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Non Ruminant Nutrition

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Growth performance and gut health of *Escherichia coli*–challenged weaned pigs fed canola meal-containing diet

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Abstract

An experiment was conducted to evaluate the effects of including canola meal (**CM**) in diets for weaning pigs challenged with a F18 strain of *Escherichia coli* on growth performance and gut health. A total of 36 individually housed weaned pigs (initial body weight [**BW**] = 6.22 kg) were randomly allotted to one of the three diets (12 pigs/diet). The three diets were corn–soybean meal (**SBM**)-based basal diet (control diet) and the basal diet with 0.3% zinc oxide, 0.2% chlortetracycline, and 0.2% tiamulin (antibiotic diet) or with 20% CM diet. The diets were fed in two phases: Phase 1: days 0 to 7 and Phase 2: days 7 to 20. All pigs were given an oral dose of 2×10^9 CFU of F18 strain of *E. coli* on day 7. Fecal score was assessed daily throughout the trial. Dietary antibiotics increased ($P < 0.05$) overall average daily gain (**ADG**) and average daily feed intake (**ADFI**) compared by 48% and 47%, respectively. Dietary CM increased ($P < 0.05$) overall ADG and ADFI by 22% and 23%, respectively; but the ADG and ADFI values for CM-containing diet did not reach those for the antibiotics-containing diet. Dietary antibiotics reduced ($P < 0.05$) fecal score; however, dietary CM unaffected fecal score. Dietary antibiotics decreased ($P < 0.05$) liver weight per unit live BW by 16% at day 20, whereas dietary CM did not affect liver weight per unit live BW (29.2 vs. 28.6). Also, dietary antibiotics increased ($P < 0.05$) serum triiodothyronine and tetraiodothyronine levels for day 14, whereas dietary CM did not affect the serum level of these hormones. Dietary antibiotics reduced ($P < 0.05$) the number white blood cells and neutrophils by 38% and 43% at day 20, respectively, whereas dietary CM tended to reduce ($P = 0.09$) the number white blood cells by 19% at day 20. The number white blood cells for CM diet tended to be greater ($P < 0.10$) than that for antibiotics diet. The dietary antibiotics decreased ($P < 0.05$) the concentration of individual volatile fatty acids and hence of total volatile fatty acid in cecum by 61% at day 20, whereas dietary CM decreased ($P < 0.05$) cecal butyric acid concentration by 61% and tended to reduce ($P < 0.10$) total volatile fatty acid concentration by 30% at day 20. In conclusion, the dietary inclusion of 20% CM improved ADG and tended to reduce white blood cell counts. Thus, inclusion of CM in antibiotics-free corn-SBM-based diets for weaned pigs that are challenged with F18 strain of *E. coli* can result in their improved performance partly through a reduction of the inflammatory response.

Key words: canola meal, *Escherichia coli*, fecal score, growth performance, weaning pigs

Abbreviations

ADF	acid detergent fiber
ADFI	average daily feed intake
ADG	average daily gain
BW	body weight
CM	canola meal
CP	crude protein
CTC	Chlortetracycline
DM	dry matter
EE	ether extract
ETEC	enterotoxigenic *E. coli*
G:F	gain-to-feed ratio
GIT	gastrointestinal tract
IDF	insoluble dietary fiber
IgA	immunoglobulin A
IgG	immunoglobulin G
IgM	immunoglobulin M
IGF-1	insulin-growth like factor-1
NDF	neutral detergent fiber
NE	net energy
NSP	nonstarch polysaccharides
SBM	soybean meal
SID	standardized ileal digestibility
SDF	soluble dietary fiber
T3	Triiodothyronine
T4	Tetraiodothyronine
TNF-α	tumor necrosis factor-α
qPCR	quantitative polymerase chain reaction
VFA	volatile fatty acid
WBC	white blood cell
ZnO	zinc oxide

Introduction

The conventional solvent-extracted Napus canola meal (**CM**) is the second most widely used protein source in the swine diets (after soybean meal; **SBM**) because it contains a well-balanced amino acid profile (Newkirk et al., 2003). However, CM contains glucosinolates and has a higher fiber content than SBM, which can limit its use in swine diets (Roth-Maier et al., 2004; Khajali and Slominski, 2012; Woyengo et al., 2014). Indeed, the inclusion of the CM in diets for growing and finishing pigs at 30% resulted in reduced growth performance (Smit et al., 2014). However, we recently observed a nonsignificant change in growth performance of weaned pigs due to an increase in dietary level of CM from 0% to 40% (Hong et al., 2020). Others also observed a nonsignificant change in growth performance of weaned pigs due to dietary inclusion of CM at 20% (Landero et al., 2011) or 40% (Parr et al., 2015). Weaned pigs, compared with growing and finishing pigs, are more susceptible to gut infections by pathogenic microorganisms such as enterotoxigenic *Escherichia coli* (**ETEC**; Fairbrother et al., 2005; Zhang et al, 2007). High proportion (>90%) of total fiber in CM is insoluble (Bell, 1993), and insoluble fiber increases digesta passage rate, thereby reducing the proliferation of *E. coli* by decreasing adhesion of the *E. coli* to the intestinal mucosa (Becker et al., 2009; Molist et al., 2014). The glucosinolates present in CM can be degraded in the gastrointestinal tract (**GIT**) by dietary and gut microbial myrosinases (Bell, 1993; Tripathi and Mishra, 2007) to yield compounds that have fungicidal and bactericidal activities (Dufour et al., 2015; Sotelo et al., 2015; Barba et al., 2016). The relative abundance of Firmicutes in feces of weaned pigs was improved by dietary inclusion of CM at 20% (Hong et al., 2020). Firmicutes have been associated with improved weight gain (Pedersen et al., 2013; Indiani et al., 2018) and improved efficiency of energy harvesting in GIT (Hildebrandt et al., 2009; Wang et al., 2019). The conventional Napus CM has a relatively low content of glucosinolates (<10 μmol/g; Woyengo et al., 2017). Thus, it has been hypothesized that, in weaned pigs, the negative effects of fiber and glucosinolates in CM on dietary nutrient utilization are partially counteracted by the positive effects of CM insoluble fiber and glucosinolates on gut health.

Previous studies (Landero et al., 2011; Parr et al., 2015; Hong et al., 2020) in which the effect of dietary CM on growth performance was determined, the animal trials were conducted in university research facilities that are considered to be clean. Also, the effects of nonstarch polysaccharide (**NSP**) products of CM on net absorption of fluid and solutes by *E. coli*-challenged pigs have been reported (Kiarie et al., 2008). However, information is lacking on the effects of dietary CM on growth performance and gut health of pigs that are raised under commercial conditions. Also, there is lack of information on the effect of dietary CM on attachment of ETEC on intestinal mucosa of weaned pigs. Pigs that are raised under commercial conditions are more prone to gut infections because of the huge pathogenic bacterial load, and hence, antibiotics or alternatives to antibiotics are added in diets to alleviate gut infections (Dirkzwager et al., 2005; Pastorelli et al., 2012). The response of pigs to feed additives or feedstuffs that improve gut health varies depending on pathogenic bacterial load in the environment in which the pigs are weaned and housed (Hermann et al., 2003). Hence, results obtained from pigs housed in an experimentally controlled environment may not be applicable to pigs housed in a commercial environment. However, results obtained from *E. coli*-challenged pigs housed in the experimentally controlled university research facilities are applicable to pigs housed in the commercial swine barns (Fairbrother et al., 2005; Adewole et al., 2016; Kiarie et al., 2008). It was hypothesized that dietary CM is as effective as dietary antibiotics in improving growth performance and gut health of *E. coli*-challenged weaned pigs. The objective of this study was to determine the effects of including CM in diets for *E. coli*-challenged weaned pigs on growth performance, fecal score, visceral organ weights, concentration of immunoglobulins and proinflammatory cytokines in blood, and attachment of ETEC on intestinal mucosa.

