Impact of *Brachyspira hyodysenteriae* on intestinal amino acid digestibility and endogenous amino acid losses in pigs¹

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ABSTRACT: *Brachyspira hyodysenteriae* (Bhyo) induces mucohemorrhagic diarrhea in pigs and is an economically significant disease worldwide. Our objectives were to determine the impact of Bhyo on apparent total tract digestibility (ATTD), ileal digestibility (AID), and ileal basal endogenous losses (BEL) in grower pigs. In addition, we assessed the effect of Bhyo on hindgut disappearance of DM, N, and GE. Thirty-two Bhyo negative gilts (38.6 ± 0.70 kg BW) were fitted with a T-cannula in the distal ileum and individually penned. In replicates 1 and 2, pigs were fed a complete diet (7 Bhyo–, 10 Bhyo+ pigs) or nitrogen-free diet (NFD; 4 Bhyo–, 11 Bhyo+ pigs), respectively. Across multiple rooms, the 21 Bhyo+ pigs (62.6 ± 1.39 kg BW) were inoculated with Bhyo on day post inoculation (dpi) 0, and the 11 Bhyo– pigs were sham inoculated. Feces were collected from 9 to 11 dpi and ileal digesta collected from 12 to 13 dpi. All pigs were euthanized at 14 to 15 dpi and intestinal tract pathology assessed. Within the complete diet and NFD treatments, data were analyzed to determine pathogen effects. All Bhyo– pigs remained Bhyo negative, and 5 Bhyo+ pigs in each replicate were confirmed Bhyo positive within 9 dpi. Infection

with Bhyo reduced ATTD of DM, N, and GE and increased AID of Gly ($P < 0.05$). No other AA AID differences were observed. Only BEL of Pro was reduced ($P < 0.05$) while Arg, Trp, and Gly tended ($P < 0.10$) to be reduced in Bhyo+ pigs. When calculated from AID and BEL, Bhyo infection reduced standardized ileal digestibility (SID) of N, Arg, Lys, Ala, Gly, Pro, and Ser ($P < 0.05$) and tended to reduce Thr SID ($P = 0.09$). In the hindgut of Bhyo+ pigs, there was generally an appearance of nutrients rather than disappearance. In Bhyo+ pigs fed a complete diet, hindgut appearance of N and GE were increased ($P < 0.05$) by 58 and nine-fold, respectively, and DM tended to be increased two-fold ($P = 0.06$). Similarly, in NFD fed pigs, hindgut appearance of N and GE was increased by 172% and 162%, respectively, although high variability led to no significance. Altogether, Bhyo infection decreases ATTD but has minimal impact on AID of AA, when corrected for BEL, SID of N, Arg, Lys and some nonessential AA are specifically reduced. Unexpectedly, BEL of several AA involved in mucin production were unaffected by Bhyo infection. This may suggest an increased need for specific AA and energy during a Bhyo challenge.

Key words: amino acid, *Brachyspira hyodysenteriae*, digestibility, endogenous loss, pig

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INTRODUCTION

Brachyspira hyodysenteriae (**Bhyo**), the classical agent of swine dysentery (**SD**), affects pigs worldwide and is a reemerging pathogen in U.S. swine (Burrough, 2017). Although infection is more prominent in finishing pigs, younger pigs can experience disease with mortality and morbidity approaching 30% and 90%, respectively (Hampson, 2012). Coupled with decreased growth performance (Wilberts et al., 2014a), Bhyo causes considerable economic loss worldwide (Hampson, 2012). The hallmark clinical sign of Bhyo infection is mucohemorrhagic diarrhea which generally appears within 14 d of experimental infection (Kinyon et al., 1977; Stanton, 2006). Lesions from Bhyo occur in the cecum and colon and are characterized by hemorrhage and necrosis (Albassam et al., 1985; Quintana-Hayashi et al., 2015). While the small intestine remains clinically unaffected (Stanton, 2006), SD has been reported to affect neuro-immunomodulatory processes in the ileum of pigs (Kaleczyc et al., 2010). This highlights gastrointestinal intramural crosstalk between the small and large intestines. However, little is known about how Bhyo infection modulates digestive tract function and nutrient and energy digestibility.

When animals are infected by different pathogens, tissue accretion rates, feed intake, and feed efficiency are reduced (Escobar et al., 2004; Curry et al., 2017; Schweer et al., 2017; Helm et al., 2018), suggesting a repartitioning of nutrients away from growth to support pathogen proliferation, host immune activation (Rauw, 2012), and barrier defense mechanisms such as mucus production (Colditz, 2008). How these insults alter digestibility and endogenous losses of AA and energy in the intestinal tract, and thus essential AA usage and requirements, is poorly understood. In nursery pigs, porcine epidemic diarrhea virus (**PEDV**) reduced apparent total tract digestibility (**ATTD**) of nutrients and energy; however, apparent ileal digestibility (**AID**) of nutrients, energy, and AA, except Lys was unaffected (Schweer et al., 2016). We have also shown that porcine reproductive and respiratory syndrome virus (**PRRSV**) reduce basal endogenous loss (**BEL**) of Arg and Ala at dpi 7 to 8 and BEL of Pro at 7 to 8 and 18 to 19 dpi (Schweer et al., 2018). In contrast, Lee (2012) reported a reduction in AID of several AA and an increase in BEL of all AA within 24 h after *Salmonella typhimurium* infection. Although the mode of action of *S. typhimurium* and Bhyo differ, it is assumed that enteric challenges that result in diarrhea would increase

BEL of AA in the intestinal tract and this would reduce SID values for AA.

