Effects of a 3 strain *Bacillus*-based direct-fed microbial and dietary fiber concentration on growth performance and expression of genes related to absorption and metabolism of volatile fatty acids in weanling pigs[1]

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ABSTRACT: Effects of a *Bacillus*-based direct-fed microbial (DFM) on growth performance, plasma tumor necrosis factor α (TNF-α), relative gene expression, and intestinal VFA concentrations in weanling pigs fed low- or high-fiber diets were evaluated. Two hundred pigs (initial BW: 6.31 ± 0.73 kg) were allotted to 1 of 4 dietary treatments (5 pigs per pen and 10 pens per treatment). Treatments were arranged in a 2 × 2 factorial design with 2 diet types [low-fiber (LF) or high-fiber (HF)] and 2 concentrations of DFM (0 or 60 g DFM/t of feed). The DFM contained 1.5×10^5 cfu/g and was obtained from Danisco Animal Nutrition-DuPont Industrial Biosciences, Marlborough, UK. Phase 1 diets were fed for 2 wk post-weaning and phase 2 diets were fed over the following 29 d. Low fiber diets contained corn and soybean meal as main ingredients and HF diets contained corn, soybean meal, corn distillers dried grains with solubles (7.5 and 15.0% in phase 1 and 2, respectively), and wheat middlings (10.0%). Pigs and feed were weighed at the start and at the end of each phase, and ADG, ADFI, and G:F were calculated. At the conclusion of phase 2, blood was collected from 1 pig per pen and 1 pig per pen was sacrificed. Cecum and rectum contents were analyzed for VFA, and tissue samples were collected from the ileum, cecum, rectum, and liver to determine expression of genes related to absorption and metabolism of VFA using quantitative reverse transcription-PCR. Results indicated that feeding HF diets reduced ($P \leq 0.05$) ADFI and ADG of pigs compared with feeding LF diets. Pigs fed DFM diets had improved ($P \leq 0.05$) G:F compared with pigs fed non-DFM diets. Pigs fed LF diets had greater ($P \leq 0.05$) BW at the end of phase 2 compared with pigs fed HF diets. The concentration of VFA in rectum contents was greater ($P \leq 0.05$) in pigs fed LF diets than in pigs fed HF diets. The expression of *monocarboxylate transporter-1* in the rectum of pigs fed HF diets was greater ($P \leq 0.05$) than for pigs fed LF diets, and pigs fed DFM-containing diets had an increased ($P \leq 0.05$) expression of *glucagon-like peptide-2 receptor* in the liver. Pigs fed HF diets had greater ($P \leq 0.05$) concentrations of urea N in plasma compared with pigs fed LF diets, but dietary fiber and DFM had no effect on plasma concentration of TNF-α. In conclusion, the *Bacillus*–based DFM improved overall G:F of weanling pigs, but pigs fed LF diets had greater final BW than pigs fed HF diets.

Key words: direct-fed microbials, glucagon-like peptide-2, swine, total dietary fiber, volatile fatty acids

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INTRODUCTION

Direct-fed microbials (**DFM**), which may be more commonly known as probiotics, are defined as "live microorganisms which, when administered in adequate amounts, confer a health benefit on the host (FAO/WHO, 2001)." Addition of DFM to swine diets may improve gut health by modifying the microbiota,

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which may enhance immune regulation, health status, and improve pig performance (Kenny et al., 2011; Cromwell, 2013). Addition of DFM to nursery diets may also reduce diarrhea (Eigel, 1989), but improved growth performance has not been consistently observed (Stavric and Kornegay, 1995).

Bacillus-based DFM are spore-forming bacteria, which allow them to survive high temperatures and low pH, but when *Bacillus*-based DFM germinate in the intestine of the pig, they may produce a wide variety of fiber-degrading enzymes (Schreier, 1993; Kenny et al., 2011). Therefore, addition of a *Bacillus*-based DFM may enhance fermentation of dietary fiber in swine diets and, subsequently, increase the available energy from the diet in the form of VFA (Davis et al., 2008). Fiber in nursery pig diets may also act as a prebiotic and stimulate beneficial gut microbiota and, therefore, reduce post-weaning diarrhea (Smith and Halls, 1968) although dietary fiber sometimes will reduce digestibility of energy and nutrients (Bindelle et al., 2008). Increasing dietary fiber may increase the efficacy of DFM, but data to confirm this hypothesis have not been reported (de Lange et al., 2010). Therefore, it may be beneficial to supplement diets containing high-fiber ingredients such as distillers dried grains with solubles (**DDGS**) and wheat middlings with a *Bacillus*-based DFM that has the ability to secrete fiber-degrading enzymes. The objective of this experiment, therefore, was to test the hypothesis that addition of a *Bacillus*-based DFM to diets fed to weanling pigs will allow pigs fed high-fiber (**HF**) diets to maintain growth performance compared with pigs fed low-fiber (**LF**) diets.