Materials and Methods

Experimental procedures were reviewed and approved by the Institutional Animal Care and Use Committee at South Dakota State University (# 18-040A).

Animals and housing

A total of 36 pigs (18 barrows and 18 gilts; initial body weight [**BW**] of 6.22 ± 0.71 kg; Yorkshire-Landrace female × TN Tempo male; Topigs Norsvin) weaned at 21 d of age were used in BSL-2 animal facilities of Animal Resource Wing, South Dakota State University (Brookings, SD). The pigs had been confirmed to be susceptible to infection by F18 strain of *E. coli* through blood testing; they all were of blood group H. Further genetic analysis of pigs with blood group H from this commercial swine barn revealed that they had genotype GG at M307 nucleotide in FUT1 gene. Pigs with genotype GG or GA at the M307 nucleotide in FUT1 gene are susceptible to infection by F18 strain of *E. coli* (Luo et al., 2010;

Bao et al., 2012). The pigs were housed individually in pens in three rooms (12 pigs balance for sex per room). Each pen had tenderfoot floor (0.65 × 0.92 m) and walls made of metallic wire mesh (0.88 m high). Also, each pen was equipped with a water nipple, a dry feeder, and a heat lamp. Room temperature was maintained at 27 ± 1 °C throughout the experiment. Air condition of the rooms was regulated automatically by the ventilation system.

Experimental diets

Three experimental diets included a corn–SBM-based basal diet without or with antibiotics or 20% CM (Table 1). The CM fed in the current study was derived from *Brassica napus* seed and had been produced by the pre-press solvent extraction method. The CM fed in the current study was the same as that fed in our previous study (Hong et al., 2020), and it was provided by South Dakota State University's feed mill, which had obtained it from a

Table 1. Ingredient composition and analyzed nutrient content of experimental diets (as-fed basis)[1]

	Phase 1 diets[2]			Phase 2 diets		
Item	Control	AB	CM	Control	AB	CM
Ingredient, % as-fed						
Corn	53.56	52.86	46.89	57.73	57.03	49.80
SBM	24.00	24.00	10.51	26.04	26.04	13.71
Canola meal	0.00	0.00	20.00	0.00	0.00	20.00
Whey powder	10.00	10.00	10.00	10.00	10.00	10.00
Soy protein[3]	3.00	3.00	3.00	2.00	2.00	2.00
Fish meal	5.00	5.00	5.00	0.00	0.00	0.00
Soybean oil	1.06	1.06	1.56	0.41	0.41	1.04
Limestone	0.92	0.92	0.73	1.19	1.19	1.03
Monocalcium phosphate	0.68	0.68	0.54	0.98	0.98	0.83
L-Lysine·HCl	0.51	0.51	0.58	0.53	0.53	0.56
DL-Methionine	0.16	0.16	0.15	0.15	0.15	0.13
L-Threonine	0.10	0.10	0.10	0.12	0.12	0.12
L-Tryptophan	0.02	0.02	0.04	0.00	0.00	0.02
Salt	0.79	0.79	0.70	0.65	0.65	0.56
Vitamin premix[4]	0.05	0.05	0.05	0.05	0.05	0.05
Mineral premix[5]	0.15	0.15	0.15	0.15	0.15	0.15
Zinc oxide[6]	0.00	0.30	0.00	0.00	0.30	0.00
CTC[7]	0.00	0.20	0.00	0.00	0.20	0.00
Tiamulin[8]	0.00	0.20	0.00	0.00	0.20	0.00
Calculated composition						
NE, Mcal/kg	2.498	2.498	2.498	2.461	2.461	2.461
SID lysine, %	1.50	1.50	1.50	1.35	1.35	1.35
SID methionine, %	0.43	0.43	0.43	0.39	0.39	0.39
SID threonine, %	0.88	0.88	0.88	0.79	0.79	0.79
SID tryptophan, %	0.25	0.25	0.25	0.22	0.22	0.22
STTD phosphorus, %	0.45	0.45	0.45	0.40	0.40	0.40
Calcium, %	0.85	0.85	0.85	0.80	0.80	0.80
Zinc, mg/kg	165	2,325	165	165	2,325	165
Chlortetracycline hydrochloride, mg/kg	0.00	220.5	0.00	0.00	220.5	0.00
Tiamulin hydrogen fumarate, mg/kg	0.00	44.1	0.00	0.00	44.1	0.00
Analyzed composition, %						
DM	88.14	88.43	88.62	87.81	87.78	88.39
CP	19.39	21.59	22.98	18.56	18.32	20.12
Crude ash	6.12	6.89	6.62	5.96	6.55	5.88
EE	2.38	2.33	3.58	1.92	1.64	2.93
NDF	8.40	9.30	11.51	9.56	9.62	12.00
ADF	4.17	4.35	7.54	4.55	4.66	7.41
Total glucosinolates, μmol/g[9]	0.00	0.00	1.23	0.00	0.00	1.23

[1]The experimental diets were fed in two phases: Phase 1 from days 0 to 7 and Phase 2 from days 7 to 20.
[2]Control, corn–SBM-based basal diet; AB, basal diet with 0.3% zinc oxide, 0.2% CTC, and 0.2% tiamulin; CM, basal diet with 20% canola meal.
[3]Soy protein was a hydrolyzed soy protein product (HP 300) from Hamlet Protein (Horsens, Denmark).
[4]Provided the following per kilogram of diet: 11,011 IU vitamin A, 1,652 IU vitamin D_3, 55 IU vitamin E, 0.04 mg vitamin B_{12}, 4.4 mg menadione, 9.9 mg riboflavin, 61 mg pantothenicacid, 55 mg niacin, 1.1 mg folic acid, 3.3 mg pyridoxine, 3.3 mg thiamine, and 0.2 mg biotin.
[5]Provided the following per kilogram of diet: 165 mg Zn as $ZnSO_4$, 23 mg Fe as $FeSO_4$; 17 mg Cu as $CuSO_4$, and 44 mg Mn as $MnSO_4$.
[6]Zinc oxide, Maximo 720 (zinc 72.0%, feed grade) from Zinc Nacional (Monterrey, N.L, Mexico).
[7]Chlortetracycline, Aureomycin 50 Granular A (chlortetracycline 10 mg per lb BW per day) from Zoetis Inc. (Kalamazoo, MI).
[8]Tiamulin: Denagard 10 (Type B medicated Swine Feed, Tiamulin hydrogen fumarate: 10 g/lb) from Elanco Animal Health (Indianapolis, IN).
[9]Total glucosinolates values for experimental diets were estimated based on total glucosinolates content (6.15 μmol/g) of canola meal fed in the current study; this total glucosinolates value of the canola meal was reported by Hong et al. (2020).

