | PEDV | Control | PEDV | SEM | trt | dpi | trt × dpi |
| Liver | | | | | | | | |
| PEPCK | 1.00 | 0.97 | 1.20 | 1.20 | 0.087 | 0.829 | 0.018 | 0.893 |
| F1,6BP | 1.56 | 1.52 | 0.62 | 0.90 | 0.202 | 0.571 | <0.001 | 0.439 |
| G6P | 0.12$^{ab}$ | 0.15$^{a}$ | 0.12$^{ab}$ | 0.06$^{b}$ | 0.023 | 0.591 | 0.042 | 0.043 |
| Jejunum | | | | | | | | |
| PEPCK | 68.74 | 65.27 | 61.10 | 64.53 | 15.127 | 0.999 | 0.784 | 0.822 |
| F1,6BP | 16.12$^{x}$ | 11.00$^{xy}$ | 13.22$^{y}$ | 12.66$^{y}$ | 1.165 | 0.021 | 0.598 | 0.060 |
| G6P | 1.13 | 1.08 | 1.16 | 1.13 | 0.107 | 0.724 | 0.699 | 0.902 |

PEPCK, phosphoenolpyruvate carboxykinase (µM ATP/min/g tissue); F1,6BP, fructose 1,6-bisphosphatase (µM NADPH/min/g tissue); G6P, glucose-6-phosphatase (mM Pi released/min/g tissue).

[1]Estimates are represented by eight observations per treatment per time.

$^{a,b}$Means without a common superscript differ (P ≤ 0.05). $^{x,y}$Means without a common superscript differ (P < 0.10).

objective was to determine the effect of PEDV on nursery pig growth performance and partitioning of energy and nutrients during peak PEDV infection and recovery.

In this study, all PEDV pigs were RT-PCR positive for PEDV in feces by dpi 5, which is consistent with previous reports (Madson et al., 2014; Jung et al., 2015; Jung and Saif, 2015). A reduction in ADFI and ADG was expected based on results from previous work (Kim and Lee, 2014; Schweer et al., 2015b; Curry et al., 2017a) and because appetite and feed intake are generally reduced during an immune response (Johnson, 1998). In swine production, there is wide variation of PEDV infection from subclinical, mild, to severe, and severe with co-infections; however, there are no data on the estimated prevalence of each severity class. Surprisingly, there was no significant overall, or initial (dpi 0 to 5) reductions in ADG or ADFI due to PEDV infection in this study. In fact, ADFI was increased overall due to PEDV challenge compared with Control pigs, and this was most evident at dpi 6 to 20. These data are contrary to what has been previously reported, which PEDV reduced ADG and ADFI from dpi 0 to 7 by 46 to 78 and 23% to 28%, respectively, compared with naïve counterparts (Schweer et al., 2015b; Curry et al., 2017a). One possible explanation could be differences in PEDV infection severity. To evaluate why there was no statistical difference between the two treatments in regard to feed intake and to confirm that the feed intake of Control pigs was as expected, the Swine National Research Council modeling module (NRC, 2012) was utilized to predict feed intake based on dpi 0 BW of each treatment group and dietary energy. Given these parameters, the Control pigs where consuming feed close to their level of predicted during dpi 0 to 5 and PEDV pigs consumed approximately 27% less than their predicted. Although ADG and ADFI were not statistically different between treatments from dpi 0 to 5, these slight reductions in PEDV pigs ADG and ADFI compared with Control pigs resulted in reduced G:F. In this study, there was a 36% reduction in PEDV pigs feed efficiency from dpi 0 to 5 compared with Controls, which is similar to the 28% reduction in feed efficiency reported in 5 wk post-weaned pigs inoculated with PEDV compared with their naïve counterparts from dpi 0 to 7 (Schweer et al., 2015b).

