A soluble and highly fermentable dietary fiber with carbohydrases improved gut barrier integrity markers and growth performance in F18 ETEC challenged pigs[1]

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ABSTRACT: This study aimed to evaluate the effects of a source of dietary soluble (**SF**) and insoluble fiber (**IF**) without or with exogenous carbohydrases (xylanase, β-glucanase, and pectinase) on diarrhea incidence, selected immune responses, and growth performance in enterotoxigenic *Escherichia coli* (**ETEC**)-challenged pigs. Sixty weaned pigs (6.9 ± 0.1 kg BW, ~23 d of age) were blocked by initial BW and placed in individual pens. Pens were randomly assigned to one of six treatments (n = 10 per treatment), including a nonchallenged control (**NC**), a positive challenge control (**PC**), the PC + a soluble fiber diet (10% sugar beet pulp) without (**SF−**) or with carbohydrases (**SF+**), and PC + an IF diet (15% corn distillers dried grains with solubles) without (**IF−**) or with carbohydrases (**IF+**). The control diet was primarily based on corn and soybean meal with 13.5% whey powder. The two sources of fiber were added at the expense of cornstarch in the control diet. Pigs were orally inoculated with 6 mL hemolytic F18 ETEC (~3.5×10^9 cfu/mL) or sham infected with 6 mL phosphate-buffered saline on day 7 (0 d postinoculation, **dpi**) postweaning. All ETEC challenged pigs were confirmed to be genetically susceptible to F18 ETEC. Pigs had free access to feed and water throughout the 14-d trial. Pig BW and feed intake were recorded on dpi −7, 0, and 7 or 8. Fecal swabs were collected on dpi −7, 0, 1, 2, 3, 5, and 7 or 8 to evaluate hemolytic *E. coli* shedding. Fecal score was visually ranked daily postchallenge to evaluate diarrhea incidence. Blood samples were collected on dpi −1, 3, and 7 or 8 at necropsy and intestinal tissues were collected at necropsy. Pigs on PC had lower dpi 1 to 7 ADG and ADFI than those on NC ($P < 0.05$). Compared with PC pigs, SF+ pigs had greater ADG during both pre- and postchallenge period ($P < 0.05$). The IF− increased postchallenge diarrhea incidence compared with PC ($P < 0.05$). Pigs on SF− had lower ileal *E. coli* attachment than PC ($P < 0.05$). The SF+ reduced haptoglobin and IF+ reduced C-reactive protein on dpi 3 compared with PC ($P < 0.05$). Compared with PC pigs, SF+ pigs tended to have lower ileal *tumor necrosis factor alpha* and greater ileal *occludin* (***OCLN***) mRNA ($P < 0.10$) and had greater ($P < 0.05$) colonic *OCLN* mRNA levels. Collectively, IF− increased incidence of diarrhea and fecal *E. coli* shedding compared with PC. The SF+ pigs had improved growth compared with PC pigs, likely due in part to a reduction in inflammatory intermediates.

Key words: diarrhea, dietary fiber, enterotoxigenic *E. coli*, enzymes, immune response, swine

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INTRODUCTION

Postweaning diarrhea (**PWD**) associated with enterotoxigenic *Escherichia coli* (**ETEC**) usually occurs between 4 and 14 d postweaning and causes economic losses for pig producers worldwide (Fairbrother and Gyles, 2012). The ETEC, mainly F4 and F18 strains, adheres to specific receptors on enterocytes through fimbria followed by colonization and secretion of enterotoxins, which ultimately lead to secretory diarrhea in weaned pigs (Zhang et al., 2007; Luppi, 2017). With the use of antibiotics in animal production being restricted, alternative nutritional strategies are needed to control PWD and improve piglet health and performance. Dietary fiber is indigestible by pig endogenous enzymes but can be degraded by intestinal microbes. It is widely accepted that feeding dietary fiber may be beneficial to gut function (in terms of physiology, mucosal immunity, and barrier integrity) and microbioal population through microbial fermentation in the hindgut of pigs (Molist et al., 2014; Jha and Berrocoso, 2015).

There is growing interest in the inclusion of dietary fiber in pig diets to improve intestinal barrier function (e.g., increased tight junction protein mRNA; Chen et al., 2013) and enhance disease resistance through modulation of intestinal microbiota, microbial metabolites, and immune response (e.g., production of pro- and anti-inflammatory cytokines). However, results regarding the impact of dietary fiber on PWD caused by ETEC have been inconsistent, probably due to differences in the sources, chemical composition, inclusion level, and physiochemical properties of the fibers used, as well as individual differences in gut microbial populations and health status of pigs (Hopwood et al., 2004; Wellock et al., 2008; Molist et al., 2010).

Exogenous carbohydrases aid in fiber degradation and liberate entrapped nutrients, thus can be used in swine diets with high fiber contents to improve growth performance (Tsai et al., 2017; Li et al., 2018). Carbohydrases, particularly xylanase, have been shown to break down nonstarch polysaccharides in fibrous feed ingredients to release low molecular weight oligosaccharides or monosaccharides (Lærke et al., 2015; Pedersen et al., 2015). This results in the release of entrapped nutrients and subsequent improvements in energy and nutrient digestibility (Patience, 2017; Zeng et al., 2018) and an associated decrease in digesta viscosity (Lærke et al., 2015). A recent study in our research team showed that a blend of xylanase, β-glucanase, and cellulase improved small intestinal markers of barrier integrity (Li et al., 2018). Thus, it is reasonable to hypothesize that adding carbohydrases to fiber containing diets could further promote intestinal barrier function, beneficially modulate intestinal microbiota, and decrease the occurrence of diarrhea, which eventually leads to improved performance. Currently, limited information is available about the potential protective effect of carbohydrase on ETEC induced PWD in pigs.

Therefore, the objectives of this study were to evaluate the effects of a source of dietary soluble (**SF**) and insoluble fiber (**IF**) without or with exogenous carbohydrases (xylanase, β-glucanase, and pectinase) on the incidence of diarrhea, immune response, and growth performance in ETEC-challenged weaned pigs. It was hypothesized that the inclusion of a source of SF or IF would mitigate PWD and the addition of carbohydrases would provide additional benefits on alleviating PWD and improving performance.

MATERIALS AND METHODS

All procedures for this experiment adhered to guidelines for the ethical and humane care of animals used for research and were approved by the Institutional Animal Care and Use Committee at Iowa State University (IACUC #6-16-8306-S and #16-I-0027-A) prior to the start of this study.

Animals, Diets, and Experimental Design

Sixty newly weaned piglets (6.9 ± 0.1 kg; ~23 d of age; L337 × Camborough; PIC Inc., Hendersonville, TN) were blocked by initial BW and allocated to individual pens. Pens were randomly assigned to one of six treatments (n = 10 per treatment), including a nonchallenged control (**NC**), a positive challenge control (**PC**), the PC + a soluble fiber diet without (**SF−**) or with carbohydrases (**SF+**), and the PC + an IF diet without (**IF−**) or with carbohydrases (**IF+**). Three feed enzymes were added on top of the SF− and IF− diets to make the SF+ and IF+ diets, with 0.01% pectinase (Pectinase ABE; 56 PE per kg of diet), 0.01% xylanase (Econase XT; 19,000 BXU per kg of diet), and 0.001% β-glucanase (Econase GT P; 23,200 BU per kg of diet), based on the manufacturer's recommendations (AB Vista, Plantation, FL). The control diet was primarily based on corn and soybean meal with 13.5% whey powder. Sugar beet pulp (**SBP**; 10%) or corn distillers dried grains with solubles (**DDGS**; 15%) were added to the control diet at the expense of cornstarch to supply the soluble or IF, respectively.