local supplier in a single batch. The antimicrobial agents used in the current study were zinc oxide (**ZnO**; 72% ZnO; Maximo 720, Zinc Nacional, Monterrey, N.L, Mexico), chlortetracycline (**CTC**; 11% chlortetracycline hydrochloride; Aureomycin 50 Granular A, Zoetis Inc., Kalamazoo, MI), and tiamulin (2.2% tiamulin hydrogen fumarate; Denagard 10, Elanco Animal Health, Indianapolis, IN). The ZnO, CTC, and tiamulin were included in the diets at 0.3%, 0.2%, and 0.2%, respectively. The experimental diets were fed for 21 d in two phases: Phase 1 for first 7 d and Phase 2 for last 14 d. The experimental diets were formulated to similar net energy (**NE**), standardized total tract digestible P, Ca, and standardized ileal digestible Lys, Met, Thr, and Trp contents. The diets were fed as mash and were formulated to meet or exceed NRC (2012) nutrient recommendations for nursery pigs.

Experimental design and procedure

The three diets were allotted to the 36 individually housed pigs in three room within sex and room, for a total of four pigs per diet per room to give a total of 12 pigs per diet for the study. Diet and fresh water were offered to pigs ad libitum during the whole experiment period. All pigs were orally challenged with F18 strain of *E. coli* (2134P; Rippinger et al., 1995) on day 7 as described by Lin et al. (2013). All pigs were monitored two times daily (0700 and 1900) before *E. coli* challenge and four times daily (0100, 0700, 1300, and 1900) after *E. coli* challenge for signs of illness including lethargy, coughing, diarrhea, and dehydration. Pig BW and feed intake were determined by days 7, 14, and 20 to calculate average daily gain (**ADG**), average daily feed intake (**ADFI**), and gain-to-feed ratio (**G:F**). Fecal score was assessed at 0700 and 1900 during the whole experiment period. Pens were cleaned each day after recording the fecal score at 0700 to avoid scoring same feces on different days. The occurrence and severity of postweaning diarrhea were assessed daily throughout the study on a pen basis by using the following fecal scoring system: 0 = firm feces, 1 = soft feces, 2 = mild diarrhea, and 3 = severe or watery diarrhea. Pigs with a fecal score of ≤1 were considered not to have diarrhea. The diarrhea incidence after *E. coli*-challenge was calculated by the number of pigs with diarrhea (fecal score ≤ 1) during first week of post-*E. coli*-challenge. At the end of each week, fresh fecal sample was collected from each pig by rectal palpation. The collected fecal samples were immediately snap-frozen in the liquid nitrogen and then stored in a −80 °C freezer for quantification of the F18 strain of *E. coli* by quantitative polymerase chain reaction (**qPCR**) method. Blood was collected from each pig via jugular vein puncture into blood serum tubes (BC Vacutainer, Plymouth, UK; 5 mL/tube) prior to the challenge (day 7) and postchallenge (days 14 and 20) for determination of serum concentration of triiodothyronine (**T3**), tetraiodothyronine (**T4**), immunoglobulin A (**IgA**), immunoglobulin G (**IgG**), immunoglobulin M (**IgM**), and tumor necrosis factor-alpha (**TNF-α**). Also, on day 21, blood was collected into EDTA-coated tubes (BD Vacutainer, Plymouth, UK) and immediately sent to the Animal Disease Research and Diagnostic Laboratory, South Dakota State University (Brookings, SD) for determination of complete blood count.

At the end of the trial (day 21), all pigs were euthanized and the following procedures took place. The GIT was divided into stomach, small intestine, cecum, and colon by using clamps to minimize digesta movement. The small intestine was stripped free of its mesentery and further divided into three sections: (1) duodenum (from pylorus to 70 cm below the pylorus), (2) ileum (from the ileal–cecal junction to 70-cm cranial to this junction), and (3) jejunum (the rest of small intestine). The five centimeters of segment for ileal tissue at 15-cm proximal to the ileo–cecal junction was cleaved off from each pig. The segment was snap-frozen in liquid nitrogen and stored at −80 °C for later quantification of the F18 strain of *E. coli* by qPCR method. Digesta from ileum, cecum, and 10-cm segment of the middle of the colon was collected and used to determine ileal, cecal, and colonic pH using a potable pH meter (AB 15, Fisher Scientific, Pittsburgh, PA), and then stored frozen at −20 °C for later determination of the volatile fatty acid (**VFA**) concentration. The stomach, all sections of small intestine, cecum, and colon were emptied of their digesta and weighed. Also, heart, spleen, kidneys, and liver were obtained, blotted dry with paper towels, and weighed.

Sample preparation and analyses

The experimental diets, SBM, and CM were ground to pass through a 0.75-mm screen using a centrifugal mill (model ZM200; Retsch GmbH, Haan, Germany). The ground diet samples were analyzed for dry matter (**DM**), crude protein (**CP**), ether extract (**EE**), crude ash, acid detergent fiber (**ADF**), and neutral detergent fiber (**NDF**). The samples were analyzed for DM by oven drying at 135 °C for 2 h (method 930.15), CP by a combustion procedure (method 990.03), EE (method 2003.06), crude ash (method 942.05) as per AOAC (2012); and for NDF and ADF on a Ankom 200 Fiber Analyzer (Ankom Technology, Fairport, NY). The ground SBM and CM were analyzed for NDF, ADF, soluble dietary fiber (**SDF**), and insoluble dietary fiber (**IDF**); SDF and IDF contents of the SBM and CM were measured by using the Megazyme Total Dietary Fiber kit (Megazyme International Ireland Ltd, Wicklow, Ireland) according to AOAC-991.43 and AACC-32-07.01 methods (AOAC, 2012; McCleary et al., 2012). The total dietary fiber was calculated as sum of SDF and IDF.

The number of white blood cells (**WBC**) was determined using manual WBC differential method (Blumenreich, 1990). The serum concentration of T3 was determined using an immunoassay analyzer (Immulite 1000, DPC, Los Angeles, CA), whereas serum T4 concentration was determined using Clinical Chemistry Auto-Analyzer System (Vet Axcel Chemistry Analyzer, Alfa Wassermann Diagnostic Technologies, West Caldwell, NJ). The serum concentration of IgG, IgA, IgM, and TNF-α were determined using the enzyme linked immunosorbent assay (ELISA; Bethyl Laboratories, Inc., Montgomery, TX).

The cecal digesta and colonic digesta samples for VFA analysis were thawed and centrifuged (>15,000 × g) for 20 min, and 500 μL of supernatant was collected in the tubes before loading into the gas chromatograph (Agilent Technologies, Santa Clara, CA) for VFA (acetate, propionate, butyrate, and branched-chain VFA) analysis.

