RESEARCH REPORT

EFFICACY OF DIETARY INCLUSION OF DIRECT-FED MICROBIALS IN PREVENTING POST-WEANING DIARRHEA CAUSED BY F18-POSITIVE *E. COLI* IN PIGS

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Summary

The objective of this study was to determine the effect of an oral $F18^+$ enterotoxigenic *Escherichia coli* (ETEC) challenge on the growth performance and gut health on newly weaned pigs, and the efficacy of direct-fed microbials (DFM, PrimaLac, Star Labs/Forage Research, Inc) in preventing of post-weaning diarrhea caused by $F18^+$ ETEC. Pigs (16 barrows and 16 gilts at 6.99 ± 0.33 kg BW) were randomly allotted to 4 treatments (2 × 2 factorial) with 32 individual pens. Pigs were fed experimental diets for 25 d immediately after weaning based on 10-d phase 1 and 15-d phase 2. First factor was oral challenge with 2×10^9 CFU $F18^+$ ETEC on d 13 after weaning and the second factor was DFM (0.15 and 0.10% for phase 1 and 2, respectively). Body weight and feed intake were measured on d 5, 9, 13, 19, and 25. Fecal scores were measured on d 2, 3, 5, 9, 12, and daily from d 13. Blood samples were taken from jugular vein on d 19 and 24. On d 25, all pigs were euthanized to obtain jejunum, ileum, and spleen. Digesta from jejunum, ileum, and colon were also obtained to measure pH. Both serum and intestinal tissues were used to measure TNFα and MDA. Intestinal tissues were used for histological evaluation. In jejunum, *E. coli* challenge decreased ($P < 0.05$) crypt depth (241 to 221 μm). On the other hand, *E. coli* increased ($P < 0.05$) crypt depth (255 to 284 μm) in ileum. Fecal scores were increased ($P < 0.05$) by orally *E. coli* challenge (0.45 to 1.03). The number of pigs with diarrhea was increased ($P < 0.05$) by orally *E. coli* challenge (1 to 6) from d 13 to 25. Direct-fed microbials increased ($P < 0.05$) BW (11.8 to 14.7 kg) and ADG (193 to 308 g/d) by increasing ADFI (354 to 491 g/d), whereas G:F was not affected. Direct-fed microbials increased ($P < 0.05$) crypt depth (223 to 239 μm) in jejunum. Interactions ($P < 0.05$) on villus height and villus height:crypt depth indicated that direct-fed microbials further increased villus height and villus height:crypt depth when pigs were challenged with *E. coli*. Interactions ($P < 0.05$) on serum TNFα concentrations on d 19 indicated that DFM further decreased TNFα when pigs were challenged with *E. coli*. Collectively, oral challenge of *E. coli*

increased occurrence of diarrhea and caused mild issues on gut morphology. Direct-fed microbial improved growth performance by increasing their ADG and ADFI.

Objective

The objective of this study was to investigate the efficacy of dietary supplementation of DFM (PrimaLac, Clarksdale, MO) in prevention of post-weaning diarrhea caused by F18-positive *E coli* in newly weaned pigs.

Materials and Methods

Animals: Thirty two newly weaned crossbred pigs (21 d of age, 16 barrows and 16 gilts) with an initial body weight of 6.99 ± 0.33 kg were used in this study. They were randomly allotted to 32 pens based on a 2 × 2 factorial arrangement. There are 4 treatments and 8 replicates in each treatment. Feeding period included 2 phases. From d 0 to d 9 pigs were fed phase 1 diet, and from d 10 to d 25 pigs were fed phase 2 diet. On d 13, half of the pigs were orally challenged with *E coli*. Body weight and feed intake were measured on d 0, 5, 9, 13, 19, and 25. Fecal score was measured daily after challenge.

ETEC Challenge Strains *Escherichia coli* F18-producting strains 2144 (O147: NM, where NM indicates nonmotile) and S1191 (O139) were used as challenge strains. Strain S1191 was isolated from a pig with edema disease, and strain 2144 was isolated from piglets with PWD. The culture of these 2 strains was followed by previous study and the concentration for each strain was 1×10^9 CFU (Cutler et al., 2007). This model was used to study F18 ETEC because of our previous experience with this infection model, which observed more than 50% incidence of diarrhea, and F18 ETEC induces comparable clinical disease, via common pathophysiologic mechanisms, in both animals and humans (Cutler et al., 2007; McLamb et al., 2013)