It was hypothesized that feed efficiency was reduced in PEDV pigs due to available nutrients in the lumen being utilized by the intestine during infection and recovery. Upon PEDV infection, enterocytes undergo apoptosis (Jung and Saif, 2015) to rid the host of virus, which can result in reduced absorptive capacity of the small intestine (Curry et al., 2017b). Previous work reported that PEDV detected by IHC is most abundant at dpi 5 and this coincides with increased crypt stem cell proliferation, DNA fragmentation, and active glutamine transport in the jejunum (Curry et al., 2017b). In agreement with this study, it has been well characterized that during peak infection PEDV reduces VH (Stevenson et al., 2013; Madson et al., 2014) and that PEDV increases villus fusion as well as immune cell infiltrates (Schweer et al., 2015a). Crypt stem cell proliferation has been assessed using Ki67 IHC, and PEDV-inoculated pigs have been shown to have increased Ki67-positive cells in small intestinal crypts (Jung et al., 2015; Curry et al., 2017b). Results from this study agree with

these two earlier findings. The increased crypt stem cell proliferation was most likely due to rapid re-epithelialization of denuded villi observed in PEDV pigs at dpi 5, which would require increased glutamine transport as the main energy substrate for enterocytes (Burrin and Stoll, 2009), or as a precursor for synthesis of purines, glutathione, or acute phase proteins (Reeds et al., 1994). The supply of energy and nutrients to the intestine is needed to regain absorptive capacity, but also needed by resident immune cells for proliferation and synthesis of effector molecules to rid the host of the pathogen (Humphrey and Klasing, 2004). If energy and nutrients ingested and absorbed by the animal are not enough for immune cell proliferation and activation, then body tissue reserves such as amino acids and fatty acids may be required to provide those substrates. This in turn would likely exacerbate decreases in feed efficiency of livestock during pathogen challenge, which is reported herein.

Enteric virus infections have been shown to increase intestinal epithelial protein translation machinery (Rhoads et al., 2007). To further understand what was contributing to the reduction in G:F, it was tested to see if there was an increase in intestinal epithelial protein synthesis translation efficiency by assessing the protein-to-RNA ratio in PEDV and Control pig jejunal scrapings at dpi 5 and 20. Although there was no difference in this ratio between treatments within dpi, PEDV pigs tended to have greater protein-to-RNA ratio at dpi 20 compared with dpi 5. This may indicate that efficiency and machinery for protein synthesis were increased in PEDV pigs, but that increase may be after dpi 5. Active (phosphorylated) $p70^{S6K}$ protein abundance was increased in jejunum of pigs inoculated with rotavirus compared with naïve counterparts (Rhoads et al., 2007). $p70^{S6K}$ is protein involved in the mammalian target of rapamycin (**mTOR**) pathway, which is understood to be involved in the initiation of protein synthesis. In malnourished and rotavirus-infected pigs, (phosphorylated) $p70^{S6K}$ protein abundance was also increased in the jejunum, but reduced in the muscle compared with naïve counterparts (Rhoads et al., 2007). Rotavirus, similar to PEDV, infects enterocytes and causes similar clinical signs of diarrhea and dehydration (Hagbom et al., 2011; Holloway and Coulson, 2013). Collectively, these data demonstrate that the intestine has to rebuild an epithelial layer after PEDV infection in order to regain absorptive capacity. The rebuilding intestinal epithelial layer requires energy and nutrients for recovery and those may be coming from muscle, jejunum, liver, or combination of the three.

Feed efficiency may also be altered due to changes in postabsorptive metabolism and nutrient partitioning. The metabolic and nutritional cost of activating the immune system has been difficult to define due to the complex nature of the immune system, differences in pathogenesis (i.e., viral and bacterial) and responses by the host (i.e., enteric, respiratory or systemic). It is generally accepted that during an infection, there is a net loss of nitrogen due to changes in nitrogen retention by the host (Coleman and Dubois, 1915). Acute phase proteins are those proteins released by the liver early in infection that help orchestrate the immune response. Reeds et al. (1994) estimated that 2.33 mg of muscle protein would need to be catabolized in order to supply the AA needed to produce 1 mg of acute phase proteins. In this study, haptoglobin, an acute phase protein, was increased in PEDV pigs at dpi 5 compared with Controls, but there was a no statistical difference at dpi 5 in serum BUN concentration. However, there was a numerical decrease in BUN at dpi 5 in PEDV pigs suggesting a more efficient use of AA.