Pelleted SBP was ground to a similar particle size as DDGS using a 2.5-mm screen to avoid the confounding effect of different particle sizes. All diets were formulated to meet or exceed NRC (2012) estimates of requirements of weaned pigs and did not contain antibiotics or pharmacological levels of copper or zinc (Table 1). Pigs had free access to feed presented in mash form and water throughout the 14-d experiment. Each pen (~4 ft^2) was equipped with a four-space polyethylene dry feeder and one nipple drinker. Analyzed nutrient composition of SBP, DDGS, and diets is shown in Table 2.

This experiment was conducted in a biosecurity level 2 facility at Iowa State University. Challenged pigs were housed in two rooms (25 pigs per room; five pigs per treatment) and nonchallenged pigs were housed in a separate room. Strict biosecurity procedures were followed to avoid ETEC

Table 1. Ingredient composition of the experimental diets (as-fed basis, %)

	Diets[1]		
Item	Control	SF	IF
Corn	41.79	42.11	42.40
Cornstarch	15.00	5.00	—
Soybean meal, 46.5% CP	15.00	15.00	15.00
DDGS[2]	—	—	15.00
Sugar beet pulp	—	10.00	—
Fish meal, menhaden select	5.00	5.00	5.00
Whey powder, >61% lactose	13.50	13.50	13.50
Casein	4.00	4.00	4.00
Soybean oil	2.50	2.50	2.50
Monocalcium phosphate	0.56	0.56	0.20
Limestone	0.95	0.73	1.10
Sodium chloride	0.25	0.25	0.25
L-Lys HCl	0.50	0.46	0.42
DL-Met	0.23	0.22	0.11
L-Thr	0.20	0.18	0.09
L-Trp	0.05	0.04	0.03
L-Val	0.07	0.05	-
Vitamin premix[3]	0.25	0.25	0.25
Trace mineral premix[4]	0.15	0.15	0.15
Calculated nutrient levels, %			
ME, Mcal/kg	3.53	3.43	3.42
NE, Mcal/kg	2.68	2.53	2.50
CP	19.96	20.71	23.67
Ether extract	4.67	4.77	5.22
Neutral detergent fiber	5.04	9.56	10.16
Acid detergent fiber	2.00	4.35	4.55
Total dietary fiber	8.24	14.99	13.03
Starch	40.07	31.17	28.30
Calcium	0.80	0.80	0.80
Total P	0.60	0.61	0.64
STTD P	0.43	0.43	0.43
SID Lys	1.44	1.44	1.44
SID Met + Cys	0.79	0.79	0.79
SID Thr	0.85	0.85	0.85
SID Trp	0.26	0.26	0.26

[1]SF = soluble fiber diets without or with carbohydrases; IF = insoluble fiber diets without or with carbohydrases; the carbohydrases were added on top of the SF and IF diets, with 0.01% pectinase (Pectinase ABE; 56 PE per kg of diet), 0.01% xylanase (Econase XT; 19,000 BXU per kg of diet), and 0.001% β-glucanase (Econase GT P; 23,200 BU per kg of diet), based on the manufacturer's recommendations (AB Vista, Plantation, FL).

[2]Distiller's dried grains with solubles.

[3]Provided per kilogram of diet: 7,656 IU vitamin A, 875 IU vitamin D, 63 IU vitamin E, 4 mg vitamin K, 70 mg niacin, 34 mg pantothenic acid, 14 mg riboflavin, and 0.06 mg vitamin B_{12}.

[4]Provided per kg of diet: 165 mg Zn (zinc sulfate), 165 mg Fe (iron sulfate), 39 mg Mn (manganese sulfate), 17 mg Cu (copper sulfate), 0.3 mg I (calcium iodate), and 0.3 mg Se (sodium selenite).

Table 2. Analyzed nutrient composition of fibrous ingredients and experimental diets (as-fed basis, %)

	Ingredient[2]		Diets[3]				
Item[1]	Beet pulp	DDGS	Control	SF−	SF+	IF−	IF+
DM	92.78	89.57	89.77	90.05	90.06	89.98	89.63
GE, Mcal/kg	3.82	4.48	4.02	4.05	4.06	4.17	4.17
CP	9.41	28.09	18.40	19.38	19.74	23.21	22.32
aEE	1.94	7.04	5.00	5.33	5.57	6.61	6.25
NDF	35.70	28.67	4.83	8.52	8.32	9.54	9.67
ADF	22.14	7.85	1.48	3.44	3.22	2.65	2.51
Hemicellulose	13.56	20.83	3.34	5.08	5.10	6.88	7.16
SDF	17.10	1.70	0.70	2.10	1.90	1.00	1.20
IDF	43.80	31.10	8.60	11.60	11.20	11.30	11.40
TDF	60.90	32.80	9.30	13.70	13.10	12.30	12.60

[1]GE = gross energy; aEE = acid ether extract; NDF = neutral detergent fiber; ADF = acid detergent fiber; SDF = soluble dietary fiber; IDF = insoluble dietary fiber; TDF = total dietary fiber; hemicellulose = NDF – ADF; TDF = SDF + IDF.

[2]Distillers dried grains with solubles.

[3]SF−: soluble fiber diet included 10% sugar beet pulp without carbohydrases; SF+: soluble fiber diet included 10% sugar beet pulp with carbohydrases; IF−: insoluble fiber diet 15% distiller's dried grains with solubles without carbohydrases; IF+: insoluble fiber diet 15% distiller's dried grains with solubles with carbohydrases; the carbohydrases were added on top of the SF and IF diets, with 0.01% pectinase (Pectinase ABE; 56 PE per kg of diet), 0.01% xylanase (Econase XT; 19,000 BXU per kg of diet), and 0.001% β-glucanase (Econase GT P; 23,200 BU per kg of diet), based on the manufacturer's recommendations (AB Vista, Plantation, FL).

contamination of the nonchallenged pigs. All pigs were given 6 mL of freshly grown F18 ETEC inoculum (~3.5 × 10^9 cfu/mL) or 6 mL of a sham inoculum of PBS via oral gavage on day 7 (0 d postinoculation, **dpi**) postweaning. Sows and piglets used in this experiment were not vaccinated against *E. coli* before this trial. None of the pigs shed hemolytic *E. coli* on dpi −7. All ETEC challenged pigs were confirmed to be genetically susceptible to F18 ETEC by genotype sequencing of the α (1,2) fucosyltransferase-1 gene according to Frydendahl et al. (2003).

Inoculum Preparation

A hemolytic F18 ETEC strain expressing heat-labile, heat-stable b, and enteroaggregative *E. coli* heat-stable enterotoxin 1 was recovered from the intestine of a nursery pig with enteric colibacillosis and used to prepare the bacterial inoculum. Briefly, a fresh bacterial culture grown (~18 h at 37 °C) on blood agar was used to inoculate two bottles (each having 50 mL) of sterile tryptic soy broth (**TSB**), which were then incubated overnight at 37 °C with shaking. The broth cultures were then transferred to two new bottles (each containing 450 mL fresh TSB) and incubated for an additional 5 h at 37 °C with shaking. The bacterial culture was centrifuged for 15 min at 3,000 × *g* and the pellet was suspended in sterile PBS. The OD_{600} of the culture in PBS was measured to be about 7.0 using a spectrometer, which had ~3.5 × 10^9 cfu/mL as determined using viable plate count.