For analysis of the F18 strain of *E. coli* in feces and ileal mucosa, total microbial DNA was extracted from fecal samples using PowerFecal Prof DNA kit (QIAGEN, MD) and from ileal mucosa samples using DNeasy Blood & Tissue Kit (QIAGEN) following the manufacturer's instructions. The quality of the DNA was determined using NanoDrop one (Thermo Fisher Scientific, DE) and quantified using Qubit Fluorometer 3.0 (Invitrogen, CA). The F18 strain of *E. coli* in feces and ileal mucosa was then quantified by qPCR method as described by Ståhl et al. (2011) with some modifications. To prepare standard curve, we used DNA extracted from pure culture of the F18 strain of *E. coli* in LB broth. Overnight culture of the F18 strain of *E. coli* was adjusted to OD_{600} to 1, and extracted DNA was prepared using a similar extraction method used for the samples. The same OD adjusted culture was serially diluted and plated to get the bacterial load in the culture as colony forming units per mL (CFU/mL). Extracted DNA was then serially

diluted and performed the qPCR in ABI 7500 Real-Time PCR system (Applied Biosystems Inc., Beverly, MA) as standards along with test samples. Primers and probe (Table 2) for the F18 strain of *E. coli* were purchased from Integrated DNA Technology Inc. (Coralville, IA). A standard curve was prepared using the cycle threshold (**CT**) value of standards and the corresponding bacterial load expressed in log CFU/mL. Quantification of the bacterial load in samples was performed using the standard curve generated that had a R^2 value of 0.9948. All amplifications were run at the same cycling conditions consisting of activation at 94 °C for 2 min followed by 40 cycles of 94 °C for 15 s and 60 °C for 60 s in JumpStart Taq Ready Mix for qPCR (Sigma-Aldrich, St. Louis, MO). The $MgCl_2$ concentration was 5.0 mM, and qPCR reaction carried out in a total volume of 25 µL containing 3 µL of sample DNA.

Statistical analysis

Data were analyzed using the MIXED procedure of SAS (ver. 9.4, SAS Institute Inc., Cary, NC) with pen as the experimental unit. The model included diet, sex, and room, which was the random term. Means were separated by the probability of difference in order to compare control diet with other diets. Data from two pigs fed the control diet and one pig fed the CM-containing diet were not included in the analysis. This is because the two pigs fed the control diet had been diagnosed as having pulmonary atelectasis, whereas one pig fed CM-containing diet had been diagnosed as having bronchopneumonia by South Dakota State University's Animal Disease Research and Diagnostic Laboratory (Brookings, SD). To test the hypotheses, $P < 0.05$ was considered significant. If pertinent, trend ($0.05 \leq P < 0.10$) was also reported.

Results

The analyzed NDF, ADF, IDF, and SDF contents of CM were greater than those of SBM (Table 3). Data on effects of dietary inclusion of antibiotics or CM on growth performance of pigs are presented in Table 4. The dietary antibiotics increased ($P < 0.05$) final BW, overall ADG, and overall ADFIT. Dietary CM also increased ($P < 0.05$) final BW, overall ADG, and overall ADFI, but the values did not reach those for antibiotics-containing diet. The dietary antibiotics increased ($P < 0.05$) the G:F ratio before *E. coli* challenge (days 0 to 7), whereas the G:F ratio for post-*E. coli* challenge was unaffected by dietary antibiotics or CM.

Data on effects of dietary inclusion of antibiotics or CM on fecal score and diarrhea incidence are presented in Table 5. The mean fecal score for pigs for 1 week before the *E. coli* challenge was lower ($P < 0.05$) than that of pigs for 1 week post-*E. coli* challenge (0.52 vs. 1.31) regardless of diet type (data not presented). Fecal score for 1 week before *E. coli* challenge was unaffected by dietary antibiotics or CM. Fecal score for weeks 1 and 2 post-*E. coli* challenge was reduced ($P < 0.05$) by dietary antibiotics, but not by dietary CM. The number of pigs with diarrhea for 1 week before *E. coli* challenge was unaffected by dietary antibiotics or CM. However, the number of pigs with diarrhea for weeks 1 and 2 post-*E. coli*-challenged and diarrhea incidence after the *E. coli* challenge were reduced ($P < 0.05$) by dietary antibiotics, but not by dietary CM.

Dietary antibiotics reduced ($P < 0.05$) relative weights of heart and liver, but did not affect the relative weights of spleen, kidneys, stomach, small intestine, cecum, and colon (Table 6). Dietary CM did not affect relative weights of organs measured in the current study. The WBC and absolute neutrophil counts in blood for week 2 postchallenge were reduced ($P < 0.05$) by dietary antibiotics, and WBC count tended to be reduced ($P = 0.09$) by dietary CM (Table 7). The CM-containing diet and antibiotics-containing diet did not differ in WBC and absolute neutrophil counts in blood. Dietary antibiotics or CM did not affect the percentage for lymphocytes, monocytes, mature neutrophils, and immature neutrophils in the blood. Dietary antibiotics increased ($P < 0.05$) serum T3 and T4 concentrations before the challenge (days 7) and 7 d postchallenge (Table 8). Dietary CM did not affect serum levels of T3 and T4 before and after the challenge. The serum T3 and T4 levels for the CM-containing diet were lower ($P < 0.05$) than those for the antibiotics-containing diet. Dietary antibiotics tended to reduce ($P = 0.08$) serum IgA for week 1 before the challenge. Dietary antibiotics or CM did not affect serum IgG concentration before and after the challenge. However, serum IgG concentration for week 1 postchallenge for CM-containing diet was less ($P < 0.05$) than that for antibiotics-containing diet. Dietary antibiotics or CM did not affect serum levels of IgM and TNF-α before and after the challenge.

The pH of ileal digesta was increased ($P < 0.05$) by dietary antibiotics or CM (Table 9). The pH of cecal digesta and colonic digesta was increased ($P < 0.05$) by dietary antibiotics, but dietary CM did not affect the pH of cecal digesta or colonic digesta. The cecal and colonic pH for CM-containing diet was less ($P < 0.05$) than that for antibiotics-containing diet. The dietary antibiotics decreased ($P < 0.05$) the concentrations of total VFA, acetic acid, propionic acid, butyric acid, and valeric acid in cecum, whereas dietary CM decreased ($P < 0.05$) butyric acid concentration and tended to decrease ($P = 0.08$) total VFA concentration in cecum (Table 10). Also, dietary antibiotics decreased ($P < 0.05$) valeric acid concentration in colon. However, dietary CM did not affect the VFA concentrations in colon.

Data on effects of dietary inclusion of antibiotics or CM on the population of the F18 strain of *E. coli* in fecal samples are presented in Table 11. The F18 strain of *E. coli* was not present in

Table 2. Primer and probe sequence 5′–3′ direction in the qPCR for F18 strain of *E. coli*[1]

Item	Sequence 5′–3′ direction	Sequence accession number
Primer F	GGC GGT TGT GCT TCC TTG T	M61713
Primer R	CCG TTC ACG GTT TTC AGA GC	
Prob	FAM-TAA CTG CCC GCT CCA AGT TAT ATC AGC TGT T-TAMRA	

[1]The information for the qPCR of F18 strain of *E. coli* was referred from Ståhl et al. (2011).