Furthermore, AA metabolism is likely altered due to SD. In pigs that developed clinical SD, glucose metabolism, likely through glycolysis, was increased (Somchit et al., 2003). Similarly, Jonasson et al. (2007) reported a decrease in serum concentrations of gluconeogenic AA during clinical SD. Further, Wilberts et al. (2014b) and Quintana-Hayashi et al. (2015) have reported an increase in colonic mucin secretion in relation to Bhyo infection and considering mucins are rich in Thr, Ser, Pro, and Cys (Lien et al., 1997), dietary supply and metabolism of these AA may alter mucin synthesis and BEL. Therefore, the objective of this study was to determine the digestibility of nutrients, energy, and AA, and to determine BEL of AA in response to Bhyo infection. We hypothesized that Bhyo would decrease AID and ATTD coefficients and increase BEL of AA.

MATERIALS AND METHODS

All animal work was approved by the Iowa State University Institutional Animal Care and Use Committee (IACUC# 1-16-8156-S) and adhered to the ethical and humane use of animals for research.

The experiment was performed in two replicates consisting of 17 and 15 pigs, respectively. In all, 32 gilts (38.6 ± 0.70 kg BW) were selected and surgically fitted with a T-cannula in the distal ileum as described by Stein et al. (1998). Thirty-five days after surgery, and following a PRRSV study (Schweer et al., 2018), pigs were utilized for this Bhyo challenge study. Pigs were individually penned (1.4×1.5 m) across two rooms. Since Bhyo is spread through feces and is not aerosolized, there were control (Bhyo-) and Bhyo challenged (Bhyo+) pigs in each room with ~7 m separating Bhyo- and Bhyo+ pens. In total, there were 11 Bhyo- pigs (7 and 4 in replicates 1 and 2, respectively) and 21 Bhyo+ (10 and 11 in replicates 1 and 2, respectively) pigs across both replicates. All pigs were weighed on day post inoculation (**dpi**) 0 and at the end of the challenge period (dpi 14 or 15).

Diets and Feeding

In the first replicate, all pigs were fed a complete corn-soybean meal-based diet (Table 1) formulated to meet or exceed nutrient and energy requirements (NRC, 2012). In the second replicate, all pigs were fed the complete diet and a nitrogen-free diet (**NFD**; formulated based on cornstarch; Table 1), during

Table 1. Diet composition, as-fed basis

Ingredient, %	Complete ¹	NFD ²
Corn	75.69	—
Cornstarch	—	78.95
Soybean meal, 46.5% CP	19.86	—
Dextrose	—	10.00
Solka floc	—	4.00
Soybean oil	—	3.00
Casein	1.09	—
Monocalcium phosphate	0.82	1.35
Limestone	1.03	1.00
Salt	0.50	0.50
L-Lys-HCl	0.22	—
L-Thr	0.07	—
L-Trp	0.02	—
Chromic oxide	0.40	0.40
Potassium carbonate	—	0.40
Vitamin premix ³	0.15	0.15
Mineral premix ⁴	0.15	0.15
Magnesium oxide	—	0.10

¹Complete = corn-soy diet.

²NFD = nitrogen-free diet.

³Provided per kilogram of diet: 6,125 IU vitamin A, 700 IU vitamin D₃, 50 IU vitamin E, 30 mg vitamin K, 0.05 mg vitamin B₁₂, 11 mg riboflavin, 56 mg niacin, and 27 mg pantothenic acid.

⁴Provided per kilogram of diet: 22 mg Cu (as CuSO₄), 220 mg Fe (as FeSO₄), 0.4 mg I (as Ca(IO₃)₂), 52 mg Mn (as MnSO₄), 220 mg Zn (as ZnSO₄), and 0.4 mg Se (as Na₂SeO₃).

the 2-wk study period to determine BEL associated with Bhyo (4 Bhyo–, 11 Bhyo+ pigs). The NFD was fed from 5 dpi through 13 dpi and pigs were fed the complete diet otherwise. Both diets contained 0.40% chromic oxide (Cr₂O₃) as an indigestible marker. All pigs were restrictively fed to ensure the entire meal was eaten during the collection period. Based on pig BW, pigs were fed 2.5 times the estimated energy requirement for maintenance (2.5×197 kcal of ME per kg BW^{0.60}; [NRC, 2012](#)).

Animal Inoculation, Clinical Evaluation, and *Brachyspira* Detection

Fresh rectal swabs were collected from all animals immediately prior to the start of each replicate and were submitted for selective anaerobic culture targeting *Brachyspira* spp. at the Iowa State University Veterinary Diagnostic Laboratory (ISU VDL; Ames, IA) to confirm negative status. At 62.6 ± 1.39 kg BW, on dpi 0 and 2, the 21 Bhyo+ pigs were inoculated with 30 mL of agar slurry containing Bhyo B204 via their terminal ileum T-cannula. The agar slurry was prepared as previously described ([Burrough et al., 2012](#)) and the inoculation dose was $\sim 1.4 \times 10^6$ cfu/mL on dpi 0 and 1.2×10^5 cfu/mL on dpi 2. The Bhyo– pigs

received a sham inoculation with 30 mL of sterile agar intra-cannularly on dpi 0 and 2.