MATERIALS AND METHODS

The Institutional Animal Care and Use Committee at the University of Illinois reviewed and approved the protocol for this experiment.

Animals, Diets, and Experimental Design. A total of 200 weanling pigs (initial BW: 6.31 ± 0.73 kg) that were the offspring of L 359 boars mated to C-46 females (Pig Improvement Company, Hendersonville, TN) were used in this experiment in 2 separate blocks of 100 pigs each. Pigs were randomly allotted in a completely randomized design to 4 dietary treatments. There were 5 pigs per pen and 10 replicate pens per treatment. Five of the pens for each treatment housed 2 gilts and 3 barrows, and the other 5 pens housed 2 barrows and 3 gilts. Pigs were housed in environmentally controlled nursery barns in 1.4 × 1.4 m pens with fully slatted floors. A 2-hole feeder and a nipple drinker were provided in each pen and feed and water were provided on an ad libitum basis throughout the experiment. Feed was provided as mash.

Treatments were arranged in a 2 × 2 factorial arrangement with 2 dietary sequence types (LF or HF) and 2 concentrations of DFM (0 or 60 g DFM/t of feed; Table 1). The *Bacillus*-based DFM was formulated to provide 1.5×10^5 cfu per gram of feed and was obtained from Danisco Animal Nutrition-DuPont Industrial Biosciences (Marlborough, UK). The DFM used in this experiment is identical to the 3-strain DFM used by Cai et al. (2015) and consisted of 1 strain of *Bacillus subtilis* and 2 strains of Bacillus amyloliquefaciens in equal proportions. Limestone and maltodextrin were the carriers. Phase 1 diets were fed for 2 wk post-weaning and phase 2 diets were provided during the following 29 d. The LF diets contained corn and soybean meal as main ingredients and HF diets contained corn, soybean meal, corn DDGS (7.5 and 15.0% in phase 1 and 2, respectively) and wheat middlings (10.0%). Phase 1 diets contained no microbial phytase, whereas phase 2 diets contained 500 units of microbial phytase (Axtra PHY; Danisco Animal Nutrition-DuPont Industrial Biosciences, Waukesha, WI) per kg of complete diet. Diets did not contain antibiotic growth promoters and diets were not formulated to be isocaloric or isonitrogenous, and therefore, HF diets contained less NE, but more CP, than LF diets. However, all diets were formulated to meet or exceed requirements for standardized ileal digestible AA, standardized total tract digestible P, vitamins, and minerals (NRC, 2012).

Individual pig weights were recorded at weaning and at the conclusion of each phase. Daily allotments of feed were recorded and feed remaining in the feeder at the end of each phase was recorded and feed intake calculated. Data were summarized and ADG and ADFI were calculated. The G:F was calculated as kg gain per kg gain per kg feed intake and as kg gain per Mcal NE intake because LF and HF diets were not formulated to be isocaloric.

Sample Collection. Blood samples (10 mL; 1 pig per pen) were collected from the same pig in each pen at weaning and at the conclusion of phases 1 and 2. At weaning, the 10 replicate pens within each treatment were randomly assigned to having either a female or a male pig bled giving a total of 5 gilts and 5 barrows being bled for each diet. The pig within pen that had the sex that was selected for that pen and a BW closest to the average for the pen was then selected and this same pig was bled at each bleeding time. Blood samples were analyzed for plasma urea nitrogen (**PUN**) on an Olympus AU680 chemistry analyzer (Olympus Life Science Research Europa GmbH, Munich, Germany) and plasma concentrations of tumor necrosis factor α (**TNF-α**) were analyzed in duplicate using a porcine sandwich ELISA kit according to the manufacturer's instructions (R&D Systems, Minneapolis, MN). Intra-assay CV was 5.5% for TNF-α.