Muscle contains approximately 60% of total body protein; therefore, changes in muscle protein degradation or synthesis can influence whole-body protein accretion and AA supply for gluconeogenesis (Snell, 1980; Humphrey and Klasing, 2004). Sepsis increased m-calpain protein abundance and release of myofilaments from the myofibrillar fraction of the rat extensor digitorum longus muscle compared with naïve controls (Williams et al., 1999). These authors hypothesized that the disruption of actin and myosin from the Z-disk was calcium-dependent and mediated by calpains, calcium-dependent proteinases. In addition, infusing rats with TNF-α or IL-1β, two pro-inflammatory cytokines, resulted in increased total muscle protein breakdown and increased myofibrillar protein breakdown (Zamir et al., 1992). Growing pigs that were inoculated PRRSV had no difference in total calpastatin activity, but had increased μ-calpain activity in muscle compared with muscle in healthy counterparts at dpi 21 (Lonergan et al., 2015). In 3-d-old pigs that were infused with 10 μg/(kg × h) of lipopolysaccharide (**LPS**) for 14 h, then 13.3 μg/(kg × h) of LPS for an additional 6 h, protein synthesis in LD was reduced by approximately 13% as determined by incorporation of L-[4-3H]phenylalanine and fractional rate of protein synthesis calculated (Orellana et al., 2004). Collectively, these studies suggest that during a disease state, there is either a dysregulation of protein synthesis or an increase

in muscle proteolysis. However, in this study, there were no differences in unautolyzed μ-calpain (80 kilodalton) and autolysis products (78 and 76 kilodalton) or calpastatin abundance in LD of PEDV pigs compared with Control pigs.

Although there was no difference in ERMs in LD of PEDV pigs compared with Control pigs, there was an increase in 20S proteasome activity at dpi 5 in LD of PEDV pigs compared with Controls, but the reverse was observed at dpi 20. However, it is hypothesized that increased release of myofilaments from myofibrillar protein is necessary in order for degradation of these myofilaments by the proteasome (Williams et al., 1999; Hasselgren, 2000). This suggests that the timing of our sampling may not have been early enough in infection period to capture increases in ERM production or that the 20S proteasome is utilizing cytosolic proteins. At dpi 20, it appears that PEDV may be conserving muscle mass as the abundance of calpastatin was numerically increased and activity of 20S proteasome was reduced compared with Control pigs. These data also support the numerical reduction of BUN in PEDV pigs at dpi 5 suggesting fewer AA being broken down to urea.

Pigs inoculated with PEDV had increased insulin-to-glucose ratios compared with Control pigs regardless of dpi, indicating that PEDV reduced insulin sensitivity. It has been demonstrated in neonatal rats that infusing insulin, while maintaining fasting levels of amino acids and glucose, resulted in increased protein synthesis in muscle, but this response decreased with developmental stage (Davis et al., 2000). In septic rats infused with increasing levels of insulin, protein synthesis increased in extensor digitorum longus muscle compared with healthy counterparts; however, protein breakdown was not inhibited as insulin increased (Hasselgren et al., 1987), indicating that response to insulin in muscle only influenced protein synthesis and not degradation. It has been suggested that pro-inflammatory cytokine IL-1β inhibits the anabolic effect of insulin in muscle (Klasing and Johnstone, 1991). Although insulin sensitivity was reduced, there appeared to be no difference in efficiency of translation in LD of PEDV and Control pigs as measured by protein-to-RNA ratios. However, as discussed previously, Rhoads et al. (2007) demonstrated a (~50%) decrease in the active state of protein translation initiation machinery in the semitendinosus muscle of pigs inoculated with rotavirus at dpi 1 and 3 compared with naïve counterparts.