Pigs were examined daily for the development of diarrhea. Feces were examined and scored 0 if normal, 1 if semi-formed, 2 if semi-liquid, 3 if watery or mucoid $\pm$ blood [mucoid to mucohemorrhagic diarrhea (MD)].

To detect Bhyo infection and shedding, in replicate one, rectal swabs were taken for culture on dpi 5, 7, 9, 12, and at necropsy (dpi 14 or 15). In the second replicate, PCR was performed on feces collected at dpi 9 and 12, and selective culture was performed on feces at necropsy (dpi 14 or 15). Freshly collected samples were submitted to the ISU VDL and were processed routinely for *Brachyspira* selective culture and/or PCR using standard methods of the laboratory ([Wilberts et al., 2014a](#)).

Pathology and Histopathology

At dpi 14 or 15, pigs were euthanized by captive bolt followed by exsanguination and a necropsy was performed to assess gross lesions and to collect samples for histopathology as previously described ([Wilberts et al., 2014a](#)). Specifically, the lungs were examined for lesions related to the prior PRRSV infection, and the jejunum, ileum, cecum, and colon were examined for gross lesions of enteric disease. Sections of lung, distal ileum, cecum, spiral colon (apex), and descending colon were fixed in 10% neutral buffered formalin and were processed routinely at the ISU VDL for histopathology. All tissues were examined after hematoxylin and eosin staining, and sections of spiral colon were also examined following Warthin–Starry silver staining to detect spirochetes.

For histopathology, sections were semi-quantitatively scored by a pathologist blinded to the treatment groups using the following guidelines. Sections of lung were evaluated for lesions of pneumonia and scored 0 if no significant lesions, 1 if mild lymphohistiocytic interstitial pneumonia, 2 if moderate lymphohistiocytic interstitial pneumonia, and 3 if moderate lymphohistiocytic interstitial pneumonia and neutrophilic alveolar infiltration. Sections of ileum were examined for the presence or the absence of proliferative and/or neutrophilic ileitis typical of *Lawsonia* or *Salmonella* infection. Sections of cecum, spiral colon, and descending colon were each examined independently and scored 0 if no significant lesions; 1 if there was mild, diffuse mucosal thickening and lymphoplasmacytic infiltration of the lamina propria; 2 if there was moderate, diffuse mucosal thickening with

neutrophilic infiltration and mucus-filled crypts; and 3 if moderate to severe diffuse mucosal thickening with a combination of neutrophil infiltration, neutrophilic exudation, superficial hemorrhage, crypt ectasia, and luminal accumulations of mucus and erythrocytes. The semi-quantitative scores from the cecum, spiral colon, and descending colon were then combined to form a composite colitis score (CC) for each pig that was then used for statistical analysis. Sections of spiral colon that had been silver-stained were examined for the presence or the absence of spirochetes with features typical of *Brachyspira* spp.

Digesta and Fecal Sample Collection, Analysis, and Calculations

A representative feed sample from the complete diet and NFD were obtained for analysis. In the first replicate, fecal collections started when ~50% of pigs started to exhibit clinical signs (loose, watery stool; ~dpi 9) of Bhyo and ileal digesta collections followed. In both replicates, feces were collected from 9 to 11 dpi and pooled by pig. Ileal digesta was collected from 0800 to 1600 hours on 12 and 13 dpi. Digesta was collected by attaching a 207-mL plastic bag (Whirl-Pak; Nasco, Fort Atkinson, WI) to the opened cannula with a cable tie. Bags were removed when filled with digesta or every 30 min, whichever occurred first. Fecal samples were stored at -20°C until further analysis. Digesta samples were stored on dry ice in each room of the BSL2 facility during collections and transferred to -20°C after each collection day.

After the collection period, ileal and fecal samples were thawed and homogenized within pig. A subsample of digesta was collected, stored at -20°C , and lyophilized (Model 10-100; Virtis Co. Ltd., Gardiner, NY). Fecal samples were dried in a mechanical convection oven at 100°C . Feed, feces, and digesta samples were ground through a 1-mm screen (Model ZM1; Retsch Inc., Newton, PA) prior to analysis. Proximate analysis was performed on feed, feces, and ileal digesta samples as previously described (Schweer et al., 2018). Briefly, all samples were analyzed for DM (AOAC method 930.15), Cr_2O_3 according to Fenton and Fenton (1979), N using TruMac N (Leco Corporation, St. Joseph, MO), and GE using bomb calorimetry (Oxygen Bomb Calorimeter 6200, Parr Instruments, Moline, IL). Amino acid composition of feed and digesta samples was determined by the Agricultural Experiment Station Chemical Laboratories (University of Missouri-Columbia,

Columbia, MO) by cation-exchange HPLC (L8900 Amino Acid Analyzer, Hitachi High-Technologies Corporation, Tokyo, Japan).