Sample Collection

Pig BW and feed intake were recorded on dpi −7, 0, and 7 or 8 at the end of the trial to calculate ADG, ADFI, and G:F. Fresh fecal swabs were collected by rectal swabbing on dpi −7, 0 (immediately before ETEC inoculation), 1, 2, 3, 5, and 7 or 8 to evaluate hemolytic *E. coli* shedding. After collecting rectal swabs for feces, the rectal temperature was obtained from every pig via rapid-response digital electric thermometers (Model V911F/V912F, KAZ, Incorporated, Hudson, NY). Fecal score was visually ranked daily using the following scale: 1 = solid, 2 = semi-solid, 3 = semi-liquid, and 4 = liquid. Fecal score ≥3 was considered diarrhea (Liu et al., 2013). The incidence of diarrhea (%) = (total pig days with a fecal score ≥ 3)/(total pig days) × 100. Blood samples were collected from the jugular vein by venipuncture into 10-mL vacuum containers (Becton Dickinson, Franklin Lakes, NJ) on dpi −1 (as baseline), 3 (expected peak response), and 7 or 8 at necropsy (recovery). The blood was centrifuged at 2,000 × *g* for 10 min at 4 °C. The resulting sera were aliquoted into 1.5-mL microcentrifuge tubes and stored at −80 °C for later analysis of acute phase proteins (**APPs**).

Each half of the pigs (five pigs per treatment) was euthanized on dpi 7 and 8 by captive bolt stunning followed by exsanguination. For the convenience of result description, dpi 7 was used to indicate necropsy days and the end of this trial. Posteuthanasia, the abdomen was opened and the entire gastrointestinal tract was removed. The ileum (30 cm from the ileal–cecal junction)

and mid-colon tissues were collected, rinsed with ice-cold PBS, snap-frozen in liquid N, and stored at −80 °C pending analysis of gene expression. Three 1-cm pieces of jejunum and ileum were fixed with 10% neutral buffered formalin for 24 h and then transferred to 75% alcohol for later microscopic assessment of *E. coli* attachment.

Chemical Analyses

Diets and the two fibrous ingredients were ground to 1 mm and analyzed in duplicate for DM (method 930.15; AOAC, 2007), nitrogen (method 990.03; AOAC, 2007; TruMac; LECO Corp., St. Joseph, MI), and acid-hydrolyzed ether extract (method 2003.06; AOAC, 2007). The EDTA was used (9.56% N; Leco Corporation) as a standard for N calibration and was determined to contain 9.56 ± 0.03% of N. CP was calculated as N × 6.25. Gross energy was determined in duplicate using an isoperibolic bomb calorimeter (Model 6200; Parr Instrument Co., Moline, IL). Benzoic acid (6,318 kcal GE/kg) was used as the standard for calibration and was determined to contain 6,316 ± 4 kcal GE/kg. Neutral and acid detergent fiber were analyzed in triplicate (Van Soest and Robertson, 1979). Soluble and IF were analyzed in duplicate (method 991.43; AOAC, 2007) using an Ankom TDF Fiber Analyzer (Ankom Technology, Macedon, NY).

Fecal Hemolytic E. coli Shedding

Fecal swabs were plated onto Remel Blood Agar (TSA with 5% sheep blood) followed by incubation at 35 °C for 24 h to determine hemolytic *E. coli* shedding using a semi-quantification method according to the growth of *E. coli* on the plates. The *E. coli* shedding was scored on a five-point scale from 0 to 4 with 0 representing no growth, 1 represens hemolytic colonies only in the primary streak, 2 represens compatible growth extending into the secondary streak, 3 represens growth into the tertiary streak, and 4 represents growth of hemolytic *E. coli* into the quaternary section of agar plate. The accumulative shedding score on each collection day was calculated by summing up the shedding scores of 10 pigs in each treatment. Identification of isolates as *E. coli* was confirmed by matching the mass spectrometry (**MS**) spectrum of the isolates with the MS spectra of *E. coli* in the open database using matrix-assisted laser desorption ionization time-of-flight mass spectrometry (MALDI-TOF MS; Singhal et al., 2015) at the Iowa State University Veterinary Diagnostic Laboratory (**ISU VDL**).

E. coli Attachment to Epithelial Cells in the Small Intestine

Formalin fixed jejunum and ileum were routinely processed and embedded in paraffin wax at the ISU VDL. Three transverse sections (5 µm) were cut from both jejunum and ileum, stained with hematoxylin and eosin, and mounted on glass slides. The *E. coli* attachment on epithelial cells was visualized using a microscope (OLYMPUS BX 53, Center Valley, PA) with a 40× power. Each section was scored as either 0 if there was no attachment (Supplemental Figure 1A) or 1 if there were ≥5 villi that had *E. coli* attached among all the villi of each section (Supplemental Figure 1B). The *E. coli* attachment frequency (%) of jejunum or ileum was calculated by summing up the scores of all three sections on each glass slide and then divided by 3.

Serum APP

Haptoglobin and C-reactive protein (**CRP**) concentration in the serum were analyzed in duplicate using commercially available porcine ELISA kits according to the manufacturer's instructions (Immunology Consultants Laboratory, Inc., Portland, OR).

RNA Isolation and Quantitative PCR

Approximately 50 to 100 mg of ileal tissue was homogenized in 1 mL of Trizol (Invitrogen, Carlsbad, CA) using the Qiagen Tissuelyser II (Germantown, MD). Total RNA was then isolated according to the manufacturer's recommendations. Concentration and RNA purity was measured using a spectrophotometer (ND-100; NanoDrop Technologies, Inc., Rockland, DE). All samples had 260:280 nm ratios above 1.8. Isolated RNA (1 µg) was used for cDNA synthesis using the QuantiTect Reverse Transcription Kit (Qiagen GmbH, Hilden, Germany) according to the manufacturer's instructions. All cDNA samples were diluted 10-fold with nuclease free water for qPCR reactions.

Real-time quantitative PCR was performed in 20-µL reactions using iQ SYBR Green Supermix (Bio-Rad Laboratories, Inc., Hercules, CA). Each reaction included 10 µL of SYBR Green Supermix, 1 µL of each forward and reverse primer (10 µM), 2 µL of cDNA and 6 µL of nuclease free water. Gene-specific primer sequences are shown in Table 3. A no-reverse transcriptase control, a water control, and a pooled cDNA reference sample was included in each 96-well plate. Each sample was performed