During an immune response, the utilization of dietary AA shifts from lean growth to production of glucose for energy (Klasing and Johnstone, 1991) and this is speculated to be contributing to the reductions in feed efficiency in PEDV challenged pigs. As gluconeogenesis is important for maintenance of blood glucose when hepatic glycogen stores begin to deplete (Watford, 2005), activities of enzymes that bypass the irreversible steps of glycolysis for gluconeogenesis were determined in liver and jejunum during PEDV infection. It was hypothesized that gluconeogenesis would provide glucose for ATP needed to combat infection and regeneration of the intestine and keto acids for metabolism. The liver is the main generator of endogenous glucose production (Mittelman and Bergman, 1998); however, the intestine is also gluconeogenic and may contribute to glucose production during various disease states. Glutamine is the main precursor for glucose production from the small intestine, which can provide approximately 20% of systemic endogenous glucose production (Croset et al., 2001). Schweer et al. (2015a) and Curry et al. (2017b) demonstrated that during PEDV infection, the jejunum increases active transport of glutamine. In addition, glutamine has been shown to increase ornithine decarboxylase activity, an important enzyme for pyrimidine synthesis, and incorporation of [3H]thymidine, an indicator of increased protein synthesis (Kandil et al., 1995). We speculate that the increased feed intake observed in PEDV pigs was sufficient in supplying energy and nutrients to the intestinal epithelium for restitution, thus explaining why we did not observe any difference between treatments within dpi in jejunum or liver gluconeogenic enzyme activities.

In conclusion, PEDV-inoculated pigs did recover the absorptive capacity that was reduced during PEDV infection by increasing proliferation of intestinal stem cells. However, the energy and nutrients needed to recover the epithelium may be originating from available luminal nutrients instead of muscle proteolysis and gluconeogenesis as hypothesized. Thus, we propose that luminal nutrients are being diverged from lean growth to intestinal epithelium recovery during PEDV infection, resulting in altered feed efficiency.

## LITERATURE CITED

Bee, G., A. L. Anderson, S. M. Lonergan, and E. Huff-Lonergan. 2007. Rate and extent of pH decline affect proteolysis of cytoskeletal proteins and water-holding capacity in pork. Meat Sci. 76:359–365.

Burrin, D. G., and B. Stoll. 2009. Metabolic fate and function of dietary glutamate in the gut. Am. J. Clin. Nutr. 90:850S–856S.

Coleman, W., and E. F. Dubois. 1915. Seventh paper calorimetric observations on the metabolism of typhoid patients with and without food. Arch. Intern. Med. XV:887–938.

Croset, M., F. Rajas, C. Zitoun, J.-M. Hurot, S. Montano, and G. Mithieux. 2001. Rat small intestine is an insulin-sensitive gluconeogenic organ. Diabetes. 50:740–746.

Cruzen, S. M., A. J. Harris, K. Hollinger, R. M. Punt, J. K. Grubbs, J. T. Selsby, J. C. M. Dekkers, N. K. Gabler, S. M. Lonergan, and E. Huff-Lonergan. 2013. Evidence of decreased muscle protein turnover in gilts selected for low residual feed intake1. J. Anim. Sci. 91:4007–4016.

Curry, S. M., K. A. Gibson, E. R. Burrough, K. J. Schwartz, K. J. Yoon, and N. K. Gabler. 2017a. Nursery pig growth performance and tissue accretion modulation due to porcine epidemic diarrhea virus or porcine deltacoronavirus challenge. J. Anim. Sci. 95:1–9.

Curry, S. M., K. J. Schwartz, K. J. Yoon, N. K. Gabler, and E. R. Burrough. 2017b. Effects of porcine epidemic diarrhea virus infection on nursery pig intestinal function and barrier integrity. Vet. Microbiol. 211:58–66.

Davies, P. R. 2012. One world, one health: the threat of emerging swine diseases. A North American perspective. Transbound. Emerg. Dis. 59:18–26.

Davis, T. A., H. V. Nguyen, A. Suryawan, J. A. Bush, L. S. Jefferson, and S. R. Kimball. 2000. Developmental changes in the feeding-induced stimulation of translation initiation in muscle of neonatal pigs. Am. J. Physiol. Endocrinol. Metab. 279:E1226–E1234.

Hagbom, M., C. Istrate, D. Engblom, T. Karlsson, J. Rodriguez-Diaz, J. Buesa, J. A. Taylor, V.-M. Loitto, K.-E. Magnusson, H. Ahlman, et al., 2011. Rotavirus stimulates release of serotonin (5-HT) from human enterochromaffin cells and activates brain structures involved in nausea and vomiting. PLoS. Pathog. 7:e1002115.