After analysis of diet, feces, and digesta components, the AID and ATTD were calculated using the following equation (Stein et al., 2007):

$$\text{AID or ATTD} = 100 - \left[100 \times \frac{\left(\frac{\text{concentration of } \text{Cr}_2\text{O}_3 \text{ in diet} \times \text{concentration of component in feces or digesta}}{\text{concentration of } \text{Cr}_2\text{O}_3 \text{ in feces or digesta} \times \text{concentration of component in diet}} \right)}{\right]$$

The BEL of N and AA (g/kg DMI) were calculated using the following equation (Stein et al., 2007):

$$\text{BEL} = \left[\frac{\text{AA or N in digesta} \times (\text{Cr}_2\text{O}_3 \text{ in diet} \div \text{Cr}_2\text{O}_3 \text{ in digesta})}{\right]$$

Standardized ileal digestibility values for N and AA were calculated by correcting AID values for BEL using the following equation (Stein et al., 2007):

$$\text{SID} = [\text{AID} + (\text{BEL} \div \text{AA in diet})]$$

The study design was not a crossover; therefore, each pig could not serve as its own control for SID determination. Consequently, statistical analysis was performed on BEL values from the second replicate, and the reported treatment averages were applied to AID values from the first replicate to determine SID values.

Hindgut disappearance of DM (g/d), N (g/d), and GE (Mcal/d) was calculated using the following equation:

$$\text{hindgut disappearance} = \text{amount at terminal ileum} - \text{amount excreted in feces.}$$

Statistical Analysis

Data were initially analyzed to determine if there was an effect of PRRSV from the prior study or if there was an interaction between PRRSV and Bhyo challenge on any parameters. There was no PRRSV effect or interaction between PRRSV and Bhyo challenge for any parameters assessed ($P > 0.10$); therefore, data were analyzed to determine the main effect of Bhyo challenge. Digestibility, BEL, and hindgut disappearance data were analyzed using the MIXED procedure of SAS version 9.4 (SAS Inst. Inc. Cary, NC). Data from control and Bhyo pigs fed a complete diet were analyzed to determine treatment effects on AID, SID, and

hindgut disappearance of nutrients and energy. Data from control and Bhyo pigs fed NFD were analyzed separately from the pigs fed the complete diet to determine the impact of Bhyo on BEL and hindgut disappearance of nutrients and energy. The CC score was analyzed using the FREQ procedure with the Fisher's exact test to assess the effect of Bhyo challenge on lesion score distribution. Pig was considered the experimental unit for all analyses. All digestibility, BEL, and hindgut disappearance data are reported as least squares means $\pm$ SEM and considered significant if $P \leq 0.05$ and a trend if $P \leq 0.10$.

RESULTS AND DISCUSSION

Swine dysentery clinically affects the large intestine and induces lesions characterized by hemorrhage, necrosis (Albassam et al., 1985; Quintana-Hayashi et al., 2015), and increased mucin secretions (Wilberts et al., 2014b; Quintana-Hayashi et al., 2015). Considering that Bhyo infection induces intramural crosstalk between the small and large intestine (Kaleczyc et al., 2010), the extent to which this modulates nutrient, particularly AA, and energy digestibility and endogenous losses is largely undefined. This is important considering mucoproteins (i.e., mucins), host secretions (i.e., saliva, gastric secretions, digestive enzymes, and bile), sloughed cells, and bacterial proteins can all contribute to apparent ileal, total tract, and hindgut digestibility, and endogenous losses (Stein et al., 2007).

Differences in digestibility and BEL impact have ramifications for pig nutrition, growth, and metabolism. However, it is unclear to what degree, if any, SID of AA requirements and BEL change during these disease states in growing pigs. In pigs, infection with systemic and enteric pathogens causes nutrient (i.e., AA) and energy repartitioning that results in reduced tissue accretion rates, growth, and feed efficiency (Escobar et al., 2004; Curry et al., 2017; Schweer et al., 2017; Curry et al., 2018; Helm et al., 2018). We have previously shown that PRRSV challenge in grower pigs reduced BEL of Pro, Arg, and Ala during peak viremia, but only Pro BEL differed at seroconversion (Schweer et al., 2018). In similar size pigs, Lee (2012) reported increased BEL of several AA within 24 h of an *S. typhimurium* challenge; however, similar to PRRSV, BEL of AA returned to pre-inoculation values by 56 h post-inoculation. It is important to note that the extent of these differences will be dependent on the pathogen, severity and duration

of infection, the extent of cell necrosis and sloughing, and where the disease manifests anatomically within the gastrointestinal tract and epithelium.