Table 3. Primer sequences used for quantitative PCR

Gene	Primer sequence	Product size, bp	GenBank accession
TNFα	F: CACCACGCTCTTCTGCCTAC	132	X57321
	R: ACGGGCTTATCTGAGGTTTGAGACG		
IL-1B	F: TGGCCCACACATGCTGAA	84	NM_214055
	R: CCTTGCACAAAGCTCATGCA		
IL-6	F: GGCTGTGCAGATTAGTACC	124	AF518322
	R: CTGTGACTGCAGCTTATCC		
IL-8	F: AGGACCAGAGCCAGGAA	172	NM_213867
	R: GTGGAATGCGTATTTATGC		
IL-10	F: TGGGTTGCCAAGCCTTGT	61	L20001
	R: GCCTTCGGCATTACGTCTTC		
CLDN1	F: GATTTACTCCTACGCTGGTGAC	199	AJ318102
	R: CACAAAGATGGCTATTAGTCCC		
CLDN3	F: TTGCATCCGAGACCAGTCC	85	NM_001160075
	R: AGCTGGGGAGGGTGACA		
OCLN	F: AACTCCCGTCAGCAGATCC	95	NM_001163647
	R: ATCAGTGGAAGTTCCTGAACCA		
ZO-1	F: CTCTTGGCTTGCTATTCG	197	XM_003353439
	R: AGTCTTCCCTGCTCTTGC		
TLR4	F: CAGATAAGCGAGGCCGTCATT	113	AB232527
	R: TTGCAGCCCACAAAAAGCA		
CD14	F: CCTCAGACTCCGTAATGTG	180	AB267810
	R: CCGGGATTGTCAGATAGG		
MYD88	F: GCTGGAACAGACCAACTAT	153	NM_001099923.1
	R: TCCTTGCTTTGCAGGTAAT		
CFTR	F: ACTATGGACCCTTCGAGCCT	123	NM_001104950
	R: CGCATTTGGAACCAGCGTAG		
RPL19	F: AACTCCCGTCAGCAGATCC	147	AF435591
	R: AGTACCCTTCCGCTTACCG		

TNFα = tumor necrosis factor alpha; *CLDN1 = claudin-1*; *CLDN3 = claudin-3; OCLN = occludin*; *ZO-1 = zonula occludens-1*; *TLR4 = toll-like receptor 4*; *CD14 = cluster of differentiation 14*; *MYD88 = myeloid differentiation primary response 88*; *CFTR = cystic fibrosis transmembrane conductance regulator*; *RPL19 = ribosomal protein L19.*

in triplicate. The PCR cycling conditions included 5-min initial denaturation at 95 °C followed by 40 PCR cycles (95 °C for 30 s, 60 °C for 30 s, and 72 °C for 30 s) and a dissociation curve to confirm amplification of a single PCR product. Optical detection was performed at 55 °C. Fluorescence of SYBR Green was quantified with the iQ5 Real-Time PCR Detection System and cycle threshold (**CT**) value for each reaction was obtained by analysis of amplification plots with the iQ5 Optical System Software version 2.0 (Bio-Rad Laboratories Inc.). Ribosomal protein L19 (**RPL19**) was used as an endogenous reference gene. The mRNA abundance for each sample was normalized to RPL19 and the pooled sample using the $2^{-\Delta\Delta CT}$method (Livak and Schmittgen, 2001).

Statistical Analysis

Data were analyzed according to the randomized complete block design using PROC GLIMMIX of SAS (9.4; SAS Institute Inc., Cary, NC). Two pigs in PC treatment were removed due to excessive loss of weight (>1 kg). Probably due to weaning stress, a few pigs had negative G:F values during the prechallenge period. All G:F < −2 were removed from the analysis. Treatment, sex, and their interaction were fixed effects. Pen was the experimental unit. Block was a random effect.

Fecal score and *E. coli* shedding score data were analyzed using a multinomial model in PROC GENMOD. The frequency of occurrence of each score was identified in the FREQ statement. Diarrhea incidence data were analyzed by a χ^2 test. The odds ratio of each treatment was reported. The *E. coli* attachment data were analyzed using PROC GLIMMIX with a binomial distribution. Preplanned contrasts were performed using the ESTIMATE statement to evaluate the effects of the ETEC challenge (NC vs. PC) and dietary treatment (SF−, SF+, IF−, or IF+ vs. PC), and to compare the effect of fiber sources (SF vs. IF), without or with enzymes, as well as fiber by enzyme interactions. Least square means of treatment were

reported. Differences were considered significant if $P \leq 0.05$ and a tendency if $0.05 < P \leq 0.10$.

RESULTS

Growth Performance

Pigs on the PC had lower ($P < 0.05$) postchallenge (dpi 1 to 7) ADG and ADFI than those on NC, resulting in lower ($P < 0.05$) final BW and overall ADG (Table 4). During the prechallenge period, SF− had no impact on pig performance parameters compared with PC, whereas SF+ improved ($P < 0.05$) pig BW and ADG, and tended ($P < 0.10$) to improve G:F. Growth performance of pigs fed IF, regardless of exogenous carbohydrases addition, was not different from those fed PC during the prechallenge period. During the postchallenge period, pigs on SF+ had greater ($P < 0.05$) ADG and a trend ($P < 0.10$) for greater ADFI than PC pigs. This resulted in improved ($P < 0.05$) final BW, ADG, and G:F in pigs fed SF+ compared with PC during the 14-d trial. The SF− pigs tended ($P < 0.10$) to have improved ADG during dpi 1 to 7 and had improved ($P < 0.05$) overall G:F compared with PC pigs. A tendency ($P < 0.10$) for greater ADG during dpi 1 to 7 and the overall period, but not ADFI or G:F, was observed in IF− pigs compared with PC pigs. Growth performance of pigs on IF+ did not differ from PC during the pre- and postchallenge or overall period.

Diarrhea Incidence, Fecal Score, and Rectal Temperature

No mortality occurred during the experiment. During the prechallenge period (dpi −7 to 0), the

Table 4. Effect of soluble or IF without or with carbohydrases on growth performance and diarrhea incidence in weaned pigs challenged with F18 ETEC[1]

	Treatment[2]							Contrast *P*-value[3]		
			SF		IF					
Item	NC	PC	SF−	SF+	IF−	IF+	SEM	Fiber	Enzyme	F × E
Pig no.	10	8	10	10	10	10				
BW, kg										
Dpi −7	6.86	6.84	7.03	6.89	6.93	6.84	0.25	0.416	0.220	0.812
Dpi 0	7.87	7.36	8.07	8.40*	8.22#	7.52	0.40	0.201	0.520	0.078
Dpi 7	9.90*	8.57	9.82#	10.34*	9.93*	9.17	0.58	0.211	0.777	0.136
Dpi −7 to 0										
ADG, kg	0.14	0.09	0.15	0.22*	0.18	0.10	0.04	0.305	0.833	0.063
ADFI, kg	0.24	0.17	0.18	0.24	0.21	0.16	0.03	0.404	0.974	0.076
G:F	0.78	0.60	0.87	0.91#	0.86	0.66	0.01	0.210	0.435	0.245
Dpi 1 to 7										
ADG, kg	0.29*	0.17	0.25#	0.28*	0.244#	0.236	0.03	0.397	0.731	0.527
ADFI, kg	0.45*	0.31	0.39	0.43#	0.37	0.36	0.05	0.294	0.806	0.590
G:F	0.69	0.55	0.53	0.64	0.62	0.69	0.08	0.341	0.196	0.775
Overall										
ADG, kg	0.22*	0.13	0.20	0.25*	0.22#	0.17	0.03	0.266	0.986	0.107
ADFI, kg	0.31#	0.23	0.27	0.32#	0.28	0.24	0.03	0.294	0.843	0.208
G:F	0.62	0.51	0.76*	0.76*	0.65	0.65	0.18	0.179	0.981	0.950
Diarrhea, %[4]										
Dpi −7 to 0	0	0	5.71	2.86	5.71	1.43	—	0.622	0.133	0.622
Dpi 1 to 7	7.14*	40.00	31.43	27.14	57.14*	40.00	—	<0.001	0.075	0.337

*Significant difference compared with PC ($P \leq 0.05$).