Hasselgren, P.-O. 2000. Catabolic response to stress and injury: implications for regulation. World. J. Surg. 24:1452–1459.

Hasselgren, P. O., B. W. Warner, J. H. James, H. Takehara, and J. E. Fischer. 1987. Effect of insulin on amino acid uptake and protein turnover in skeletal muscle from septic rats. Evidence for insulin resistance of protein breakdown. Arch. Surg. 122:228–233.

Hoang, H., M. L. Killian, D. M. Madson, P. H. Arruda, D. Sun, K. J. Schwartz, and K. J. Yoon. 2013. Full-length genome sequence of a plaque-cloned virulent porcine epidemic diarrhea virus isolate (USA/Iowa/18984/2013) from a Midwestern U.S. swine herd. Genome. Announc. 1:e01049–01013.

Holloway, G., and B. S. Coulson. 2013. Innate cellular responses to rotavirus infection. J. Gen. Virol. 94:1151–1160.

Humphrey, B., and K. Klasing. 2004. Modulation of nutrient metabolism and homeostasis by the immune system. Worlds. Poult. Sci. J. 60:90–100.

Johnson, R. W. 1998. Immune and endocrine regulation of food intake in sick animals. Domest. Anim. Endocrinol. 15:309–319.

Jung, K., T. Annamalai, Z. Lu, and L. J. Saif. 2015. Comparative pathogenesis of US porcine epidemic diarrhea virus (PEDV) strain PC21A in conventional 9-day-old nursing piglets vs. 26-day-old weaned pigs. Vet. Microbiol. 178:31–40.

Jung, K., and L. J. Saif. 2015. Porcine epidemic diarrhea virus infection: etiology, epidemiology, pathogenesis and immunoprophylaxis. Vet. J. 204:134–143.

Kandil, H. M., R. A. Argenzio, W. Chen, H. M. Berschneider, A. D. Stiles, J. K. Westwick, R. A. Rippe, D. A. Brenner, and J. M. Rhoads. 1995. L-glutamine and L-asparagine stimulate ODC activity and proliferation in a porcine jejunal enterocyte line. Am. J. Physiol. 269:G591–G599.

Kim, Y., and C. Lee. 2014. Porcine epidemic diarrhea virus induces caspase-independent apoptosis through activation of mitochondrial apoptosis-inducing factor. Virology. 460–461:180–193.

Klasing, K. C., and B. J. Johnstone. 1991. Monokines in growth and development. Poult. Sci. 70:1781–1789.

Lonergan, S. M., E. Huff-Lonergan, K. Schwartz, W. Schweer, and N. K. Gabler. 2015. Viral challenges augment skeletal muscle proteolysis in growing pigs. In: 61st International Congress of Meat Science and Technology, DigICoMST, Helsinki, Finland. Available from http://icomst-proceedings.helsinki.fi/papers/2015_06_56.pdf [accessed June 20, 2017].

Madson, D. M., D. R. Magstadt, P. H. Arruda, H. Hoang, D. Sun, L. P. Bower, M. Bhandari, E. R. Burrough, P. C. Gauger, A. E. Pillatzki, et al., 2014. Pathogenesis of porcine epidemic diarrhea virus isolate (US/Iowa/18984/2013) in 3-week-old weaned pigs. Vet. Microbiol. 174:60–68.

Mittelman, S., and R. N. Bergman. 1998. Liver glucose production in health and diabetes. Curr. Opin. Endocrinol. Diabetes. Obes. 5:126–135.

Murray, M. J., and A. B. Murray. 1979. Anorexia of infection as a mechanism of host defense. Am. J. Clin. Nutr. 32:593–596.

Neti, G., S. M. Novak, V. F. Thompson, and D. E. Goll. 2009. Properties of easily releasable myofilaments: are they the first step in myofibrillar protein turnover? Am. J. Physiol. Cell. Physiol. 296:C1383–1390.

NRC. 2012. Nutrient requirements of swine. 11th rev. ed. Washington, DC: Natl. Acad. Press.