Table 1. Ingredient composition and calculated and analyzed composition of experimental diets (as-fed basis)

	Phase 1				Phase 2			
Dietary fiber concentration:	Low		High		Low		High	
Direct-fed microbial:	-	+	-	+	-	+	-	+
Ingredient, %								
Corn	51.44	51.38	37.09	37.03	54.10	54.04	35.24	35.18
Soybean meal, 48% CP	20.00	20.00	17.00	17.00	27.00	27.00	21.00	21.00
Whey, dried	15.00	15.00	15.00	15.00	10.00	10.00	10.00	10.00
DDGS[1]	–	–	7.50	7.50	–	–	15.00	15.00
Wheat middlings	–	–	10.00	10.00	–	–	10.00	10.00
Fish meal	5.00	5.00	5.00	5.00	4.00	4.00	4.00	4.00
Blood plasma	4.00	4.00	4.00	4.00	–	–	–	–
Soybean oil	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00
Limestone	1.00	1.00	1.25	1.25	1.07	1.07	1.42	1.42
Dicalcium phosphate	0.40	0.40	–	–	0.50	0.50	–	–
L-Lys HCl	0.30	0.30	0.33	0.33	0.40	0.40	0.45	0.45
DL-Met	0.10	0.10	0.08	0.08	0.12	0.12	0.09	0.09
L-Thr	0.06	0.06	0.05	0.05	0.10	0.10	0.09	0.09
Sodium chloride	0.40	0.40	0.40	0.40	0.40	0.40	0.40	0.40
Vitamin-mineral premix[2]	0.30	0.30	0.30	0.30	0.30	0.30	0.30	0.30
DFM premix[3]	–	0.06	–	0.06	–	0.06	–	0.06
Calculated composition[4]								
NE, kcal/kg	2,525	2,525	2,463	2,463	2,483	2,483	2,414	2,414
CP, %	21.80	21.80	22.81	22.81	20.95	20.95	22.29	22.29
SID[5] Lys, %	1.43	1.43	1.43	1.43	1.36	1.36	1.35	1.35
Ca, %	0.85	0.85	0.85	0.85	0.85	0.85	0.85	0.85
STTD[6] P, %	0.46	0.46	0.46	0.46	0.39	0.39	0.39	0.39
Analyzed energy and nutrients								
GE, kcal/kg	3,986	3,947	4,067	4,103	3,963	3,949	4,045	4,077
DM, %	87.1	87.1	86.7	86.2	87.8	87.5	87.8	87.9
Ash, %	6.3	5.6	6.0	6.0	5.0	5.2	5.4	5.7
ADF, %	4.3	4.5	5.5	4.5	4.7	4.0	6.1	5.8
NDF, %	7.1	8.7	12.6	12.3	11.4	10.5	14.0	14.1
Insoluble dietary fiber, %	9.5	10.7	15.4	14.7	13.5	13.5	17.0	17.0
Soluble dietary fiber, %	1.1	1.4	0.2	0.6	0.4	0.5	3.0	3.0
Total dietary fiber, %	10.6	12.1	15.6	15.3	13.9	14.0	20.0	20.0
Physical characteristics								
Water binding capacity, g/g	0.93	1.04	1.23	1.22	1.21	1.19	1.35	1.32
Bulk density, g/L	754.3	760.7	683.7	695.0	760.3	758.3	680.7	683.0

[1]DDGS = distillers dried grains with solubles.

[2]Provided the following quantities of vitamins and micro-minerals per kilogram of complete diet: vitamin A as retinyl acetate, 11,136 IU; vitamin D3 as cholecalciferol, 2208 IU; vitamin E as DL-alpha tocopheryl acetate, 66 IU; vitamin K as menadione dimethylprimidinol bisulfite, 1.42 mg; thiamin as thiamine mononitrate, 0.24 mg; riboflavin, 6.59 mg; pyridoxine as pyridoxine hydrochloride,0.24 mg; vitamin B12, 0.03 mg; D-pantothenic acid as D-calcium pantothenate, 23.5 mg; niacin, 44.1 mg; folic acid, 1.59 mg; biotin, 0.44 mg; Cu, 20 mg as copper sulfate and copper chloride; Fe, 126 mg as ferrous sulfate; I, 1.26 mg as ethylenediamine dihydriodide; Mn, 60.2 mg as manganese sulfate; Se, 0.3 mg as sodium selenite and selenium yeast; and Zn, 125.1 mg as zinc sulfate.

[3]DFM = direct-fed microbial. The premix consisted of 30 g of DFM mixed with 270 g of corn.

[4]Calculated from NRC (2012).

[5]SID = standardized ileal digestible.

[6]STTD = standardized total tract digestible.