#Tendency for difference compared with PC ($0.05 < P \leq 0.10$).

[1]n = 10 pigs per treatment except for PC with eight pigs.

[2]SF = soluble fiber diets included 10% sugar beet pulp without or with carbohydrases; IF = insoluble fiber diets 15% distiller's dried grains with solubles without or with carbohydrases; SF− = soluble fiber diet without carbohydrases; SF+ = soluble fiber diet with carbohydrases; IF− = insoluble fiber diet without carbohydrases; IF+ = insoluble fiber diet with carbohydrases; the carbohydrases contained 0.01% pectinase (Pectinase ABE; 56 PE per kg of diet), 0.01% xylanase (Econase XT; 19,000 BXU per kg of diet), and 0.001% β-glucanase (Econase GT P; 23,200 BU per kg of diet), based on the manufacturer's recommendations (AB Vista, Plantation, FL).

[3]Fiber = dietary fiber type effect (SF vs. IF); Enzyme = carbohydrases effect (without vs. with); F × E = fiber type by carbohydrases interaction effect.

[4]Diarrhea incidence (%) = (total number of pigs with diarrhea score ≥ 3)/(total number of pigs) × 100; statistical analysis was conducted by a χ^2 test.

incidence of diarrhea and the fecal scores were not different among treatments (Table 4; Figure 1A). During the postchallenge period, pigs on PC had a greater incidence of diarrhea than NC, but a lower incidence of diarrhea than IF− ($P < 0.01$). Meanwhile, PC decreased ($P < 0.01$) the odds ratio of the lower fecal score compared with NC, but increased the odds ratio of the lower fecal score compared with the IF− treatment (Figure 1B). In addition, the main effect of SF reduced ($P < 0.05$) the incidence of diarrhea compared with IF; enzyme supplementation tended ($P < 0.10$) to decrease the incidence of diarrhea compared with the two fiber diets without enzymes (SF− and IF−). There were no differences in rectal temperature among dietary treatments during the pre- or the postchallenge period (data not shown).

E. coli Shedding Score

Pigs on NC had no hemolytic *E. coli* shedding and thus greater ($P < 0.01$) odds ratio of lower hemolytic *E. coli* shedding score compared with PC (Figure 2A). Pigs fed SF, regardless of enzyme addition, did not differ from PC in their postchallenge *E. coli* shedding score. The IF− reduced ($P < 0.01$) and IF+ tended ($P < 0.10$) to reduce the odds ratio of lower *E. coli* shedding score compared with PC. Pigs fed the SF diets had greater odds ratio of lower hemolytic *E. coli* shedding score than IF diets ($P < 0.01$). No hemolytic *E. coli* shedding was detected in NC pigs during dpi 1 to 7 before the ETEC challenge (Figure 2B).

E. coli Attachment to Epithelial Cells

No differences were observed for *E. coli* attachment on jejunal epithelium among treatments (data not shown). As expected, pigs on NC had no *E. coli* attachment in the ileum (Figure 3). Compared with PC pigs, SF− pigs had decreased ($P < 0.05$) *E. coli* attachment in the ileum. No differences in *E. coli* attachment in the ileum were observed between pigs fed PC and those fed SF+, IF−, or IF+.

Serum APPs

There were no differences in serum haptoglobin or CRP between PC pigs and NC pigs on any collection day (Table 5). Serum haptoglobin concentration was similar among treatments before challenge. There was a fiber × enzyme interaction for CRP on dpi −1, such that enzyme addition decreased ($P < 0.01$) CRP concentration in IF diets, but not in SF diets. On dpi 3, enzyme supplementation decreased ($P < 0.05$) haptoglobin concentration, regardless of fiber source. Serum haptoglobin on dpi 3 was lower ($P < 0.05$) in SF+ pigs, but not SF− pigs, compared with those on PC. The IF+ tended ($P < 0.10$) to decrease haptoglobin on dpi 7 and decreased ($P < 0.05$) CRP concentration on dpi 3 compared with PC. No diet effects were observed for CRP on dpi 7.

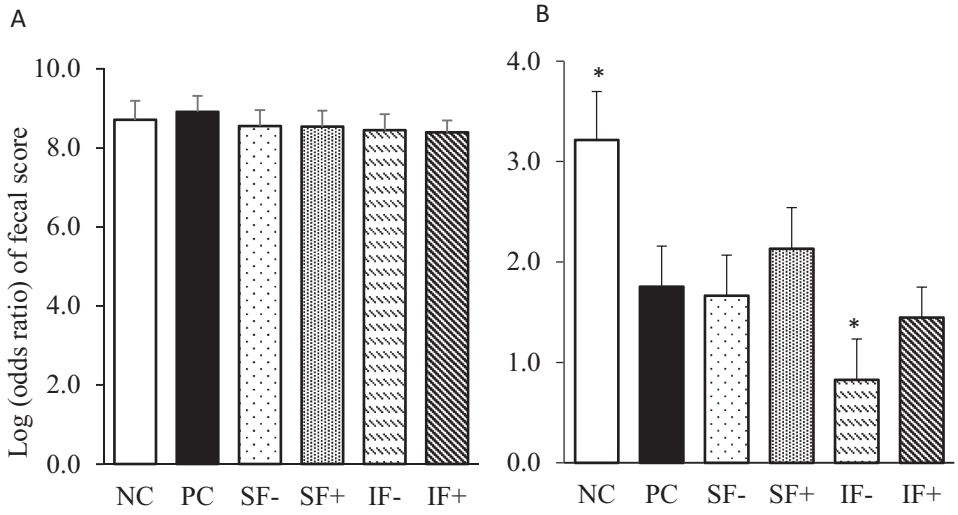

Figure 1. Effects of soluble or IF without or with carbohydrases on the fecal score of pigs challenged with ETEC. (**A**) Prechallenge log (odds ratio) of fecal score, (**B**) postchallenge log (odds ratio) of fecal score. NC = nonchallenge control; PC = positive challenge control; SF− = soluble fiber diet included 10% sugar beet pulp without carbohydrases; SF+ = soluble fiber diet included 10% sugar beet pulp with carbohydrases; IF− = insoluble fiber diet 15% distiller's dried grains with solubles without carbohydrases; IF+ = insoluble fiber diet 15% distiller's dried grains with solubles with carbohydrases. The carbohydrases contained 0.01% pectinase (Pectinase ABE; 56 PE per kg of diet), 0.01% xylanase (Econase XT; 19,000 BXU per kg of diet), and 0.001% β-glucanase (Econase GT P; 23,200 BU per kg of diet) according to the manufacturer's recommendations (AB Vista, Plantation, FL). P (IF vs. SF) < 0.001, P (enzyme) = 0.013, P (fiber × enzyme) > 0.10; *significant difference between NC vs. PC and IF− vs. PC ($P < 0.05$).