Experimental Diets In this study, DFM (PrimaLac, Star Labs/Forage Research, Inc) was the source of microorganisms, it contains *Lactobacillus acidophilus*, *Lactobacillus casei*, *Bifidobacterium thermophilum* and *Enterococcus faecium* with the concentration of 0.25×10^8 CFU/g for each strain. Concentration of DFM in the diets was changed based on different phases, phase 1 (0.15%) and phase 2 (0.10%). Experimental diets were based on a 2 × 2 factorial arrangement of the treatments. The first factor was oral challenge with $F18^+$ ETEC (0 or 2×10^9 CFU on d 13 after weaning), and the second factor was direct-fed microbial (0 or 1×10^8 CFU/g). Direct-fed microbial was mixed with control diet prior to feeding. During the 25 d of feeding period, all pigs had free access to feed and water. Concentrations of nutrients met the requirements suggested by NRC (1998). The calculated value and analyzed value were showed in Table 1.

Sampling On d 19 and 24, all the pigs were bled two times from jugular vein to obtain blood samples. Blood was collected in vacutainers without anticoagulant (BD, Franklin Lakes, NJ). Serum samples were collected after centrifuging (3,000 × *g* for 15 min at 4°C) and stored at -80°C until they were analyzed for concentration of malonedialdehyde (MDA) and tumor necrosis factor α (TNFα). On d 25, all pigs were euthanized by electric device. Then the gastric intestinal tract was quickly removed, and the small intestine was dissected. Middle section of jejunum and ileum were isolated, flushed with distilled water. Half of the sections was fixed in 10% formaldehyde-phosphate buffer, and kept for microscopic assessment of mucosal morphology. The other half of the sections was then opened for scraping mucosal layer of intestine. Mucosa of jejunum and ileum was scraped into a microassay tube and frozen in liquid nitrogen. Mucosa samples were then stored in -80°C until analyzing for MDA and TNFα concentration. One tube of digesta sample (50 mL) from jejunum, ileum and colon were also collected, and digesta pH was measured using pH meter immediately. Digesta were directly put on ice,

and then stored in -20°C until analyzing. Spleen weight was also measured as an indicator of the expression of pro-inflammatory cytokines such as TNFα and interleukin-β (Touchette et al., 2002).

Fecal Scores and Occurrence of Diarrhea Fecal Scores were measured using FC score on d 2, 3, 5, 9, 12, and daily from d 13. FC score is the mean fecal consistency score: 0, normal feces; 1, soft feces; 2, mild diarrhea; 3, severe diarrhea. Pigs with a fecal score of ≤ 1 were considered not to have diarrhea (Ronald et al., 1999). The occurance of diarrhea was difined as maintaining the fecal score which is greater than 1 for 2 consecutive days (Liu et al., 2008). And the No. of pigs with diarrhea was observed from d 13 to 19, d 19 to 25, and d 13 to 25.

Small Intestinal Morphology The segments of the 2 small intestinal sections were send to the North Carolina State University Histopathology Laboratory (College of Veterinary Medicine, Raleigh, NC, USA) to prepare polylysine-coated slides with hematoxylin and eosin (H&E) staining. Then the slides were examined under a Sony CCD color video camera attached to a Olympus Van-ox S microscope (Opelco, Washington, DC). Villus height (from the tip of the villi to the villous-crypt junction), villus width (width of the villus at the middle of the villus height), and crypt depth (from villous-crypt junction to the base of the crypt) were determined (Shen et al., 2009). Lengths of 10 well-oriented intact villi and their associated crypt were measured in each slide. One person complemented all the analysis of small intestinal morphology.

Cytokine Measurement The concentration of TNFα was measured in serum and mucosa. Jejunum and ileum mucosa was homogenized in PBS containing protease inhibitors and the supernatant was collected and analyzed for protein content using a BCA assay (Peace et al., 2011). Then the supernatant and serum were measured using a Porcine TNFα Colorimetric ELISA Kit (Pierce Biotechnology, Inc., Rockford, IL) as an indicator of inflammation and acute phase reaction. Briefly, 50 µL of assay diluent

plus 50 µL of standard or sample were added to microplate wells which were already coated with capture antibody in conjunction with biotinylated antibody reagent. Detection occurred by the use of horseradish peroxidase, TMB substrate, and a stop solution of 0.18 M H2SO4. Absorbance was read at 450 nm and 540 nm by an ELISA plate reader and the KC4 data analysis software. Detection limit for TNFα was 5 pg/mL.