At the conclusion of phase 2, 1 pig per pen was sacrificed using captive bolt penetration. In pens with 3 gilts, the gilt that had a BW closest to the pen average was sacrificed, and in pens with 3 barrows, the barrow that had a BW closest to the pen average was sacrificed. Thus, for each treatment, 5 gilts and 5 barrows were sacrificed. Ileal digesta were collected from the distal 20 cm of the ileum and cecal digesta were collected from the center of the cecum. Rectal contents were collected as close to the anus as possible. The pH of each sample was measured immediately after collection using a pH meter (Accumet Basic, Fisher

Scientific, Pittsburgh, PA). After the pH was measured, 10 mL cecal and 10 mL rectal samples were separately mixed with 2*N* HCl in a 1:1 ratio and stored at -20°C until analyzed for concentrations of VFA. The remaining ileal and cecal digesta and rectal contents (50 to 100 mg) were also stored at -20°C for further analysis.

A 5-cm tissue sample was collected from the ileum 10 cm cranial to the ileo-cecal sphincter, from the tail of the cecum, from the rectum 10 cm cranial to the internal anal sphincter, and from the left lateral lobe of the liver. After collection, tissue samples, with the exception of liver tissue, were opened at the mesentery, rinsed with ice-cold PBS, snap-frozen in liquid nitrogen, and stored at -80°C.

RNA Extraction and Quantitative Reverse Transcription-PCR. Total RNA was isolated from 100 mg of frozen tissue samples according to the PureLink RNA Mini Kit (Life Technologies, Grand Island, NY) manufacturer's instructions. Total RNA was quantified using a NanoDrop ND-1000 spectrophotometer (NanoDrop Technologies, Wilmington, DE). The RNA quality was determined using a 2100 Bioanalyzer (Agilent Technologies, Santa Clara, CA) and all RNA samples used for reverse transcription had an RNA integrity number greater than 8.

Total RNA (100 ng/μL) was reverse transcribed by means of a SuperScript III First-Strand Synthesis SuperMix kit (Life Technologies) to synthesize the double-stranded cDNA. Double-stranded cDNA was diluted and used for quantitative reverse transcription (**qRT-PCR**). Each 10 μL reaction consisted of 5 μL SYBR Green (Applied Biosystems, Foster City, CA), 4 μL diluted cDNA sample, 0.4 μL of 10 μM forward and reverse primer, and 0.2 μL DNase/RNase free water. The reactions were performed in an ABI Prism 7,900 HT (Applied Biosystems, Foster City, CA) using the following conditions: 2 min at 50°C, 10 min at 95°C, 40 cycles of 15 s at 95°C, and 1 min at 60°C. An additional dissociation stage was added to verify the presence of a single PCR product. All reactions were run in triplicate. Data were analyzed using the 7,900 HT Sequence Detection Systems Software (version 2.2.1, Applied Biosystems).

Two internal control genes, glyceraldehyde 3-phosphate dehydrogenase (***GAPDH***) and hydroxymethylbilane synthase (***HMBS***), were used to normalize the expression of tested genes (Vigors et al., 2014). The *GAPDH* gene was selected as an internal control gene because it is constitutively expressed at high levels in most tissues and it was expected that glycolysis would not be different among pigs fed experimental diets. The *HMBS* gene was selected as an internal control gene because it was expected that heme synthesis would not be different among pigs fed experimental diets.

The tested genes included mucin 2 (***MUC2***), monocarboxylate transporter 1 (***MCT1***), basigin (***CD147***), phosphoenolpyruvate carboxykinase 1 (***PEPCK1***), and glucagon-like peptide-2 receptor (***GLP-2R***). Mucin 2 is responsible for the production of mucin and was selected because previous research has indicated that high-fiber diets may increase mucin production (de Lange et al., 1989). Monocarboxylate transporter 1 is a proton-coupled transporter of VFA and *CD147* is responsible for translocation and function of *MCT1* (König et al., 2010) and, therefore, these 2 genes were selected to aid in the explanation of intestinal concentrations of VFA. Phosphoenolpyruvate carboxykinase 1 is the rate-controlling enzyme of gluconeogenesis in pigs (Shulman and Petersen, 2012) and was selected because we hypothesized that HF fed pigs would have less absorption of dietary glucose and, therefore, have increased gluconeogenesis in the liver. Glucagon-like peptide- 2 receptor is a G protein-coupled, transmembrane receptor for the peptide glucagon-like peptide- 2, which has been indicated to control gastrointestinal growth and function (Guan et al., 2006). Ileum, cecum, and rectum tissue were tested for mRNA expression of *MUC2*, *MCT1*, *CD147*, and *GLP-2R*, whereas liver tissue was tested for *MCT1*, *CD147*, *GLP-2R*, and *PEPCK1*. Primers used for amplification of target genes are provided in Table 2. To obtain the relative gene expression, the average quantity of triplicate samples was calculated and divided by the geometric mean of the 2 internal control genes.