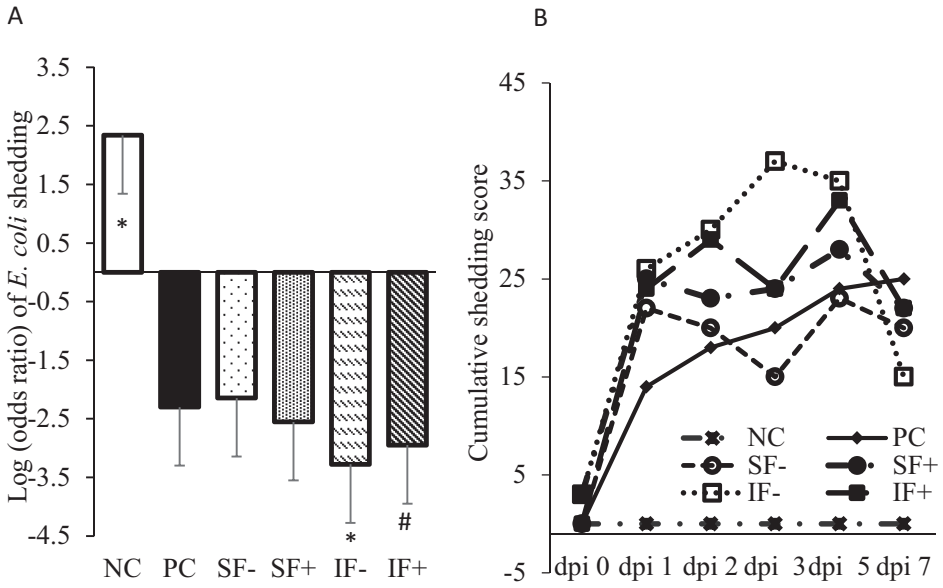

Figure 2. Effects of soluble or IF without or with carbohydrases on hemolytic *E. coli* shedding score of pigs challenged with ETEC. (A) Postchallenge log (odds ratio) of *E. coli* shedding score; (**B**) postchallenge cumulative *E. coli* shedding score. The accumulative shedding score on each collection day was calculated by summing up the shedding scores of 10 pigs in each treatment. NC = nonchallenge control; PC = positive challenge control; SF− = soluble fiber without carbohydrases; SF+ = soluble fiber with carbohydrases; IF− = insoluble fiber without carbohydrases; IF+ = insoluble fiber with carbohydrases. The carbohydrases contained 0.01% pectinase (Pectinase ABE; 56 PE per kg of diet), 0.01% xylanase (Econase XT; 19,000 BXU per kg of diet), and 0.001% β-glucanase (Econase GT P; 23,200 BU per kg of diet) according to the manufacturer's recommendations (AB Vista, Plantation, FL). *P* (IF vs. SF) < 0.01, *P* (enzyme) > 0.10, *P* (fiber × enzyme) > 0.10. *Significant difference between NC vs. PC and IF− vs. PC (*P* < 0.01). #Trends between IF+ vs. PC (*P* < 0.10).

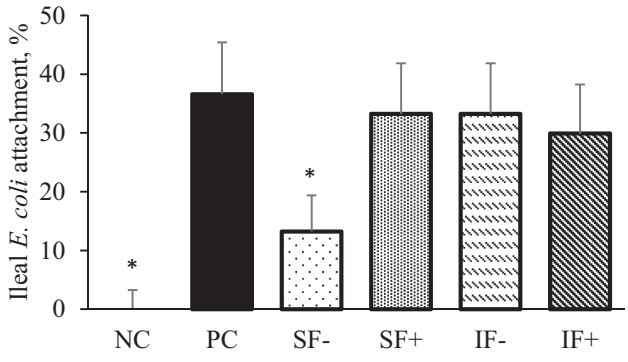

Figure 3. Effects of soluble or IF diet without or with carbohydrases on ileal *E. coli* attachment of pigs challenged with ETEC. NC = nonchallenge control; PC = positive challenge control; SF− = soluble fiber without carbohydrases; SF+ = soluble fiber with carbohydrases; IF− = insoluble fiber without carbohydrases; IF+ = insoluble fiber with carbohydrases. The *E. coli* attachment frequency (%) was calculated by summing up the scores (*E. coli* attachment as 1 and no attachment as 0) of all three ileal sections on the glass slide and then divided by 3. The carbohydrases contained 0.01% pectinase (Pectinase ABE; 56 PE per kg of diet), 0.01% xylanase (Econase XT; 19,000 BXU per kg of diet), and 0.001% β-glucanase (Econase GT P; 23,200 BU per kg of diet) according to the manufacturer's recommendations (AB Vista, Plantation, FL). *Significant difference between NC vs. PC and SF− vs. PC (*P* < 0.05).

Ileal and Colonic Gene Expression

Pigs on NC had lower mRNA abundance for ***IL****-8*, *toll-like receptor 4* (***TLR4***), *cluster of differentiation 14* (***CD14***), and greater mRNA abundance of *claudin-1* (***CLDN1***) in the ileum than those on PC (*P* < 0.05; Table 6). There were no differences in mRNA levels of *IL-1B, IL-6*, *claudin-3* (***CLDN3***), *myeloid differentiation factor 88,* and *cystic fibrosis transmembrane conductance regulator* in both ileum and colon among any treatments (data not shown). The ileal mRNA levels of *tumor necrosis factor alpha* (***TNF***α), *IL-10*, *occludin* (***OCLN***), and *zonula occludens 1* (***ZO-1***) did not differ between NC and PC. The SF− pigs tended (*P* < 0.10) to have lower *IL-10* and had greater (*P* < 0.05) *CLDN1* mRNA in the ileum compared with PC pigs. A trend (*P* < 0.10) for lower *TNFα* and greater *OCLN* mRNA abundance in the ileum was observed in SF+ pigs than PC pigs. The IF+ tended (*P* = 0.052) to decrease *TLR4* and decreased (*P* < 0.05) *CD14* mRNA in the ileum compared with PC. In the colon, greater (*P* < 0.05) mRNA abundance of *ZO-1* was observed in NC pigs than those on PC. Pigs fed SF+ tended (*P* = 0.051) to have greater *ZO-1* and had greater (*P* < 0.05) mRNA abundance of *OCLN* than pigs on PC. The IF− diet did not impact mRNA levels of any genes in the ileum and colon. The main effect of the SF diet increased (*P* < 0.05) mRNA expression of *CLDN1* in the ileum and *OCLN* and *ZO-1* in the colon compared with IF diet. A fiber × enzyme interaction was observed for colonic *IL-10* mRNA abundance; IF− increased (*P* < 0.05) *IL-10* mRNA compared with SF−, but IF+ did not differ from SF+.

Table 5. Effect of soluble or IF without or with carbohydrases on serum haptoglobin and C-reactive protein (**CRP**) in weaned pigs challenged with F18 ETEC[1]

	Treatment[2]							Contrast *P*-value[3]		
			SF		IF					
Item	NC	PC	SF−	SF+	IF−	IF+	SEM	Fiber	Enzyme	F × E
Haptoglobin, mg/mL										
dpi −1	1.81	1.74	1.35	1.74	1.35	1.86	0.53	0.712	0.259	0.953
dpi 3	0.52	0.65	0.29	0.34*	0.52	0.29	0.21	0.035	0.042	0.287
dpi 7	0.58	0.86	0.57	0.40	0.83	0.41#	0.36	0.344	0.565	0.948
CRP, μg/mL										
dpi −1	304	382	328	379	485	190	116	0.579	0.136	0.012
dpi 3	200	423	346	353	333	189*	160	0.358	0.681	0.217
dpi 7	184	149	153	150	385	122	183	0.947	0.246	0.184

*Significant difference compared with PC ($P \leq 0.05$).

#Tendency for difference compared with PC ($0.05 < P \leq 0.10$).

[1]n = 10 pigs per treatment except for PC with eight pigs; P (day) < 0.05 and P (day × trt) > 0.10 for haptoglobin and CRP; least square means and pooled SEM were from data before log transformation.