Apparent Total Tract and Ileal Digestibility

To examine the extent to which Bhyo infection modulates nutrient and energy digestibility, AID and ATTD of AA and energy were determined during peak disease (dpi 9–15). The calculated and analyzed nutrient concentrations for the NFD and complete diet are presented in Table 2. Analyzed nutrient and energy composition of both diets were similar to calculated values. At the start of fecal (dpi 9) and ileal collections (dpi 12), a similar number of pigs were Bhyo positive and had developed SD in both replicates; therefore, it was assumed that a similar number of pigs had been infected prior to the diet change (NFD at 5 dpi). Apparent total tract digestibility of DM, N, and GE were determined from 9 to 11 dpi (Table 3) by fecal grab sampling. Compared to the Bhyo– pigs, Bhyo+ decreased DM, N, and GE ATTD coefficients by 3%, 8%, and 4%, respectively ($P < 0.02$). These reductions in ATTD coefficients are likely related to diarrhea and shortened passage time through the gut. The increased rate of passage can result in the insufficient enzymatic breakdown of feedstuffs leading to poor absorption (Blaxter and Wood, 1953; Bush et al., 1963). Further, diarrhea scores have been negatively correlated with ATTD in pigs (Entringer et al., 1975). Although we did not statistically compare diarrhea scores in the current study, Bhyo+ pigs had increased CC scores compared to Bhyo– pigs (Table 7) that can lead to increased diarrhea. We also report that Bhyo+ pigs had reduced ATTD, indicating increased endogenous losses and this is especially true if there is increased bacterial growth.

To assess the modulatory impact the Bhyo challenge may have in the small intestine, AID of DM, N, AA, and GE were assessed from 12 to 13 dpi. Although there were differences in ATTD, no differences were noted for AID of DM, N, total AA, or GE over this period. An increase in the AID of Gly ($P = 0.039$) was the only difference observed. This may not be surprising as the primary target of Bhyo is the cecum and colon, while small intestinal absorptive function may remain unaffected (Argenzio, 1980). Therefore, it is reasonable that the challenge did not alter AID coefficients while ATTD coefficients were reduced. This reduction in Gly AID may be a result of the need for Gly in purine and protein synthesis and the fact that Gly is readily metabolized by enterocytes (Wang et al., 2013;

Table 2. Calculated and analyzed nutrient composition of experimental diets, as-fed basis

Parameter	Calculated		Analyzed	
	Complete	NFD	Complete	NFD
DM, %	88.6	—	94.8	96.3
Energy, Mcal/kg ¹	3.69	3.71	3.90	3.82
CP, %	16.7	0.20	15.6	0.73
Indispensable AA, %				
Arg	0.87	0.01	0.86	0.01
His	0.39	0.01	0.39	0.02
Ile	0.59	0.01	0.69	0.02
Leu	1.30	0.03	1.41	0.05
Lys	0.90	0.00	1.07	0.03
Met	0.24	0.00	0.31	0.01
Phe	0.69	0.01	0.78	0.02
Thr	0.57	0.01	0.63	0.01
Trp	0.19	0.00	0.20	0.00
Val	0.66	0.01	0.76	0.02
Dispensable AA, %				
Ala	—	—	0.77	0.03
Asp	—	—	1.41	0.03
Cys	0.24	0.00	0.24	0.09
Glu	—	—	2.74	0.06
Gly	—	—	0.58	0.01
Pro	—	—	1.08	0.03
Ser	—	—	0.62	0.02
Tyr	0.47	0.01	0.53	0.01

Complete = corn-soy-based diet; NFD = nitrogen-free diet.

¹Calculated composition = Mcal ME/kg; analyzed composition = Mcal GE/kg.

Wang et al., 2014). Glycine also serves a precursor for glutathione and can prevent oxidative stress and cytokine response which are induced during Bhyo challenges (Naresh et al., 2009; Chmielewska et al., 2013; Wang et al., 2014).

Basal Endogenous Losses

Currently, BEL of N and AA are not well characterized in pathogen challenged livestock species. As infection with Bhyo results in increased mucin secretion (Wilberts et al., 2014b; Quintana-Hayashi et al., 2015), it was anticipated that endogenous secretions of mucin-related AA, namely Cys, Ser, and Thr, would be increased. Thus, we hypothesized that Bhyo infection would increase total tract N and ileal AA losses, particularly Cys, Ser, and Thr. Surprisingly, there was a 55% reduction in BEL of Pro ($P = 0.046$) and a tendency for BEL of Arg ($P = 0.092$), Trp ($P = 0.087$), Gly ($P = 0.096$), and total AA ($P = 0.081$) to be reduced in Bhyo+ pigs (Table 4). However, the AA abundant in mucin (i.e., Cys, Ser, and Thr) was unaffected. In contrast, nursery and growing pigs challenged with *S. typhimurium* had increased BEL of all AA within 24 h of infection (Lee, 2012). This discrepancy between

pathogen challenges may be a result of pathogen site of colonization as *S. typhimurium* can colonize both the small and large intestine (Côté et al., 2004), while Bhyo colonization is confined to the cecum and colon. Although total AA BEL tended to be reduced, BEL of N were numerically reduced compared to controls (4.40 vs. 2.71 g/kg DMI). Swine dysentery is characterized by a mucohemorrhagic diarrhea (Burrough, 2017). Expectedly then, total tract endogenous N loss was increased by Bhyo infection (4.89 vs. 1.03 g/kg DMI), although due to high variability and small sample size this was only numerically different. In the context of SD, this supports the localized impact of disease to the colon and suggests that the observed increase in mucin production is regulated locally as a diffuse increase in intestinal mucin production should manifest as an increase in BEL. Interestingly, reduced BEL and increased total tract N loss has also been reported during a PRRSV systemic infection (Schweer et al., 2018).

Although the use of NFD tends to result in overestimation of Pro and Gly BEL (Moughan et al., 1992), and when fed for extended periods Pro BEL increases (Jansman et al., 2002), BEL of Pro was drastically reduced due to Bhyo infection.