Chemical Analyses. Prior to analysis, ileal and cecal digesta and rectal contents were freeze-dried and ground through a 1-mm screen in a Wiley Mill (Model 4; Thomas Scientific, Swedesboro, NJ). All main ingredients used in the diets and all diets, cecal, and rectal samples were analyzed for DM (Method 930.15; AOAC International, 2007; Table 3). All diets and main ingredients were analyzed for ADF and NDF using Ankom Technology method 12 and 13, respectively (Ankom2000 Fiber Analyzer, Ankom Technology, Macedon, NY), whereas insoluble and soluble dietary fiber were analyzed according to method 991.43 (AOAC International, 2007) using the AnkomTDF Dietary Fiber Analyzer (Ankom Technology). Total dietary fiber was then calculated as the sum of insoluble and soluble dietary fiber. All diets and main ingredients also were analyzed for ash (Method 942.05; AOAC International, 2007) and for GE using bomb calorimetry (Model 6300; Parr Instruments, Moline, IL).

All cecal digesta samples and rectal samples that were stabilized in 2*N* HCl were analyzed for concentrations of VFA by gas chromatography according to Erwin et al. (1961) using a gas chromatograph (Hewlett-Packard 5890A Series II, Palo Alto, CA) and a glass column (180 cm × 4 mm i.d.) packed with 10% SP-1200/1% H3PO4 on 80/100 + mesh Chomosorb WAW (Supelco Inc., Bellefonte, PA). Nitrogen was the carrier gas with a

Table 2. Primers utilized for quantitative reverse transcription-PCR

Gene	Acc. No	Direction[1]	Primer	BP[2]	Reference
MUC2[3]	XM_013992271	F	CAACGGCCTCTCCTTCTCTGT	312	Leonard et al. (2011)
		R	GCCACACTGGCCCTTTGT		
MCT1[4]	AM286425	F	GGTGGAGGTCCTATCAGCAG	169	Metzler-Zebeli et al. (2012)
		R	TGAAGGCAAGCCCAAGAC		
CD147[5]	NM_001123086	F	CCTCGGAGACCAAGACAGAG	901	König et al. (2010)
		R	TCATTCACGTGGTGTCCACT		
PEPCK1[6]	NM_001123158.1	F	CCCTGCCTTTGAAAAAGCCC	2591	Qu et al. (2015)
		R	GGAGATGATTTCTCGGCGGT		
GLP-2R[7]	NM_001246266.1	F	TGTCCTACGTGTCGGAGATGTC	1671	Guan et al. (2006)
		R	TAATTGGCGCCCACGAA		

[1]Direction of primer (F = forward; R = reverse).

[2]Amplicon size in base pair (BP).

[3]MUC2 = mucin 2.

[4]MCT1 = monocarboxylate transporter 1.

[5]CD147 = basigin.

[6]PEPCK1 = phosphoenolpyruvate carboxykinase 1.

[7]GLP-2R = glucagon-like peptide-2 receptor.

flow rate of 75 mL/min. Oven, detector, and injector temperatures were 125, 175, and 180°C, respectively. The physical characteristics of the main ingredients and diets were determined by measuring the water binding capacity (Robertson et al., 2000; Cervantes-Pahm et al., 2014) and bulk density (Cromwell et al., 2000).

Statistical Analysis. Normality of residuals were determined by the UNIVARIATE procedure of SAS (SAS Inst. Inc., Cary, NC). Outliers were determined using the BOXPLOT procedure of SAS (SAS Inst. Inc., Cary, NC) and any value that deviated from the treatment mean by 1.5 times the interquartile range was removed; 6 outliers were identified and removed. Gene expression data were log-10 transformed to align measures to a normal distribution. Growth performance, intestinal concentrations of VFA, and log-scale relative gene expression data were analyzed as a 2 × 2 factorial arrangement of treatments with dietary fiber concentration and DFM as the 2 factors and block as the random effect using the MIXED procedure of SAS (SAS Inst. Inc., Cary, NC). Initial BW was used as a covariate for growth performance. Relative gene expression data presented were back-transformed using antilog. The pen was the experimental unit for all analyses.