[2]SF = soluble fiber diets included 10% sugar beet pulp without or with carbohydrases; IF = insoluble fiber diets 15% distiller's dried grains with solubles without or with carbohydrases; SF− = soluble fiber diet without carbohydrases; SF+ = soluble fiber diet with carbohydrases; IF− = insoluble fiber diet without carbohydrases; IF+ = insoluble fiber diet with carbohydrases; the carbohydrases contained 0.01% pectinase (Pectinase ABE; 56 PE per kg of diet), 0.01% xylanase (Econase XT; 19,000 BXU per kg of diet), and 0.001% β-glucanase (Econase GT P; 23,200 BU per kg of diet), based on the manufacturer's recommendations (AB Vista, Plantation, FL).

[3]Fiber = dietary fiber type effect (SF vs. IF); Enzyme = carbohydrases effect (without vs. with); F × E = fiber type by carbohydrases interaction effect.

Table 6. Effect of soluble or IF without or with carbohydrases on ileal and colonic gene mRNA abundance in weaned pigs challenged with F18 ETEC[1]

	Treatment[3]							Contrast *P*-value[4]		
			SF		IF					
Gene[2]	NC	PC	SF−	SF+	IF−	IF+	SEM	Fiber	Enzyme	F × E
Ileum										
TNFα	1.56	1.66	1.48	1.17#	1.45	1.46	0.25	0.357	0.420	0.328
IL-8	0.59*	1.40	1.27	1.50	1.50	1.59	0.56	0.588	0.270	0.804
IL-10	0.47	0.86	0.45#	0.53	0.61	0.47	0.21	0.581	0.934	0.868
CLDN1	2.32*	0.96	3.11*	1.14	1.22	0.77	0.93	0.026	0.086	0.757
OCLN	0.76	0.78	1.56	2.11#	1.79	1.17	0.53	0.219	0.481	0.787
ZO-1	1.76	1.32	1.47	1.41	1.45	1.21	0.27	0.423	0.497	0.466
TLR4	1.35*	1.68	1.62	1.42	1.49	1.38#	0.18	0.471	0.314	0.934
CD14	1.00*	1.32	1.22	1.22	1.13	1.01*	0.18	0.252	0.925	0.441
Colon										
TNFα	1.30	1.30	1.15	1.38	1.35	1.28	0.23	0.711	0.367	0.323
IL-8	1.49	0.90	1.07	1.40	1.04	1.43	0.32	0.724	0.167	0.978
IL-10	1.21	1.36	1.05	1.59	2.04	1.31	0.34	0.237	0.797	0.038
CLDN1	1.19	1.49	2.10	0.92	0.90	0.66	1.00	0.487	0.567	0.436
OCLN	1.16	0.92	1.18	1.29*	0.88	0.93	0.18	0.011	0.385	0.615
ZO-1	1.18*	0.87	0.93	1.13#	0.81	0.87	0.11	0.024	0.172	0.525

*Significant difference compared with PC ($P \leq 0.05$).

#Tendency for difference compared with PC ($0.05 < P \leq 0.10$).

[1]n = 10 pigs per treatment except for PC with eight pigs; least square means of fold change were reported.

[2]*TNFα = tumor necrosis factor alpha*; *IL = interleukin*; *CLDN1 = claudin-1*; *OCLN = occludin*; *ZO-1 = zonula occludens-1*; *TLR4 = toll-like receptor 4*; *CD14 = cluster of differentiation 14.*

[3]SF = soluble fiber diets included 10% sugar beet pulp without or with carbohydrases; IF = insoluble fiber diets 15% distiller's dried grains with solubles without or with carbohydrases; SF− = soluble fiber diet without carbohydrases; SF+ = soluble fiber diet with carbohydrases; IF− = insoluble fiber diet without carbohydrases; IF+ = insoluble fiber diet with carbohydrases; the carbohydrases contained 0.01% pectinase (Pectinase ABE; 56 PE per kg of diet), 0.01% xylanase (Econase XT; 19,000 BXU per kg of diet), and 0.001% β-glucanase (Econase GT P; 23,200 BU per kg of diet), based on the manufacturer's recommendations (AB Vista, Plantation, FL).

[4]Fiber = dietary fiber type effect (SF vs. IF); Enzyme = carbohydrases effect (without vs. with); F × E = fiber type by carbohydrases interaction effect.

DISCUSSION

PWD induced by ETEC impairs growth performance of piglets due to decreased feed intake, intestinal villus atrophy, and an elevated inflammatory response. Previous research evaluating the impact of dietary fiber (different solubility, viscosity, and fermentability) in ETEC-challenged pigs have reported inconsistent results (Hopwood et al., 2004; Wellock et al., 2008; Molist et al., 2010). This study evaluated the effect of two fibrous ingredients (DDGS and SBP) that are commonly available and used in North American swine diets without or with carbohydrases in pigs challenged with F18 ETEC.

Postinoculation, pigs on PC presented an increased incidence of diarrhea, shedding of hemolytic *E. coli*, and adhesion of *E. coli* on ileal enterocytes compared with pigs on NC, confirming that the challenge model was successful. As expected, the ETEC challenge decreased pig BW and ADG during dpi 1 to 7 and the overall period, in agreement with previous research (Song et al., 2012; Liu et al., 2013). This was partly due to the reduction in ADFI and increased diarrhea postchallenge. Compared with PC pigs, while SF− pigs tended to increase ADG during dpi 1 to 7, SF+ pigs significantly improved ADG during both dpi 1 to 7 and the overall experimental period. This indicates that the inclusion of SF− alleviated the growth depression caused by ETEC infection and the addition of exogenous carbohydrases further mitigated the negative effects of ETEC infection on growth. Limited research has evaluated the effect of carbohydrase supplementation in diets containing SBP in pigs. Nevertheless, under nonchallenged conditions, carbohydrase addition in poultry diets containing 7.5% SBP improved ADG and ADFI compared with diets without carbohydrases (Abdel-Hafeez et al., 2018). Taken together, the data suggest that the addition of multiple carbohydrases degraded the fiber components (mainly pectin) in the SBP diet to liberate the cell wall entrapped nutrients (starch and protein) for digestion and absorption in the small intestine, which will be used by the pig with greater efficiency than if fermented in the hindgut (Patience, 2017). Furthermore, the released oligosaccharides from fiber degradation may enhance gut barrier function and reduce intestinal immune activation (Chen et al., 2012). It is also possible that enzyme supplementation in SBP-diets decreased endogenous losses of AA (from mucin secretion and sloughed epithelial cells) in the small intestine compared with SBP-diets without enzymes (Cowieson and Bedford, 2009).