Table 3. Composition of SBM and canola meal (as-fed basis)[1]

Item	SBM	Canola meal
Fiber components, %		
Neutral detergent fiber	14.94	28.44
Acid detergent fiber	11.51	21.71
Total dietary fiber	22.17	37.56
Insoluble dietary fiber	20.36	34.15
Soluble dietary fiber	1.81	3.41
Total glucosinolates, µmol/g[2]	—	6.15

[1]Canola meal had been produced by the prepress solvent extraction method.
[2]Source of the total glucosinolates value of canola meal is Hong et al. (2020).

Table 4. Effect of a diet containing canola meal or antibiotics on growth performance of weaning pigs challenged with F18 strain of *E. coli*

Item[1]	Diet[2]				
	Control	AB	CM	SEM	P-value
BW, kg					
Initial	6.22	6.22	6.22	0.194	
Day 7	7.12	7.51	7.12	0.139	0.096
Day 14	8.72[b]	10.51[a]	9.35[b]	0.263	<0.001
Day 20	11.77[c]	14.57[a]	13.06[b]	0.388	<0.001
ADG, g					
0–7 d	128	184	129	19.9	0.096
7–14 d	245[c]	435[a]	326[b]	25.0	<0.001
14–20 d	508[b]	676[a]	619[a]	31.8	0.006
7–20 d	379[b]	590[a]	451[b]	36.2	0.005
0–20 d	293[c]	434[a]	357[b]	16.0	<0.001
ADFI, g					
0–7 d	160	175	146	14.8	0.391
7–14 d	315[b]	536[a]	397[b]	27.9	<0.001
14–20 d	579[b]	818[a]	752[a]	44.9	0.005
7–20 d	446[b]	736[a]	568[ab]	53.2	0.008
0–20 d	350[c]	513[a]	430[b]	20.8	<0.001
G:F					
0–7 d	0.787[b]	1.088[a]	0.842[ab]	0.0858	0.049
7–14 d	0.808	0.819	0.820	0.0298	0.960
14–20 d	0.884	0.819	0.837	0.0322	0.402
7–20 d	0.833	0.832	0.828	0.0254	0.990
0–20 d	0.821	0.907	0.836	0.0349	0.181

[1]Data are means of 10 pigs for control diet, 11 pigs for AB diet, and 11 pigs for CM diet.
[2]Control, corn–SBM-based basal diet; AB, basal diet with 0.3% zinc oxide, 0.2% CTC, and 0.2% tiamulin; CM, basal diet with 20% canola meal.
[a–c]Within a row, means without a common superscript differ ($P < 0.05$).

Table 5. Effect of a diet containing canola meal or antibiotics on fecal score and diarrhea incidence of weaning pigs challenged with F18 strain of *E. coli*

Item[1]	Diet[2]				
	Control	AB	CM	SEM	P-value
Fecal score					
Before *E. coli* challenge	0.58	0.51	0.47	0.066	0.497
Week 1 of post-*E. coli* challenge	1.69[a]	0.74[b]	1.49[a]	0.131	<0.001
Week 2 of post-*E. coli* challenge	1.38[a]	0.57[b]	1.12[a]	0.117	<0.001
Number of pigs with diarrhea					
Before *E. coli* challenge	0.33	0.17	0.33	0.207	0.807
Week 1 of post-*E. coli* challenge	7.14[a]	1.71[b]	5.43[a]	0.973	0.001
Week 2 of post-*E. coli* challenge	4.00[a]	0.86[b]	2.43[ab]	0.873	0.052
Diarrhea incidence after *E. coli* challenge, %	75.0[a]	33.3[b]	58.3[ab]	14.06	0.124

[1]Data are means of 10 pigs for control diet, 11 pigs for AB diet, and 11 pigs for CM diet.
[2]Control, corn-SBM-based basal diet; AB, basal diet with 0.3% zinc oxide, 0.2% CTC, and 0.2% tiamulin; CM, basal diet with 20% canola meal.
[a,b]Within a row, means without a common superscript differ ($P < 0.05$).

fecal samples collected from pigs before the challenge, but was present in fecal samples that were collected from pigs during days 7 and 14 postchallenge. However, dietary antibiotics or CM did not affect the population of F18 strain of *E. coli* in feces of pigs during days 7 and 14 postchallenge. Also, F18 strain of *E. coli* was not detected on ileal mucosa of pigs at day 14 postchallenge.

Discussion

Postweaning diarrhea is economically one of the major challenges facing the swine industry because of mortality and growth retardation that are associated with the postweaning diarrhea (Rhouma et al., 2017). Although antibiotics have been added in diets for weaned at subtherapeutic levels to manage postweaning diarrhea, their use is being discouraged because they can contribute to the development of antibiotic-resistant bacteria such as resistance to colistin or tetracycline (Morales et al., 2012; Sneeringer et al., 2019). Thus, there has been a need for alternatives to antibiotics in diets for weaned pigs. In our previous study (Hong et al., 2020), inclusion of CM at 20% in antibiotics-free diets for weaned pigs housed in a university research facility that is considered to be clean resulted in improved growth. However, as previously mentioned, the effects of dietary treatment on performance of weaned pigs housed in a "clean" environment can be different from the effects of the

Table 6. Effect of a diet containing canola meal or antibiotics on relative organ weight per live BW of weaning pigs challenged with F18 strain of *E. coli*

Item[1]	Diet[2]				
	Control	AB	CM	SEM	P-value
Relative organ weight per live BW, g/kg					
Heart	6.18[a]	5.51[b]	5.96[a]	0.154	0.027
Liver	29.16[a]	24.36[b]	28.56[a]	1.099	0.016
Spleen	2.18	2.13	2.08	0.090	0.681
Kidneys	6.93	6.38	6.58	0.285	0.476
Stomach	8.19	7.48	7.93	0.212	0.119
Small intestine	42.08	38.13	39.68	1.571	0.283
Cecum	1.88	1.91	1.82	0.116	0.838
Colon	14.62	13.74	15.38	0.815	0.371

[1]Data are means of 10 pigs for control diet, 11 pigs for AB diet, and 11 pigs for CM diet.
[2]Control, corn–SBM-based basal diet; AB, basal diet with 0.3% zinc oxide, 0.2% CTC, and 0.2% tiamulin; CM, basal diet with 20% canola meal.
[a,b]Within a row, means without a common superscript differ ($P < 0.05$).

Table 7. Effect of a diet containing canola meal or antibiotics on WBC count of weaning pigs challenged with F18 strain of *E. coli*

Item[1]	Diet[2]				
	Control	AB	CM	SEM	P-value
WBC, k/μL[3]	11.93[a]	7.45[b]	9.66[a,b]	0.891	0.006
Mature neutrophils, %	64.40	59.73	63.27	3.564	0.630
Lymphocytes, %	37.20	39.10	35.63	3.437	0.770
Monocytes, %	0.500	0.636	0.636	0.225	0.888
Immature neutrophils, %	0.400	0.182	0.546	0.219	0.494
Absolute neutrophil count, cells/μL	7,838[a]	4,443[b]	6,182[a,b]	716	0.009

[1]Data are means of 10 pigs for control diet, 11 pigs for AB diet, and 11 pigs for CM diet.
[2]Control, corn–SBM-based basal diet; AB, basal diet with 0.3% zinc oxide, 0.2% CTC, and 0.2% tiamulin; CM, basal diet with 20% canola meal.
[3]WBC count for CM-containing diet tended to be less ($P = 0.09$) than that for control diet and tended to be greater ($P = 0.10$) than that for AB diet.
[a,b]Within a row, means without a common superscript differ ($P < 0.05$).

same dietary treatment on performance of weaned pigs raised under commercial conditions. Also, postweaning diarrhea can be variable among pigs because it is affected by many factors including the genetics of the pigs (Madec et al., 1998; Rhouma et al., 2017). A standardized *E. coli*-challenged pig model can be used to study the effects of dietary treatments on gut health and performance of pigs weaned and raised under commercial conditions (Heo et al., 2013; Adewole et al., 2016). The objective of the current study was to determine growth performance and indicators of gut health of *E. coli*-challenged weaned pigs fed diet containing 20% CM in comparison with diet containing antibiotics.