Oxidative Damage Status Measurement Malondialdehyde content in serum and mucosa was measured using an OxiSelect TBARS Assay Kit (Cell Biolabs, Inc., San Diego, CA) as an index of lipid peroxidation (Yeum and Krinsky, 2007). All the procedures followed manufacturer's instruction. Concentration of MDA in serum was expressed as µmol/L, and concentration of MDA in mucosal tissue was expressed as µmol/g.

Statistical Analysis This study used a randomized complete block design based on 2 × 2 factorial arrangement treatments. Initial BW and sex were blocks. The first factor was oral challenge of $F18^+$ ETEC, and the second factor was DFM. One pig was considered as the experimental unit. Data, except for No. of pigs with diarrhea were analyzed using the Mixed procedures (SAS Inst. Inc., Cary, NC). The No. of pigs with diarrhea was analyzed using Chi-square test in SAS software. Probability values less than 0.05 were considered statistically significant and between 0.05 and 0.10 as trends.

Results

Growth Performance Initial BW of pigs did not differ among treatments (Table 2). During the entire feeding period, body weight was not affected by the factor of *E. coli*. From d 9 to 13 pig's BW was increased ($P < 0.05$) in treatments with DFM by 13.7% and 8.4%, respectively, without or with *E. coli*. From d 13 to 19 the supplementation of DFM increased ($P < 0.05$) BW by 20.1% and 17.6%, respectively, without or with *E. coli*. From d 19 to 25 the supplementation of DFM increased ($P < 0.05$) BW by 28.8% and 20.1%, respectively, without or with *E. coli*.

Average daily gain of pigs was not affected by the factor of *E. coli* during the entire feeding period. The factor of DFM did not affect ADF from d 0 to 9 and d 0 to d 13. However, from d 9 to 13 the supplementation of DFM increased ($P < 0.05$) ADG by 104.1% and 131.4%, respectively, without or with *E. coli*. From d13 to 25, the supplementation of DFM increased ($P < 0.05$) ADG by 58.5% and 44.7%, respectively, without or with *E. coli*. During the whole feeding period, the supplementation of DFM increased ($P < 0.05$) ADG by 71.4% and 47.9%, respectively, without or with *E. coli*.

Average daily feed intake of pigs was not affected by the factor of *E. coli* during the entire feeding period. The factor of DFM did not affect ADFI during the first 13 days. However, from d 13 to 19, the supplementation of DFM increased ($P < 0.05$) ADFI by 54.3% and 41.3%, respectively, without or with *E. coli*. From d 19 to 25, the supplementation of DFM increased ($P < 0.05$) ADFI by 43.4% and 39.7%, respectively, without or with *E. coli*. From d 13 to 25, the supplementation of DFM increased ($P < 0.05$) ADFI by 47.7% and 40.5%, respectively, without or with *E. coli*. And during the whole feeding period, the supplementation of DFM increased ($P < 0.05$) ADFI by 49.0% and 29.0%, respectively, without or with *E. coli*. The G:F of pigs during the whole feeding period did not affected by the factor of *E. coli* or DFM.

Fecal Scores and Occurrence of Diarrhea After challenge, the orally challenge of *E. coli* increased ($P < 0.05$) mean fecal consistency (FC) score (Table 6). From d 13 to 19, mean FC scores were increased ($P < 0.05$) by 358.1% and 159.1%, respectively, without or with DFM. From d 19 to 25, mean FC scores were increased ($P < 0.05$) by 40.8% and 232.1%, respectively, without or with DFM. From the whole challenge period, mean FC scores were increased ($P < 0.05$) by 166.0% and 178.9%, respectively, without or with DFM. From the whole feeding period, mean FC scores were increased ($P < 0.05$) by 174.4% and 92.2%, respectively, without or with DFM. Before challenge, mean FC scores of pigs were decreased ($P < 0.05$) in DFM groups (0.88 and 0.86 vs. 0.25 and 0.63, respectively) from d 0 to 9. However, from d 9 to 13, mean FC score of pigs were increased ($P < 0.05$) in DFM groups (0.38 and 0.14 vs. 0.83 and 0.50, respectively).

From d 13 to 19 and d13 to 25, the No. of pigs with diarrhea increased ($P < 0.05$) with the orally challenge of *E. coli*, and from d 19 to 25, the No. of pigs with diarrhea tended ($P = 0.070$) to increase with the orally challenge of *E. coli* (Table 7).