A repeated measures analysis was conducted for TNF-α and PUN data (Littell et al., 1998). Appropriate covariance structures were chosen based on the Akaike information criterion. Data were subjected to a 3-way ANOVA that included dietary fiber concentration, DFM, and d, as well as the interactions among these factors using PROC MIXED. Block was considered the random effect. The SLICE option of PROC MIXED was used to evaluate the main effects and interaction of dietary fiber concentration and DFM at each d. For all outcomes, a P-value ≤ 0.05 was used to determine significance among dietary treatments and a P-value > 0.05, but < 0.10 was considered a tendency.

RESULTS

Ingredient and Diet Analysis

Phase 1 LF diets contained 1.3 and 10.1% soluble and insoluble dietary fiber, respectively, and phase 1 HF diets contained 0.4 and 15.1% soluble and insoluble dietary fiber, respectively (Table 1). Phase 2 LF diets contained 0.5 and 13.5% soluble and insoluble dietary fiber, respectively, and phase 2 HF diets contained 3.0 and 17.0% soluble and insoluble dietary fiber, respectively.

Table 3. Analyzed energy and nutrient composition and physical characteristics of ingredients containing dietary fiber (as-fed basis)

	Ingredient			
Item	Corn	Soybean meal	DDGS[1]	Wheat middlings
GE, kcal/kg	3773	4175	4421	4027
DM, %	84.5	88.4	86.9	86.7
Ash, %	1.1	6.1	4.5	4.8
ADF, %	3.8	6.1	11.6	10.8
NDF, %	12.2	7.0	24.1	38.4
Insoluble dietary fiber, %	12.1	15.0	29.0	37.1
Soluble dietary fiber, %	0.6	1.4	1.9	6.3
Total dietary fiber, %	12.7	16.4	30.9	43.4
Physical characteristics				
Water binding capacity, g/g	0.97	2.79	1.74	3.11
Bulk density, g/L	683.0	807.3	601	363.7

[1]DDGS = distillers dried grains with solubles.

Table 4. Growth performance of weanling pigs fed low- or high-fiber diets without or with a *Bacillus*-based direct-fed microbial

Dietary fiber:	Low		High			*P*-value		
Direct-fed microbial:	-	+	-	+	SEM	Dietary fiber	DFM[1]	Dietary fiber × DFM
Phase 1 (d 0 to 14)								
Initial BW, kg	6.336	6.276	6.298	6.309	0.268	0.802	0.048	0.006
ADG, g/d	189	187	178	168	12.35	0.178	0.613	0.744
ADFI, g/d	240	219	206	172	23.30	0.033	0.138	0.726
G:F, g/g	0.802	0.923	0.830	1.048	0.084	0.350	0.043	0.555
G:F, kg/Mcal NE	0.317	0.366	0.337	0.426	0.032	0.227	0.041	0.536
Final BW, kg	8.988	8.900	8.785	8.664	0.350	0.176	0.514	0.918
Phase 2 (d 14 to 43)								
ADG, g/d	619	629	598	599	38.31	0.025	0.600	0.694
ADFI, g/d	922	936	924	875	71.55	0.127	0.335	0.099
G:F, g/g	0.672	0.676	0.649	0.678	0.015	0.298	0.118	0.240
G:F, kg/Mcal NE	0.271	0.272	0.269	0.281	0.006	0.436	0.113	0.228
Final BW, kg	26.929	27.127	26.117	26.037	1.202	0.024	0.883	0.730
d 0 to 43								
ADG, g/d	479	485	461	459	24.24	0.025	0.835	0.665
ADFI, g/d	700	702	691	646	43.94	0.048	0.192	0.153
G:F, g/g	0.685	0.695	0.667	0.702	0.013	0.592	0.022	0.192
G:F, kg/Mcal NE	0.275	0.279	0.276	0.290	0.005	0.138	0.020	0.179

[1]DFM = direct-fed microbial.

Water binding capacity was 0.99 and 1.23 g/g and bulk density was 757.5 and 689.4 g/L in phase 1 LF and HF diets, respectively, and water binding capacity was 1.20 and 1.34 g/g and the bulk density was 759.3 and 681.9 g/L in phase 2 LF and HF diets, respectively. Corn, soybean meal, DDGS, and wheat middlings contained 0.6, 1.4, 1.9, and 6.3% soluble dietary fiber, respectively, and 12.1, 15.0, 29.0, and 37.1% insoluble dietary fiber, respectively (Table 3). Water binding capacity was 0.97, 2.69, 1.74, and 3.11 g/g for corn, soybean meal, DDGS, and wheat middlings, respectively, and bulk density was 683.0, 807.3, 601.0, and 363.7 g/L, respectively.