The presence of F18 strain of *E. coli* in feces of pigs after the challenge with the same strain of *E. coli*, but not before the challenge, and an increase of fecal score of pigs after 7 days of *E. coli* challenge indicates that the challenge of the pigs with ETEC was successful. The improvement in ADG, and ADFI of pigs observed in the current study due to dietary inclusion of antibiotics was attributed to reduced diarrhea (as evidenced by reduced fecal score), reduced inflammatory response (as evidenced by reduced WBC count and serum IgA level), reduced metabolic activities in liver and heart (as evidenced by reduced relative size of the liver and heart), and increased level of thyroid hormones in blood by the dietary antibiotics. Diarrhea of piglets due to ETEC infection results in dehydration and digestive dysfunction, leading BW loss (Fairbrother et al., 2005). An increase in inflammatory response results in increased uptake of energy by immune organs for various metabolic activities such as synthesis of immunoglobulins (Pastorelli et al., 2012; Humphrey et al., 2019), leading to increased utilization of dietary energy by pigs for maintenance at expense of growth (Bray et al., 1997; Kyriazakis and Sandberg, 2006; Patience et al., 2015; Huntley et al., 2017). Moreover, an increase in metabolic activities in the liver and heart due to *E. coli* infection, results in increased energy expenditure in these organs at the expense of growth (Michiels et al., 2013). Finally, the increase in concentration of thyroid hormones in blood can result in increased secretion of growth hormone and insulin-growth factor-1 (IGF-1), thereby improving growth performance of pigs (Cabello and Wrutniak, 1989).

Torrallardona et al. (2003) and Lei and Kim (2020) similarly observed increased ADG and ADFI of *E. coli*-challenged pigs due to dietary inclusion of antimicrobial agents such as ZnO and colistin. The improvement in growth performance of pigs due to dietary inclusion of CM observed in the current study could partly be attributed to reduced inflammatory response as evidenced by the tendency of WBC count to reduce by the dietary CM. The CM used in the current study has a higher content of insoluble fiber than that of SBM. Dietary insoluble fiber can improve growth performance of weaned pigs by enhancing maturation of digestive system and feed intake (Molist et al., 2014). Indeed, dietary CM increased overall ADFI of

Table 8. Effect of a diet containing canola meal or antibiotics on blood profiles of weaning pigs challenged with F18 strain of *E. coli*

Item[1]	Diet[2]				
	Control	AB	CM	SEM	P-value
T3, ng/dL					
Day 7	97.2[b]	137.4[a]	92.8[b]	9.45	0.004
Day 14	129.8[b]	184.1[a]	150.2[b]	12.04	0.013
Day 20	136.0	137.2	155.4	9.59	0.299
T4, µg/dL					
Day 7	3.05b	3.90[a]	2.75[b]	0.281	0.021
Day 14	2.68[b]	3.86[a]	2.56[b]	0.221	<0.001
Day 20	1.99	2.52	2.84	0.610	0.630
IgA, µg/mL					
Day 7[3]	249.0	171.5	188.9	29.75	0.181
Day 14	169.1	164.1	178.8	22.36	0.892
Day 20	349.2	333.5	315.2	46.79	0.880
IgG, µg/mL					
Day 7	7,515	5,341	7,506	1,045	0.243
Day 14[3]	4,865[a,b]	5,707[a]	4,019[b]	532.4	0.092
Day 20	4,480	4,911	4,015	506.6	0.462
IgM, µg/mL					
Day 7	892.6	933.6	886.1	146.2	0.842
Day 14	795.6	841.3	940.0	83.65	0.470
Day 20	1,174.0	1,197.5	1,150.8	150.1	0.975
TNF-α, pg/mL					
Day 7	92.6	77.6	77.0	7.36	0.263
Day 14	86.7	87.7	92.4	9.16	0.897
Day 20	36.5	28.6	35.6	3.94	0.314

[1]Data are means of 10 pigs for control diet, 11 pigs for AB diet, and 11 pigs for CM diet.
[2]Control, corn–SBM-based basal diet; AB, basal diet with 0.3% zinc oxide, 0.2% CTC, and 0.2% tiamulin; CM, basal diet with 20% canola meal.
[3]Serum IgA concentration on day 7 for AB diet tended to be less ($P = 0.08$) than that for control diet.
[a,b]Within a row, means without a common superscript differ ($P < 0.05$).

Table 9. Effect of a diet containing canola meal or antibiotics on pH of digesta for ileum, cecum, and midcolon in weaning pigs challenged with F18 strain of *E. coli*

Item[1]	Diet[2]				
	Control	AB	CM	SEM	P-value
pH					
Ileal digesta	6.08[b]	6.51[a]	6.65[a]	0.142	0.024
Cecal digesta	5.77[b]	6.09[a]	5.74[b]	0.064	0.001
Colonic digesta	6.01[b]	6.31[a]	5.92[b]	0.086	0.008

[1]Data are means of 10 pigs for control diet, 11 pigs for AB diet, and 11 pigs for CM diet.
[2]Control, corn–SBM-based basal diet; AB, basal diet with 0.3% zinc oxide, 0.2% CTC, and 0.2% tiamulin; CM, basal diet with 20% canola meal.
[a,b]Within a row, means without a common superscript differ ($P < 0.05$).

pigs in the current study. Thus, the improvement in overall ADG of pigs observed in the current study due to dietary inclusion of CM could also partly be attributed increased overall ADFI by the dietary CM. The lack of effect of dietary CM on ADG for days 0 to 7 is in agreement in with results from our previous study (Hong et al., 2020) in which inclusion of CM in diet at 20% did not affect ADG of unchallenged weaned pigs during the first 7 d after weaning. Also, the magnitude by which dietary CM improved ADG of pigs for days 7 to 20 (72 g) is similar to the magnitude by which dietary CM at 20% improved ADG of unchallenged pigs for days 7 to 21 (79 g) in our previous study (Hong et al., 2020). Thus, it appears that the effect of dietary CM on growth performance of weaned pigs is not influenced by infection of the pigs with F18 strain of *E. coli*. The ADG for antibiotic-containing diet was greater than that for CM-containing diet, which could partly be attributed to the fact that dietary antibiotics increased ADFI, reduced diarrhea, inflammatory response and energy expenditure in liver, and improved the level of thyroid hormones in blood; whereas dietary CM only improved ADFI and tended to reduce inflammatory response.