Histological Evaluation In jejunum, the supplementation of *E. coli* decreased ($P < 0.05$) pig's crypt depth by 8.4% and 8.0%, respectively, without or with DFM, and it also tended to decrease ($P = 0.052$) the villus height (Table 3). In ileum, the supplementation of *E. coli* increased ($P < 0.05$) pig's crypt depth by 3.7% and 19.6%, respectively, without or with DFM. In jejunum, the supplementation of DFM increased ($P < 0.05$) pig's crypt depth by 6.6% and 7.3%, respectively, without or with *E. coli*. In jejunum, the interaction term of *E. coli* and DFM affected ($P < 0.05$) the villus height and VCR where the presence of DFM further incrased their levels when pigs were challenged with *E. coli.* In ileum, the interaction term of *E. coli* and DFM tended to affect ($P = 0.073$) the crypt depth where the presence of DFM further incrased its level when pigs were challenged with *E. coli.*

Inflammatory Cytokine and Oxidative Damage Status The interaction term of *E. coli* and DFM affected ($P < 0.05$) TNFα level from the serum sample taken at d 6 after challenge where the presence of DFM further decrease its level when pigs were challenged with *E. coli.* (Table 4).

Digesta pH and Organ Weight The interaction term of *E. coli* and DFM tended to affect ($P = 0.067$) the digesta pH from jejunum where the presence of DFM further incrased its level when pigs were challenged with *E. coli* (Table 5).

Conclusion

Based on the results of this study, oral challenge of F18+ ETEC increased occurrence of diarrhea without influencing growth performance of pigs. Supplementation of DFM increased ADG of pigs with increased ADFI. It also led to a greater crypt depth in jejunum. However, this study could not answer reasons of increased ADFI by DFM which warrents further research.

Table 1. Composition of control diets (P1 Early-wean: first 10 d post-weaning; P2 Pre-starter: 15 d after P1)

Ingredient, %	Early-wean	Pre-starter
Yellow corn	37.11	54.41
DairyLac 80	25	8
Soybean meal	25	30
Plasma protein	3	1
Fish meal, menhaden	4	2
L-Lys HCl	0.23	0.12
DL-Met	0.16	0.06
L-Thr	0.11	0.02
Poultry fat	3.4	2.2
Salt	0.22	0.22
Vitamin premix[1]	0.03	0.03
Mineral premix[2]	0.15	0.15
Dical P	1	1.15
Limestone	0.6	0.65
Total	100	100
Calculated composition:		
DM, %	91.8	90.4
ME, kcal/kg	3503.2	3437.5
CP, %	21	21.2
SID Lys, %	1.34	1.19
SID Met + Cys, %	0.78	0.68
SID Trp, %	0.24	0.23
SID Thr, %	0.85	0.73
Ca, %	0.92	0.81
P, avail, %	0.56	0.4
Analyzed composition:		
DM, %	92.83	90.93
CP, %	19.71	19.75
ADF, %	2.20	2.42
Ca, %	0.75	0.71
P, %	0.73	0.69

[1]: Vitamin premix) provided the following per kilogram of complete diet: 22,045,000 IU of vitamin A; 3,306,900 IU of vitamin D_3; 66,138 IU of vitamin K; 88 mg of vitamin B_{12}; 15,432 mg of riboflavin; 88,184 mg of niacin; 61,729 mg of d-pantothenic acid; 8,818 mg of menadione; 220 mg of biotin.

[2]: Mineral premix provided the following composition: 1.10% of Cu; 198.0 mg/kg of I; 11.02% of Fe; 2.64% of Mn; 198.4 mg/kg of Se; 11.02% of Zn.

Table 2. Growth performance of pigs fed diets supplemented with *E. coli* (0 or 2 × 10^9 CFU) and DFM (0 or 1 × 10^8 CFU/g)