Table 3. Apparent total tract and ileal digestibility coefficients in non-infected and *Brachyspira hyodysenteriae* infected pigs

Parameter	Bhyo ⁻¹	Bhyo ⁺¹	SEM	P-value
ATTD ² , %				
DM	88.16	85.16	0.79	0.017
N	84.60	78.13	1.73	0.019
GE	86.58	83.32	0.78	0.012
AID ³ , %				
DM	86.86	86.55	0.71	0.759
N	85.98	85.74	0.60	0.788
Total AA	88.61	88.63	0.57	0.979
GE	86.83	86.24	0.66	0.541
Indispensable AA, %				
Arg	90.88	91.10	0.62	0.805
His	89.90	90.47	0.48	0.413
Ile	89.01	89.36	0.44	0.589
Leu	90.56	90.87	0.42	0.604
Lys	91.63	91.08	0.40	0.341
Met	92.93	92.75	0.37	0.734
Phe	89.54	89.89	0.43	0.576
Thr	86.32	86.51	0.49	0.796
Trp	88.98	89.90	0.42	0.146
Val	86.72	86.92	0.52	0.789
Dispensable AA, %				
Ala	87.12	86.88	0.63	0.793
Asp	88.05	88.69	0.40	0.278
Cys	83.50	84.51	0.65	0.289
Glu	91.67	92.16	0.50	0.506
Gly	76.78	80.14	1.04	0.039
Pro	86.97	87.92	1.08	0.543
Ser	87.67	87.82	0.44	0.806

¹Bhyo⁻ = control, non-challenged; Bhyo⁺ = *Brachyspira hyodysenteriae* challenged pigs.

²ATTD = apparent total tract digestibility.

³AID = apparent ileal digestibility.

Proline, its derivative hydroxyproline, and Gly are primary components of collagen (Eyre and Muir, 1975), which is involved in healing of the colonic wall after an insult (Hesp et al., 1984). Although Bhyo typically resides in the colonic crypts and luminal mucus, during severe infection colonocytes can become infected, detached, and slough away (Albassam et al., 1985), thus increasing the need for collagen for colonic wall reassembly. Arginine can also aid in healing of the colon (Shashidharan et al., 1999) and can improve antioxidant status (Ma et al., 2010), both of which are important for Bhyo resolution. Similarly, serotonin, a derivative of Trp, can mediate the stress response after infection by reducing glucocorticoid concentrations (Le Floch and Seve, 2007). Since increased glucocorticoid concentrations can stimulate *Brachyspira* spp. growth in culture (Naresh and Hampson, 2011),

Table 4. Basal endogenous loss of AA (g/kg DMI) in non-infected and *Brachyspira hyodysenteriae* infected pigs

Parameter	Bhyo ⁻¹	Bhyo ⁺¹	SEM	P-value ²
Fecal N	1.03	4.89	2.84	0.355
Ileal N	4.40	2.71	0.70	0.113
Total AA	23.32	13.47	3.69	0.081
Indispensable AA				
Arg	1.31	0.69	0.24	0.092
His	0.21	0.17	0.03	0.361
Ile	0.31	0.27	0.04	0.562
Leu	0.52	0.45	0.07	0.499
Lys	0.47	0.40	0.06	0.393
Met	0.08	0.07	0.01	0.776
Phe	0.33	0.29	0.05	0.555
Thr	0.58	0.49	0.08	0.460
Trp	0.15	0.10	0.02	0.087
Val	0.51	0.43	0.08	0.482
Dispensable AA				
Ala	0.82	0.53	0.14	0.165
Asp	0.87	0.69	0.13	0.325
Cys	0.23	0.18	0.03	0.286
Glu	0.98	0.78	0.13	0.294
Gly	3.04	1.72	0.52	0.096
Pro	11.95	5.38	2.11	0.046
Ser	0.60	0.45	0.09	0.238
Tyr	0.29	0.25	0.04	0.486

¹Bhyo⁻ = control, non-challenged; Bhyo⁺ = *Brachyspira hyodysenteriae* challenged pigs.

decreased BEL of Trp may be used for the synthesis of serotonin.

Standardized Ileal Digestibility

Standardized ileal digestibility of AA was determined by correcting the AID values for BEL (Table 5). The SID of N, Arg, Lys, Ala, Gly, Pro, and Ser were reduced ($P < 0.05$) in Bhyo⁺ pigs, and Thr SID tended to be reduced ($P = 0.088$). The greatest reductions occurred in SID of Pro and Gly, which were reduced by 32% and 16%, respectively. Few studies have determined BEL during infection in livestock species, and therefore, AA SID values during enteric infection are scarce. As mentioned, BEL of some AA can be overestimated when using an NFD, leading to overestimation of some SID values. Although only BEL of Pro was significantly reduced, reductions in SID of N, Pro, Arg, Ala, and Gly are likely due to decreased BEL.