Orellana, R. A., S. R. Kimball, H. V. Nguyen, J. A. Bush, A. Suryawan, M. C. Thivierge, L. S. Jefferson, and T. A. Davis. 2004. Regulation of muscle protein synthesis in neonatal pigs during prolonged endotoxemia. Pediatr. Res. 55:442–449.

Pringle, T. D., C. R. Calkins, M. Koohmaraie, and S. J. Jones. 1993. Effect over time of feeding a β-adrenergic agonist to wether lambs on animal performance, muscle growth, endogenous muscle proteinase activities, and meat tenderness. J. Anim. Sci. 71:636–644.

Reeds, P. J., C. R. Fjeld, and F. Jahoor. 1994. Do the differences between the amino acid compositions of acute-phase and muscle proteins have a bearing on nitrogen loss in traumatic states? J. Nutr. 124:906–910.

Rhoads, J. M., B. A. Corl, R. Harrell, X. Niu, L. Gatlin, O. Phillips, A. Blikslager, A. Moeser, G. Wu, and J. Odle. 2007. Intestinal ribosomal p70(S6K) signaling is increased in piglet rotavirus enteritis. Am. J. Physiol. Gastrointest. Liver. Physiol. 292:G913–922.

Schweer, W. P., S. C. Pearce, E. R. Burrough, K. Schwartz, K. J. Yoon, J. C. Sparks, and N. K. Gabler. 2015a. The effect of porcine reproductive and respiratory syndrome virus and porcine epidemic diarrhea virus challenge on growing pigs II: intestinal integrity and function. J. Anim. Sci. 94:523–532.

Schweer, W. P., K. Schwartz, E. R. Burrough, K. J. Yoon, J. C. Sparks, and N. K. Gabler. 2015b. The effect of porcine reproductive and respiratory syndrome virus and

porcine epidemic diarrhea virus challenge on growing pigs I: growth performance and digestibility. J. Anim. Sci. 94:514–522.

Scrimshaw, N. S. 1977. Effect of infection on nutrient requirements. Am. J. Clin. Nutr. 30:1536–1544.

Snell, K. 1980. Muscle alanine synthesis and hepatic gluconeogenesis. Biochem. Soc. Trans. 8:205–213.

Stevenson, G. W., H. Hoang, K. J. Schwartz, E. R. Burrough, D. Sun, D. Madson, V. L. Cooper, A. Pillatzki, P. Gauger, B. J. Schmitt, et al., 2013. Emergence of porcine epidemic diarrhea virus in the United States: clinical signs, lesions, and viral genomic sequences. J. Vet. Diagn. Invest. 25: 649–654.

Ueda, I., and T. Wada. 1970. Determination of inorganic phosphate by the molybdovanadate method in the presence of ATP and some interfering organic bases. Anal. Biochem. 37: 169–174.

Watford, M. 2005. Is the small intestine a gluconeogenic organ? Nutr. Rev. 63:356–360.

Williams, A. B., G. M. Decourten-Myers, J. E. Fischer, G. Luo, X. Sun, and P. O. Hasselgren. 1999. Sepsis stimulates release of myofilaments in skeletal muscle by a calcium-dependent mechanism. FASEB J. 13:1435–1443.

Wimmer, M. 1988. A bioluminescent assay for the determination of phosphoenolpyruvate carboxykinase activity in nanogram-sized tissue samples. Anal. Biochem. 170:376–381.

Woo, P. C., S. K. Lau, C. S. Lam, C. C. Lau, A. K. Tsang, J. H. Lau, R. Bai, J. L. Teng, C. C. Tsang, M. Wang, et al., 2012. Discovery of seven novel Mammalian and avian coronaviruses in the genus deltacoronavirus supports bat coronaviruses as the gene source of alphacoronavirus and betacoronavirus and avian coronaviruses as the gene source of gammacoronavirus and deltacoronavirus. J. Virol. 86: 3995–4008.

Zamir, O., P.-O. Hasselgren, T. Higashiguchi, J. A. Frederick, and J. E. Fischer. 1992. Tumour necrosis factor (TNF) and interleukin-1 (IL-1) induce muscle proteolysis through different mechanisms. Mediators. Inflamm. 1:247–250.