Owusu-Asiedu et al. (2003) and Pan et al. (2017) reported reduced fecal score of *E. coli*-challenged pigs due to dietary inclusion of antimicrobial agents, which is in agreement with the result of the current study. Dietary antibiotics reduces diarrhea partly by reducing proliferation of pathogenic microorganisms in the GIT (Dibner and Richards, 2005; Li, 2017). However, in the current study, dietary antibiotics did not affect the concentration of F18 strain of *E. coli* in feces.

Table 10. Effect of a diet containing canola meal or antibiotics on VFA production of cecum and midcolon in weaning pigs challenged with F18 strain of *E. coli*

Item[1]	Diet[2]				
	Control	AB	CM	SEM	P-value
Cecum					
VFA concentration, mmol/g DM					
Total VFA[3]	2.67[a]	1.03[b]	1.87[a]	0.296	0.004
Acetic acid	1.48[a]	0.55[b]	0.89[a,b]	0.219	0.039
Propionic acid	1.21[a]	0.40[b]	0.76[a,b]	0.175	0.021
Butyric acid	0.49[a]	0.08[b]	0.19[b]	0.087	0.018
Valeric acid	0.02[a]	0[b]	0.02[a]	0.004	<0.001
Branched-chain VFA	ND[4]	ND	ND		
Molar ratio of VFA, %					
Acetic acid	48.4	48.7	45.8	6.39	0.932
Propionic acid	37.1	42.8	44.6	5.57	0.662
Butyric acid[5]	13.6[a]	8.5[b]	10.1[a,b]	1.280	0.046
Valeric acid	0.8[a]	0[b]	1.1[a]	0.157	<0.001
Branched-chain VFA	ND	ND	ND		
Colon					
VFA concentration, mmol/g DM					
Total VFA	1.45	1.45	1.77	0.226	0.534
Acetic acid	0.73	0.75	0.85	0.123	0.773
Propionic acid	0.51	0.57	0.72	0.098	0.356
Butyric acid	0.20	0.12	0.18	0.035	0.263
Valeric acid	0.01[a]	0[b]	0.01[a]	0.002	<0.001
Branched-chain VFA	0	0.02	0.03	0.017	0.660
Molar ratio of VFA, %					
Acetic acid	50.0	53.9	47.9	3.12	0.358
Propionic acid[6]	35.0	37.3	40.7	2.06	0.195
Butyric acid	11.0	9.1	9.9	1.42	0.638
Valeric acid	0.9[a]	0.2[b]	1.1[a]	0.18	0.003
Branched-chain VFA	0.3	0.9	1.7	0.68	0.447

[1]Data are means of 10 pigs for control diet, 11 pigs for AB diet, and 11 pigs for CM diet.
[2]Control, corn–SBM-based basal diet; AB, basal diet with 0.3% zinc oxide, 0.2% CTC, and 0.2% tiamulin; CM, basal diet with 20% canola meal.
[3]Total VFA concentration for control diet tended to be greater ($P = 0.08$) than that for CM diet.
[4]ND, not detected.
[5]Molar proportion of butyric acid for control diet tended to be greater ($P = 0.08$) than that for CM.
[6]Molar proportion of propionic acid for CM diet tended to be greater ($P = 0.08$) than that for control diet.
[a,b]Within a row, means without a common superscript differ ($P < 0.05$).

Table 11. Effect of a diet containing canola meal or antibiotics on concentration of F18 strain of *E. coli* in feces and ileal mucosa in weaned pigs challenged with F18 strain of *E. coli*

Concentration of F18 strain of *E. coli*, LogCFU/mL[1]	Diet[2]				
	Control	AB	CM	SEM	P-value
Feces					
Day 7	ND[3]	ND	ND		
Day 14	4.19	4.16	4.24	0.294	0.983
Day 20	4.28	3.26	3.44	0.433	0.325
Ileal mucosa					
Day 20	ND	ND	ND		

[1]Data are means of 10 pigs for control diet, 11 pigs for AB diet, and 11 pigs for CM diet.
[2]Control, corn–SBM-based basal diet; AB, basal diet with 0.3% zinc oxide, 0.2% CTC, and 0.2% tiamulin; CM, basal diet with 20% canola meal.
[3]ND, not detected.
[a,b]Within a row, means without a common superscript differ ($P < 0.05$).

Notably, concentration of ETEC in fecal samples was not corrected for moisture content in feces. Fecal score for pigs on the diet with antibiotics was lower than that for pigs on the control diet, implying that feces of pigs on the control diet had higher moisture than that from pigs on the antibiotic-containing diet, and hence the feces associated with the control diet could have had a greater content of F18 strain of *E. coli* per unit weight of DM. Thus, the content of the F18 strain of *E. coli*

in feces could have been lower for the diet with antibiotics than for the control diet if the values were expressed per unit weight of dry feces. Dietary antibiotics could also have reduced the proliferation of pathogenic microorganisms other than the F18 strain of *E. coli* in GIT of the pigs. Dietary CM reduced fecal score and incidence of diarrhea, but the reductions were not significant. Also, dietary CM did not affect the proliferation of the F18 strain of *E. coli* in GIT of the pigs as evidenced by the lack of effect of dietary CM on fecal concentration of the F18 strain of *E. coli*. The CM consists of high insoluble NSP, and an increase in dietary level of insoluble NSP has been associated with improved gut health of pigs (Molist et al., 2014; Agyekum and Nyachoti, 2017). Insoluble fiber such as that present in CM is expected to reduce proliferation of pathogenic microorganisms such as F18 strains of *E. coli* in weaned pigs by binding to the microorganisms and reducing the retention time of digesta in GIT (Kim et al., 2012; Heo et al., 2013; Molist et al., 2014). This is because the binding of the microorganisms and reduction in the retention time of digesta in GIT can reduce the adherence of bacteria to the intestinal mucosa and prevent the initiation of the infection (Shoaf-Sweeney and Hutkins, 2009; Becker et al., 2009). However, the effectiveness of insoluble fiber with regard to binding to the microorganisms and reducing the retention time of digesta in GIT may be dependent on fiber source and particle size (Molist et al., 2014). For instance, wheat bran (that has high insoluble fiber) that was coarsely ground (1,088 µm) was more effective in reducing diarrhea in weaned pigs than wheat bran that was finely ground (445 µm) because course fibrous particles have high water retention capacity (Molist et al., 2010). In the current study (data not presented), more than 80% of particles in CM had a size of less than 750 µm. Thus, the limited effect of dietary CM on fecal score and concentration of F18 strain of *E. coli* in feces of pigs in the current study could partly be attributed to fine particle size of the CM. The absence of the F18 strain of *E. coli* on ileal mucosa of all pigs on day 14 postchallenge could be attributed to the fact that pigs had recovered from the challenge by the time that they were sacrificed to determine the attachment of *E. coli* to mucosa. Fairbrother et al. (2005) reported that the ETEC proliferate rapidly to colonize the epithelial tissue in the mid-jejunum to the ileum, and that diarrhea of pigs due to ETEC generally lasts between 1 and 5 days. Bertschinger et al. (2000) observed that the number of *E. coli* in the feces of pigs challenged with a F18 strain of *E. coli* declined 7 d postchallenge. Molist et al. (2010) reported that dietary antibiotics did not affect the number of *E. coli* (4.7 Log CFU/g) attached to the ileal mucosa of *E. coli*-challenged pigs at 7 d of postchallenge.