E coli[1]	No		Yes			*P* value		
DFM[2]	No	Yes	No	Yes	SEM	E coli[1]	DFM[2]	Interaction
BW, kg								
Initial BW	6.99	6.97	6.98	7.03	0.12	0.432	0.681	0.364
D 9	7.35	7.92	7.57	7.70	0.54	0.991	0.311	0.520
D 13	7.83	8.90	7.98	8.65	0.82	0.908	0.080	0.669
D 19	9.45	11.43	9.32	10.96	1.42	0.715	0.038	0.835
D 25	11.79	15.19	11.82	14.20	2.22	0.703	0.030	0.684
ADG, g								
D 0 to 9	40	106	66	74	64	0.933	0.314	0.437
D 9 to 13	121	247	102	236	77	0.775	0.018	0.937
D 0 to 13	65	149	77	124	66	0.853	0.077	0.605
D 13 to 19	270	420	223	285	100	0.594	0.054	0.943
D 19 to 25	390	636	417	541	137	0.747	0.057	0.535
D 13 to 25	330	523	320	463	117	0.616	0.024	0.717
D 0 to 25	192	329	194	287	90	0.683	0.030	0.662
ADFI, g								
D 0 to 9	145	207	185	190	51	0.705	0.301	0.381
D 9 to 13	258	421	344	383	84	0.704	0.121	0.332
D 0 to 13	180	273	234	250	60	0.694	0.185	0.340
D 13 to 19	400	617	431	609	133	0.884	0.023	0.811
D 19 to 25	624	895	597	834	185	0.689	0.029	0.875
D 13 to 25	512	756	514	722	158	0.863	0.022	0.842
D 0 to 25	339	505	369	476	106	0.992	0.035	0.637
Gain:feed								
D 0 to 9	-0.547	0.470	0.439	0.439	0.585	0.359	0.331	0.331
D 9 to 13	0.576	0.595	0.354	0.588	0.109	0.263	0.219	0.292
D 0 to 13	0.263	0.536	0.400	0.534	0.188	0.642	0.175	0.634
D 13 to 19	0.733	0.692	0.467	0.660	0.104	0.146	0.447	0.251
D 19 to 25	0.659	0.701	0.742	0.649	0.049	0.742	0.597	0.166
D 13 to 25	0.699	0.697	0.674	0.658	0.030	0.183	0.712	0.770
D 0 to 25	0.634	0.651	0.603	0.626	0.024	0.231	0.386	0.916

[1]: Oral *E. coli* challenge on d 13 with the concentration of 2.0 × 10^9 CFU.

[2]: Direct-fed microbials which is the source of DFM with the concentration of 1.0 × 10^8 CFU per gram, and were added 0.15 % and 0.1 % in phase 1 and phase 2 diet.

Table 3. Fecal Score of pigs fed diets supplemented with *E. coli* (0 or 2×10^9 CFU) and DFM (0 or 1×10^8 CFU/g)

E coli[1]	No		Yes			*P* value		
DFM[2]	No	Yes	No	Yes	SEM	E coli[1]	DFM[2]	Interaction
FC score[3]								
D 0 to 9	0.25	0.88	0.63	0.86	0.18	0.305	0.022	0.261
D 9 to 13	0.83	0.38	0.50	0.14	0.19	0.141	0.041	0.785
D 0 to 13	0.67	0.55	0.60	0.46	0.19	0.664	0.483	0.943
D 13 to 19	0.31	0.44	1.42	1.14	0.26	0.002	0.774	0.418
D 19 to 25	0.71	0.28	1.00	0.93	0.22	0.039	0.248	0.404
D 13 to 25	0.47	0.38	1.25	1.06	0.21	0.002	0.478	0.799
D 0 to 25	0.39	0.51	1.07	0.98	0.17	0.003	0.917	0.520

[1]: Oral *E. coli* challenge on d 13 with the concentration of 2.0×10^9 CFU.
[2]: Direct-fed microbials which is the source of probiotics with the concentration of 1.0×10^8 CFU per gram, and were added 0.15 % and 0.1 % in phase 1 and phase 2 diet.
[3]: Mean fecal consistency score: 0, normal feces; 1, soft feces; 2, mild diarrhea; 3, severe diarrhea, whereas the FC score greater than 1 is considered as diarrhea.

Table 4. Number of pigs with diarrhea fed diets supplemented with *E. coli* (0 or 2×10^9 CFU) and DFM (0 or 1×10^8 CFU/g)

E coli[1]	No		Yes		*P* value	
DFM[2]	No	Yes	No	Yes	E coli[1]	DFM[2]
No. of pigs with diarrhea after challenge (FC Score[3])						
D 13 to 19	0/8 (0.31)	0/8 (0.44)	5/8 (1.42)	4/8 (1.14)	< 0.001	0.694
D 19 to 25	1/8 (0.71)	0/8 (0.28)	2/8 (1.00)	3/8 (0.93)	0.070	1.000
D 13 to 25	1/8 (0.39)	0/8 (0.38)	5/8 (1.25)	5/8 (1.06)	0.001	0.710

[1]: Oral *E. coli* challenge on d 13 with the concentration of 2.0×10^9 CFU.
[2]: Direct-fed microbials which is the source of probiotics with the concentration of 1.0×10^8 CFU per gram, and were added 0.15 % and 0.1 % in phase 1 and phase 2 diet.
[3]: Mean fecal consistency score: 0, normal feces; 1, soft feces; 2, mild diarrhea; 3, severe diarrhea, whereas the FC score greater than 1 is considered as diarrhea.