Hindgut Disappearance

Bhyo targets the cecum and colon resulting in mucohemorrhagic colitis and cell sloughing

Table 5. Standardized ileal digestibility coefficients in non-infected and *Brachyspira hyodysenteriae* infected pigs

Parameter	Bhyo ⁻¹	Bhyo ⁺¹	SEM	P-value
N	103.59	96.49	0.60	<0.001
Total AA	103.21	97.06	0.57	<0.001
Indispensable AA, %				
Arg	105.27	98.72	0.62	<0.001
His	95.07	94.56	0.48	0.464
Ile	93.27	93.12	0.44	0.805
Leu	94.05	93.87	0.42	0.764
Lys	95.79	94.58	0.40	0.047
Met	95.23	94.92	0.37	0.565
Phe	93.55	93.43	0.43	0.836
Thr	94.98	93.41	0.61	0.088
Trp	95.33	94.77	0.50	0.439
Val	93.05	92.27	0.52	0.300
Dispensable AA, %				
Ala	97.25	93.40	0.63	0.001
Asp	93.89	92.90	0.51	0.189
Cys	92.39	91.66	0.65	0.440
Glu	95.07	94.85	0.50	0.753
Gly	126.51	106.47	1.77	<0.001
Pro	191.85	131.03	3.63	<0.001
Ser	96.84	94.71	0.44	0.004
Tyr	94.30	93.89	0.40	0.475

¹Bhyo⁻ = control, non-challenged; Bhyo⁺ = *Brachyspira hyodysenteriae* challenged pigs.

(Argenzio et al., 1980; Albassam et al., 1985). Therefore, it was anticipated that hindgut nutrient disappearance would decrease, resulting in a net nutrient appearance. Hindgut disappearance of DM, N, and GE was calculated from AID and ATTD values (Table 6). When pigs were fed a complete diet, Bhyo reduced the disappearance of N and GE ($P < 0.04$) and showed a strong tendency ($P = 0.055$) to reduce the disappearance of DM in the hindgut. However, when pigs were fed an NFD, only numerical reductions in DM, N, and GE disappearance were observed and were reduced by 99%, 172%, and 162%, respectively, but due to small sample size causing high variation, differences were not significant. Reduced disappearance, or increased appearance, of DM, N, and GE during Bhyo infection is expected due to hemorrhagic diarrhea, increased secretion of mucus, and cell sloughing (Argenzio et al., 1980; Albassam et al., 1985; Wilberts et al., 2014b). This could also be a reflection of altered microbial populations, and therefore microbial metabolites, commonly associated with Bhyo. Although microbial richness and diversity are reduced in pigs that develop clinical SD (Burrough et al., 2017), bacterial metabolism may be accelerated based on increased volatile fatty acid

production (Siba et al., 1996). This increase in volatile fatty acid production and ATP in the hindgut correlated to increased clinical presentation of SD (Siba et al., 1996; Durmic et al., 2002) which likely contributes to the increased appearance of energy in the hindgut. Energy utilization by the hindgut can contribute to maintenance energy and improve feed efficiency (Dierick et al., 1990); however, an increased loss of energy in the hindgut of Bhyo⁺ pigs could suggest an increased energy demand for the pigs.

Clinical Examination, *Brachyspira* Detection, and Pathology

Although the inoculum was administered into a T-cannula positioned in the distal ileum in this study, progression of disease was not markedly different from that of pigs that have been orally inoculated with the same Bhyo isolate, where clinical signs are typically observed within 7 to 10 dpi (Kinyon et al., 1977; Wilberts et al., 2014a; Wilberts et al., 2014b). End BW was not different Bhyo⁻ and Bhyo⁺ pigs in replicate 1 (67.6 ± 2.35 vs. 69.0 ± 3.12 kg BW) or replicate 2 (61.4 ± 4.20 vs. 61.2 ± 2.13 kg BW). Gross and microscopic lung lesions consistent with prior PRRSV infection were predominantly unapparent to mild (scores 0 to 1) in both Bhyo⁻ and Bhyo⁺ pigs. A total of four animals had microscopic lung scores of 2, and a single animal scored 3. The small intestines were grossly unremarkable and microscopic lesions were not observed in the sections of ileum examined.

All pigs were confirmed culture negative for *Brachyspira* spp. prior to dpi 0 and the challenge model was confirmed via fecal PCR and culture for Bhyo (Table 7). Expectedly, Bhyo⁻ pigs in both replicates remained negative for Bhyo throughout the 2-wk study and clinical scores of feces were normal. In the first replicate, MD was first observed on dpi 8, and 50% (5/10) of Bhyo⁺ pigs were culture positive for Bhyo by dpi 9 when fecal collections began. At necropsy, 70% (7/10) of Bhyo⁺ pigs were still culture positive, and two were exhibiting MD, suggesting full resolution had not occurred and there were persistent shedders. In the second replicate, MD was first observed on dpi 5, 4 Bhyo⁺ pigs exhibited MD by dpi 8, and 45% (5/11) of Bhyo⁺ pigs were positive for Bhyo by 9 dpi based on PCR. Interestingly, MD had completely resolved by dpi 10 in the second replicate, and at necropsy, only one Bhyo⁺ pig was Bhyo positive by culture. These data suggest the NFD reduced the disease duration and helped to eliminate infection and shedding as the

Table 6. Hindgut nutrient and energy disappearance in non-infected and *Brachyspira hyodysenteriae* infected pigs fed complete or nitrogen-free diet

Parameter	Complete ¹				NFD ²			
	Bhyo- ³	Bhyo+ ³	SEM	P-value	Bhyo-	Bhyo+	SEM	P-value
DM, g/d	21.44	-21.04	14.36	0.055	127.49	0.25	70.84	0.226
N, g/d	-0.05	-2.93	0.74	0.036	5.87	-4.28	5.50	0.215
GE, Mcal/d	-20.2	-188.2	50.65	0.033	462.5	-289.4	456.13	0.265

Positive value denotes disappearance, negative value denotes appearance.