Soluble fiber from SBP, irrespective of enzyme addition, did not reduce the incidence of diarrhea and the shedding of hemolytic *E. coli* compared with PC. Soluble fiber from pear barley and carboxymethylcellulose in nursery diets has previously been reported to increase digesta viscosity and stimulate ETEC proliferation in the intestine, thereby exacebating PWD (Hopwood et al., 2004; Montagne et al., 2004). However, Van Nevel et al. (2006) reported no increase in digesta viscosity by feeding 12% SBP in nursery diets. Because digesta viscosity was not measured, it remains unknown whether the 10% SBP used in this study resulted in increased viscosity compared with the control diet and whether the increase caused negative effects in the pigs. Despite the fact that the exact mechanisms of soluble fiber from SBP on performance and ETEC proliferation are not yet known, the inclusion of SBP in pig diets was reported to beneficially modulate intestinal microbial populations, such as reduces pathogenic bacteria and increases *Bifidobacterium* and *Lactobacillus* counts (Thomson et al., 2012; Laitat et al., 2015; Yan et al., 2017). Thus, the decreased attachment of *E. coli* on ileal epithelium in SF− group herein may result from increased beneficial bacteria (e.g., *Lactobacillus*) and decreased pathogenic bacteria in the small intestine (Roselli et al., 2007). It would also be possible that some soluble fibrous fractions in SBP directly bind to intestinal ETEC (González-Ortiz et al., 2014), leading to the decreased *E. coli* attachment in SF−.

In contrast, the inclusion of IF from DDGS, regardless of enzyme addition, increased hemolytic *E. coli* shedding compared with PC, suggesting increased pathogenic *E. coli* proliferation in the intestine. Furthermore, IF− increased the fecal score and the incidence of diarrhea compared with PC, in contradiction to results reported by Mateos et al. (2006) and Molist et al. (2010). This indicates the sources and physiochemical properties, in addition to solubility, of dietary fiber affect the pig's response to bacterial infection (Molist et al., 2014). The exact reason for increased diarrhea and *E. coli* shedding in IF− remains unclear. One potential contributing factor for the diarrhea could be the increased CP content of the IF diet by 4.2% compared with the control diet (Kim et al., 2011; Opapeju et al., 2015). Additionally, feeding 30% DDGS decreased the relative abundance of *Lactobacillus* spp. in the colonic microbiota of pigs relative to pigs fed a standard corn–soy diet (Burrough et al., 2015). Such a reduction in *Lactobacilli* may lead to increased *E. coli* shedding

because *Lactobacillus* can limit ETEC adhesion (Roselli et al., 2007).

Besides causing diarrhea, ETEC infection is also known to increase intestinal permeability through disruption of the tight junction structure (reduction of *OCLN* expression, delocalization of ZO-1, and dissociation of occludin from intercellular junctions; Berkes et al., 2003; Mukiza and Dubreuil, 2013). The decreased ileal *CLDN1* and colonic *ZO-1* mRNA abundance following an ETEC challenge observed herein agrees with previous findings (Gao et al., 2013). Tight junctions play a critical role in maintaining intestinal epithelial barrier integrity, which prevents bacteria and bacterial products (e.g., endotoxin) translocation across the intestinal tissues and the subsequent activation of an immune response (Roselli et al., 2007). The greater mRNA abundance of ileal and colonic *OCLN* and colonic *ZO-1* in pigs fed SF+ compared with the PC suggests improved small and large intestinal paracellular barrier integrity, which in turn can reduce translocation of luminal pathogenic bacteria and bacterial products (e.g., endotoxins) and result in decreased markers of inflammation (ileal *TNFα* and serum haptoglobin; Cutler et al., 2007). The greater mRNA abundance for tight junction proteins in pigs fed SF compared with those fed IF may result from enhanced microbial fermentation and therefore increased production of fermentation products, especially acetate and butyrate (unpublished data in this study; Schiavon et al., 2004); these have been demonstrated to promote intestinal epithelial barrier integrity and regulate the inflammatory response (Wang et al., 2018).

APPs are liver-derived proteins and their concentrations in the blood are modulated by pro-inflammatory cytokines (e.g., TNF-α, IL-1β, and IL-6) after an immune challenge (Carroll et al., 2004; Jain et al., 2011). Elevated serum APP concentrations on day 3 and up to day 11 post-ETEC infection were previously reported (Houdijk et al., 2007; Liu et al., 2013; Kim et al., 2016). In contrast, the ETEC challenge in PC did not increase haptoglobin or CRP on dpi 3 and 7 compared with NC, suggesting a lack of systemic inflammation elicited by ETEC. The discrepancy in inflammatory response to an ETEC challenge is associated with the severity of the infection, the strain or dose of the inoculum, and type of enterotoxins expressed by the ETEC strain (Pavlova et al., 2008; Zhou et al., 2012; Loos et al., 2012). Another explanation might be that collecting blood on dpi 3 may have already missed the peak response because maximum serum APP concentration is typically reached within 24 to 48 h after the inflammation (Jain et al., 2011). Nevertheless, pigs on SF+ had lower haptoglobin and IF+ had lower CRP than PC pigs on dpi 3, which may indicate the addition of enzymes in fiber diets reduced systemic inflammation activation of pigs challenged with ETEC (Kiarie et al., 2009). This in turn potentially diverts more nutrients and energy toward growth production (Huntley et al., 2018; Li et al., 2018).

An experimental ETEC challenge induces not only systemic but also a local inflammatory response (Liu et al., 2013). In addition to recognition by antigen-presenting cells (M cells or dendritic cells) in the lamina propria, luminal bacteria can also be taken up by enterocytes through endocytosis, causing increased release of pro-inflammatory cytokines (Snoeck et al., 2005). The ETEC challenge in the current study increased mRNA levels of *TLR4*, *CD14*, and *IL-8*, indicating immune activation. These findings agree with Liu et al. (2014) who reported upregulated mRNA expression of genes related to immune activation after an ETEC infection. The increased *IL-8* mRNA with ETEC infection was also reported by Roselli et al. (2007). Increased production of IL-8 was associated with pathogen-induced disruption of the epithelial barrier (Otte and Podolsky, 2004; Roselli et al., 2007). The decreased mRNA levels for ileal *CLDN1* and colonic *ZO-1* in pigs fed PC compared with those fed NC agrees with the notion of perturbed barrier function. Despite the observation that SF− enhanced *CLDN1* and SF+ increased *OCLN* and *ZO-1* compared with PC, no significant differences were observed in pro-inflammatory cytokines mRNA abundance except for *TNFα* (tended to be decreased by SF+). One possible explanation would be that the inclusion of dietary fiber accelerated the rate of recovery of pigs from an ETEC infection, thus resulting in no differences in cytokine expression levels when tissues were collected at the end of the trial (Correa-Matos et al., 2003).

In conclusion, the F18 ETEC challenge resulted in colonization of hemolytic *E. coli* as evidence by fecal shedding and adherence of *E. coli* to ileal epithelial cells, which consequently increased the incidence of diarrhea and caused decreases in ADFI and ADG during dpi 1 to 7. Compared with PC, SF+ improved ADG during both the pre- and postchallenge period. This may be partly due to increased ADFI and markers of gut barrier integrity (*OCLN* and *ZO-1* mRNA), and reduced markers of inflammation (*TNFα* mRNA and serum haptoglobin). The SF− tended to have improved ADG during dpi 1 to 7, which was likely associated with decreased

ileal *E. coli* attachment and increased ileal *CLDN1* mRNA levels, compared with PC. Pigs on IF− had a greater incidence of diarrhea and *E. coli* shedding than pigs on PC without negatively affecting growth performance. Collectively, inclusion of SBP, a soluble and highly fermentable fiber combined with a carbohydrase complex, may be used to improve growth performance in pigs under moderate F18 ETEC challenge. The use of corn DDGS, an insoluble and less fermentable fiber, should be avoided in nursery pig diets if they are at risk of PWD. Future studies are warranted to explore the exact mechanisms whereby carbohydrase supplementation in SBP containing diets improved growth performance.

Conflict of interest statement. None declared.

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