Table 5. Villus height, width, crypt depth and villus height to crypt depth ratio from jejunum and ileum of pigs fed diets supplemented with *E. coli* (0 or 2×10^9 CFU) and DFM (0 or 1×10^8 CFU/g)

E coli[1]	No		Yes			*P* value		
DFM[2]	No	Yes	No	Yes	sem	E coli[1]	DFM[2]	Interaction
Jejunum (μm)								
Villus height	452.62	411.64	376.49	416.54	20.68	0.052	0.979	0.030
Villus width	120.90	111.82	110.48	111.34	4.28	0.144	0.365	0.180
Crypt depth	232.83	248.28	213.34	228.86	8.74	0.012	0.039	0.996
VCR[3]	1.95	1.66	1.77	1.82	0.07	0.908	0.092	0.017
Ileum (μm)								
Villus height	326.96	319.36	346.07	333.43	15.68	0.268	0.493	0.864
Villus width	100.58	97.21	98.67	105.10	5.40	0.501	0.728	0.286
Crypt depth	265.10	244.55	275.03	292.45	10.35	0.010	0.876	0.073
VCR[3]	1.24	1.31	1.26	1.15	0.06	0.224	0.721	0.107

[1]: Oral *E. coli* challenge on d 13 with the concentration of 2.0×10^9 CFU.
[2]: Direct-fed microbials which is the source of probiotics with the concentration of 1.0×10^8 CFU per gram, and were added 0.15 % and 0.1 % in phase 1 and phase 2 diet.
[3]: Villus height to crypt depth ratio.

Table 6. Inflammatory cytokines and oxidative status in serum and mucosa tissue of pigs fed diets supplemented with *E. coli* (0 or 2×10^9 CFU) and DFM (0 or 1×10^8 CFU/g)

E coli[1]	No		Yes			*P* value		
DFM[2]	No	Yes	No	Yes	SEM	E coli[1]	DFM[2]	Interaction
Jejunum								
MDA, μmol/g	30.46	33.45	30.46	27.16	4.04	0.426	0.967	0.426
TNFα, pg/mg	65.43	58.07	60.95	73.34	10.2	0.586	0.799	0.324
Ileum								
MDA, μmol/g	33.56	28.99	34.13	25.68	4.22	0.726	0.11	0.624
TNFα, pg/mg	85.04	91.15	72.84	73.37	12.49	0.171	0.753	0.796
Serum								
MDA[3], μmol/L	9.4	9.86	11.21	10.27	0.79	0.127	0.726	0.332
MDA[4], μmol/L	10.16	9.77	9.91	9.36	0.66	0.605	0.454	0.894
TNFα[3], pg/mL	81.02	90.94	87.46	66.17	6.37	0.147	0.361	0.019
TNFα[4], pg/mL	54.3	50.06	51.88	50.15	4.93	0.769	0.46	0.753

[1:] Oral *E. coli* challenge on d 13 with the concentration of 2.0×10^9 CFU.

[2]: Direct-fed microbials which is the source of DFM with the concentration of 1.0×10^8 CFU per gram, and were added 0.15 % and 0.1 % in phase 1 and phase 2 diet.

Table 7. Digesta pH and spleen weight of pigs fed diets supplemented with *E. coli* (0 or 2×10^9 CFU) and DFM (0 or 1×10^8 CFU/g)

E coli[1]	No		Yes			*P* value		
DFM[2]	No	Yes	No	Yes	SEM	E coli[1]	DFM[2]	Interaction
pH								
Jejunum	6.85	6.42	6.71	6.84	0.19	0.354	0.321	0.067
Ileum	6.83	6.54	6.45	6.54	0.13	0.138	0.420	0.146
Colon	6.31	6.30	6.20	6.02	0.16	0.202	0.505	0.571
Weight, g								
Spleen	21.11	24.17	21.18	20.67	2.09	0.388	0.520	0.371

[1]: Oral *E. coli* challenge on d 13 with the concentration of 2.0×10^9 CFU.

[2]: Direct-fed microbials which is the source of DFM with the concentration of 1.0×10^8 CFU per gram, and were added 0.15 % and 0.1 % in phase 1 and phase 2 diet.