¹Complete = corn-soy-based diet.

²NFD = nitrogen-free diet.

³Bhyo- = control, non-challenged; Bhyo+ = *Brachyspira hyodysenteriae* challenged pigs.

Table 7. Pigs positive for and lesions frequency of *Brachyspira hyodysenteriae*

		Day post-inoculation					CC ¹ score frequency			
	Pre-inoculation	5	7	9	12	14/15	0 to 2	3 to 5	6 to 9	<i>P</i> -value
Complete diet ²										
Bhyo ⁻³ (<i>n</i> = 7 pigs)	0/7	0/7	0/7	0/7	0/7	0/7	7/7	0/7	0/7	0.258
Bhyo ⁺³ (<i>n</i> = 10 pigs)	0/10	0/10	0/10	5/10	4/10	7/10	3/10	4/10	3/10	
NFD ^{4,5}										
Bhyo ⁻ (<i>n</i> = 4 pigs)	0/4	—	—	0/4	0/4	0/4	4/4	0/4	0/4	
Bhyo ⁺ (<i>n</i> = 11 pigs)	0/11	—	—	5/11	4/11	1/11	8/11	3/11	0/11	

¹CC score = composite colitis score reflecting the combined inflammatory scores from the cecum, spiral colon, and descending colon (max score = 9; detailed methods in the text).

²Positive by fecal culture for *Brachyspira hyodysenteriae*.

³Bhyo- = control, non-challenged; Bhyo+ = *Brachyspira hyodysenteriae* challenged pigs.

⁴NFD = nitrogen-free diet.

⁵PCR positive for *Brachyspira hyodysenteriae*.

number of Bhyo+ pigs still culture positive at necropsy was reduced from 70% to 9%. Regardless of diet, no Bhyo- pigs had a CC score >2; while 70% (7/10) and 27% (3/11) of Bhyo+ pigs fed complete diet or NFD, respectively, had a score of 3 or more (Table 7). Further, Bhyo+ pigs fed a complete diet had an increased incidence of severe scores (scores 6 to 9) compared to NFD fed, Bhyo+ pigs (30% vs. 0%, respectively). This further supports the protective effect of the NFD diet on SD expression in this study. Given that the NFD was based on cornstarch, and thereby highly digestible, this finding is consistent with a previous experiment where feeding a highly digestible, rice-based diet was protective against Bhyo infection and SD expression (Pluske et al., 1996).

The relationship between diet and clinical presentation and shedding of *Brachyspira* has been explored with somewhat contradicting results. While there is general agreement that diet can significantly alter SD expression, the specific substrates responsible for these differences are not entirely clear. It is generally agreed that limiting fermentation in the hindgut reduces Bhyo colonization and SD expression (Siba et al., 1996; Pluske

et al., 1998; Wilberts et al., 2014a); however, there is disagreement as to which types of fiber have the greatest impact with studies showing soluble fiber sources may increase disease (Pluske et al., 1998), soluble fiber sources such as inulin can decrease disease (Hansen et al., 2010; Hansen et al., 2011), and insoluble fibers sources such as lignins can increase SD expression (Wilberts et al., 2014a). Additionally, Durmic et al. (2000), Kirkwood et al. (2000), and Durmic et al. (2002) were unable to demonstrate the protective effect of reducing soluble non-starch polysaccharides or resistant starch content of the diets suggesting the dietary effect is likely multifactorial. Although a cornstarch-based NFD is non-physiological and not commercially relevant, it further supports the notion that highly digestible and poorly fermentable diets are beneficial for resolution of Bhyo infection as the NFD contained minimal lignin or cellulose.

CONCLUSION

The objective of this study was to determine the extent to which Bhyo modulates nutrient, energy, and AA digestibility and BEL of AA. Expectedly, Bhyo infection and associated diarrhea reduced

ATTD relative to controls. However, contrary to what was hypothesized, the results of this study suggest that Bhyo has minimal impact on small intestinal BEL of AA. Therefore, Bhyo did not impact AA digestibility in the same way as other enteric pathogens and the results were more similar to a systemic challenge. This is not unexpected given that Bhyo infection is limited to the colon, whereas other enteric pathogens typically affect the small intestine directly and are more likely to affect digestibility. This suggests that while many pathogens cause diarrhea, not all diseases act similarly with regard to AA digestibility and metabolism and consideration should be given to pathogens individually when considering dietary interventions in sick pigs. In addition, increased appearance of N and GE in the hindgut of Bhyo+ pigs is likely associated with decreased N and energy balance which attributes to reduced growth performance commonly seen with Bhyo infection. Therefore, we assume that the AA and energy needs are likely increased from decreased SID of N, Arg, Lys, and some nonessential AA, and a decreased energy contribution from the hindgut associated with Bhyo infection.

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