Growth Response

Phase 1 ADG and BW at the conclusion of phase 1 were not affected by dietary fiber concentration or DFM (Table 4), but phase 1 ADFI was greater ($P \leq 0.05$) for pigs fed LF diets compared with pigs fed HF diets. Pigs fed diets containing DFM had greater ($P \leq 0.05$) G:F in phase 1 compared with pigs fed diets without DFM. During phase 2, ADFI and G:F of pigs were unaffected by dietary fiber concentration or DFM, but ADG and BW of pigs fed LF diets were greater ($P \leq 0.05$) compared with pigs fed HF diets. Overall, pigs fed LF diets had greater ($P \leq 0.05$) ADG, ADFI, and final BW compared with pigs fed HF diets and pigs fed diets containing DFM had greater ($P \leq 0.05$) G:F than pigs fed no DFM.

Intestinal Concentrations of VFA and pH

The pH of ileal digesta was not affected by dietary fiber concentration or DFM, but cecal digesta pH tended to be greater ($P < 0.10$) in pigs fed LF diets compared with pigs fed HF diets (Table 5). In contrast, rectal content pH tended to be greater ($P < 0.10$) in pigs fed HF diets compared with pigs fed LF diets. Concentrations of individual VFAs in cecal digesta were not affected by dietary fiber concentration or DFM. The concentrations of acetate, propionate, and isovalerate were greater ($P \leq 0.05$) and the concentration of isobutyrate tended to be greater ($P < 0.10$) in rectal contents of pigs fed LF diets compared with pigs fed HF diets. Total short-chain fatty acid concentration in rectal contents of pigs fed LF diets was greater ($P \leq 0.05$) compared with pigs fed HF diets and the concentration of total branched-chain fatty acids tended to be greater ($P < 0.10$) in the rectal contents of pigs fed LF diets.

Plasma Urea Nitrogen and TNF-α

No effects of dietary fiber concentration or DFM addition on plasma concentrations of TNF-α were observed (Fig. 1). However, the effect of sampling d impacted the concentration of TNF-α because on d 0, concentration of TNF-α was the least ($P \leq 0.05$) and on d 14 the greatest ($P \leq 0.05$), whereas the concentration of TNF-α on d 43 was between d 0 and d 14 concentrations. The PUN of pigs fed DFM-containing diets was less on d 0 compared with pigs fed diets

Table 5. pH in ileal, cecal, and rectal contents and VFA concentrations, expressed as μmol/g DM, in cecal and rectal contents of pigs fed a low- or high-fiber diet without or with a *Bacillus*-based direct-fed microbial

Dietary fiber concentration:	Low		High			*P*-value		
Direct-fed microbial:	-	+	-	+	SEM	Dietary fiber	DFM[1]	Dietary fiber × DFM
Ileal digesta pH	6.78	6.77	6.83	7.04	0.18	0.216	0.444	0.413
Cecal digesta								
pH	6.01	6.01	5.84	5.89	0.10	0.053	0.696	0.767
Acetate	100.73	100.17	102.38	102.67	4.59	0.470	0.962	0.883
Propionate	27.02	28.24	29.59	29.75	1.99	0.156	0.629	0.709
Butyrate	14.30	12.88	16.64	13.37	1.66	0.343	0.121	0.536
Total SCFA[2]	142.06	141.29	149.51	145.79	7.85	0.233	0.650	0.765
Valerate	2.08	1.76	2.15	1.93	0.36	0.715	0.397	0.878
Isovalerate	0.48	0.50	0.35	0.61	0.09	0.913	0.101	0.180
Isobutyrate	0.52	0.60	0.58	0.71	0.08	0.322	0.206	0.747
Total BCFA[3]	3.08	2.87	3.07	3.25	0.39	0.618	0.961	0.612
SCFA:BCFA	53.58	55.47	48.98	49.84	5.21	0.312	0.785	0.919
Rectal contents								
pH	6.56	6.53	6.77	6.63	0.09	0.093	0.373	0.561
Acetate	112.99	111.44	100.78	93.95	5.59	0.006	0.416	0.608
Propionate	25.94	24.43	21.21	19.43	2.00	0.021	0.418	0.949
Butyrate	12.94	11.54	10.95	11.56	1.08	0.367	0.716	0.355
Total SCFA	151.71	147.41	141.19	129.79	7.20	0.027	0.204	0.563
Valerate	2.10	2.35	2.03	2.01	0.20	0.321	0.582	0.507
Isovalerate	2.79	2.71	2.18	2.21	0.39	0.043	0.920	0.825
Isobutyrate	2.29	2.06	1.81	1.71	0.23	0.059	0.445	0.749
Total BCFA	6.86	6.89	5.62	5.90	0.60	0.065	0.787	0.834
SCFA:BCFA	21.68	21.91	23.91	21.84	3.13	0.616	0.669	0.590

[1]DFM = direct-fed microbial.