Enterotoxins produced by ETEC strains that cause diarrhea in pigs can be transported to the liver where they can increase activity of detoxification enzymes (Farrar and Corwin, 1966; Nolan, 1979; Fox et al., 1990). The increase in metabolic activity in the liver can result in its increased size (Huff et al., 2002; Mejicanos et al., 2016). In the current study, dietary antibiotics reduced the relative weight of liver, which could partly have been due to reduced inflammatory response as evidenced by reduced WBC count and serum IgG and IgA levels by dietary antibiotics. However, it is not clear how dietary antibiotics could have decreased the size of the heart in the current study. Glucosinolate degradation products from dietary CM are detoxified in the liver and can lead to increased activity of detoxification enzymes in hepatic tissues, resulting in the increased weight of liver (Munday and Munday, 2002; Tanii et al., 2005; Hong et al., 2020). The CM fed in the current study was the same source of Hong et al. (2020), which contained 6.15 µmol/g of total glucosinolates. Thus, the diet with 20% CM contained ~1.23 µmol/g of total glucosinolates. Pigs can tolerate up to 2.50 µmol/g of total glucosinolates in diets (Woyengo et al., 2017). Thus, the lack of effect of dietary CM on relative liver weight in the current study could be attributed to the fact that the amounts of glucosinolates in diets that contained CM were not sufficient to impact liver size. Similarly, Hong et al. (2020) did not observe change in relative weight of liver of weaned pigs due to inclusion of CM in diets at 20%.

In the current study, pigs fed the diet with antibiotics had greater serum T3 and T4 concentrations on days 7 and 14 than those of pigs fed the control diet. Although the several mechanisms by which antimicrobial agents promote growth including modification of microbial population or bile salt hydrolase have been proposed (Partridge, 1991; Lin, 2014), it was noted that antimicrobial agents also promote growth by increasing the secretion of growth factors like growth hormone, IGF-1, and thyroid hormones (Zhao et al., 2015). In broiler chickens, serum thyroid hormones level was positively correlated with the serum IGF-1 concentration (Tsukada et al., 1998). However, the actual mechanisms by which dietary antibiotics could increase serum T3 and T4 levels have not been reported. Glucosinolate degradation products can reduce iodine uptake by the thyroid gland for the synthesis of thyroid hormones, leading to reduced thyroid hormones secretion (Tripathi and Mishra, 2007). The lack of effect of dietary CM on serum level of T3 and T4 could be attributed to the fact that amount of glucosinolates in the CM-containing diet was not sufficient to impact the synthesis of T3 and T4. Hong et al. (2020) also reported that inclusion of CM in diets for weaned pigs at 20% did not affect serum T3 and T4 levels.

White blood cells that consist of neutrophils, lymphocytes, eosinophils, monocytes, and basophils are an important part of the immune system that helps fight infections in the body. The count of WBC is considered as traditional diagnostic indicators of infection (Blumenreich, 1990). When animals were challenged with *E. coli*, the level of WBC in blood was increased (Duan et al., 2016). The demand for WBC, and hence the blood concentration of the WBC decreases with decrease in infection or antigen load (Honda et al., 2016). Thus, in the current study, the reduction of WBC count of pigs due to dietary antibiotics implies that the latter reduced antigen load. Dietary antibiotics can reduce antigen load by reducing proliferation of pathogenic microorganisms in the GIT (Garrett et al., 1966; Garrett and Won, 1973). The tendency of reduction in WBC count due to dietary inclusion of CM in the current study could be attributed to presence of insoluble fiber and glucosinolates in the CM. As previously mentioned, insoluble fiber and glucosinolates can reduce proliferation of pathogenic microorganisms, leading to reduced antigen load in GIT. Weaning stress can lead to oxidative stress in the GIT mucosa, which in turn can lead to increased permeability of gut mucosa to toxins and hence immune response (Wijtten et al., 2011). Thus, glucosinolates (that have antioxidant activity) present in CM can reduce oxidative stress leading to reduced translocation of toxins and hence immune response. The magnitude of reduction in WBC count of pigs by dietary antibiotics was greater than magnitude by which dietary CM reduced the WBC count (4.46 vs. 2.29 k/µL), which could be explained by the greater effect of dietary antibiotics than of dietary CM on antigen load in GIT as evidenced by greater effect of dietary antibiotics than of dietary CM on fecal score and incidence of diarrhea.

The serum IgA, IgG, and IgM are serum antibodies and major components of humoral immunity in pigs. Also, the TNF-α is a proinflammatory cytokine that is involved in the inflammatory process, and *E. coli* infection upregulated the expression of TNF-α in the CD34+ cells (Kim et al., 2004; Kaech et al., 2006). The IgA secretion is induced by presence of feed or microbial antigens (Van den Broeck et al., 1999; Sun et al., 2008), and hence the IgA production is related to the amount of antigens in the GIT (Gutzeit et al., 2014; Boyaka, 2017). The tendency of decreased serum IgA before *E. coli* infection due to dietary antibiotics could partly be attributed to the fact that dietary antibiotics reduced the antigen load in the GIT of pigs. However, it is not clear why dietary antibiotics did not affect the serum IgA after *E. coli* infection.

Dietary antibiotics increased pH in ileum, cecum, and colon and reduced VFA concentration in cecum, which could be attributed antimicrobial effect of antibiotics. Dietary CM increased pH in the ileum, but did not affect pH in the cecum and colon. In the current study, the inclusion of CM in diets was achieved by a partial replacement of SBM with the CM. SBM has a relatively higher content of soluble fiber and a relatively lower content of insoluble fiber than CM. For instance, the soluble NSP constituted 29% and 25% of total NSP in SBM and CM, respectively (Knudsen, 1997). Some of the soluble fiber can be fermented in the lower part of small intestine, whereas most of the insoluble fiber is fermented in hindgut (Freire et al., 2000; Molist et al., 2014). Thus, the lower ileal pH for the control diet than for the CM-containing diet could be attributed to the higher content of the insoluble fiber in the CM than in the SBM. However, the lack of effect of dietary CM on cecal and colonic pH could be attributed to the fact that some of the insoluble fiber in CM was fermented in the hindgut. The tendency of reduction in cecal VFA concentration due to dietary CM could also be attributed to the relatively higher content of insoluble fiber in CM than in SBM. Insoluble fiber is fermented at slower rate than soluble fiber (Freire et al., 2000; Montagne et al., 2003). Also, fiber in CM is more lignified than fiber in SBM (Khajali and Slominski, 2012). Fermentation of fiber is negatively related to its degree of lignification (Van Soest, 1994; Slominski et al., 2012; Woyengo et al., 2016).

In conclusion, inclusion of CM in the corn–SBM-based diet at 20% improved the growth performance of weaned pigs challenged with F18 strain of *E. coli*, likely by improving feed intake, and by reducing inflammatory response from the infection of ETEC as evidenced by the tendency of dietary CM to reduce WBC count. Thus, CM can be included in antibiotics-free corn–SBM-based diets for weaned pigs that are challenged with F18 strain of *E. coli* to improve their performance.

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Conflict of interest statement

The authors disclose that there was no conflict of interest.

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