[2]SCFA = short chain fatty acids.

[3]BCFA = branched-chain fatty acids.

without DFM (Fig. 2). On d 14, PUN was not different among pigs fed experimental diets. However, HF diets increased ($P \leq 0.05$) PUN of pigs on d 43, but an interaction ($P \leq 0.05$) between dietary fiber concentration and DFM also was observed on d 43 because DFM addition to LF diets increased ($P \leq 0.05$) PUN of pigs, whereas DFM addition to HF diets decreased ($P \leq 0.05$) PUN. As the experiment progressed from d 0 to14 and d 43, the PUN of pigs increased ($P \leq 0.05$), which is illustrated by the main effect of d ($P \leq 0.05$).

Gene Expression

The expression of *MCT1* was decreased ($P \leq 0.05$) in the ileum of pigs due to DFM addition to the diets (Table 6). The expression of genes tested from cecum

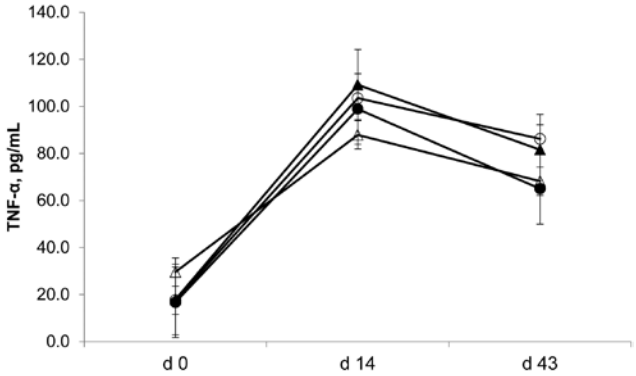

Figure 1. Tumor necrosis factor-α (TNF-α) of nursery pigs fed a low- (Δ) or high-fiber (○) diet without or with a Bacillus-based direct-fed microbial (DFM; ▲ or ●). Error bars indicate standard error of the mean. Main effect of d ($P \leq 0.05$) was observed, but no differences were detected among treatments on d 0, 14, or 43.

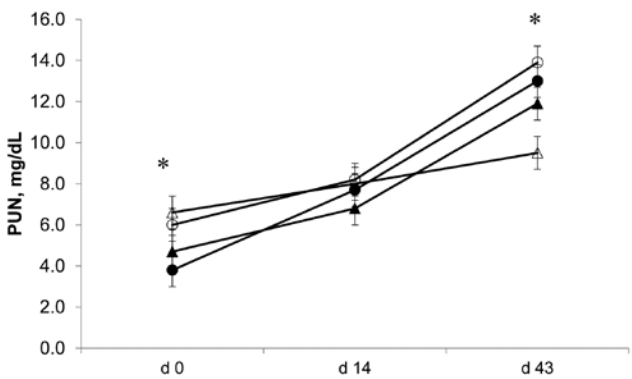

Figure 2. Plasma urea nitrogen (PUN) of nursery pigs fed a low- (Δ) or high-fiber (○) diet without or with a Bacillus-based direct-fed microbial (DFM;▲or ●). Error bars indicate standard error of the mean. *d 0 DFM ($P = 0.015$), dietary fiber × DFM ($P = 0.081$). *d 43 dietary fiber ($P = 0.001$), dietary fiber × DFM ($P = 0.002$). Main effect of d ($P \leq 0.05$) was also observed.

Table 6. Effects of dietary fiber concentration and addition of a *Bacillus*-based direct-fed microbial on relative mRNA expression of genes

Dietary fiber concentration:	Low		High			*P*-value		
Direct-fed microbial:	-	+	-	+	SEM	Dietary fiber	DFM[1]	Dietary fiber × DFM
Ileum								
MCT1[2]	2.14	1.48	1.98	1.70	1.13	0.777	0.017	0.300
CD147[3]	0.97	1.18	1.12	1.11	1.10	0.687	0.318	0.276
MUC2[4]	0.82	0.70	1.09	0.82	1.29	0.265	0.268	0.762
GLP-2R[5]	0.85	0.84	0.76	0.65	1.24	0.378	0.710	0.741
Cecum								
MCT1	0.66	0.58	0.64	0.55	1.19	0.801	0.424	0.969
CD147	3.40	2.46	2.52	2.66	1.12	0.344	0.245	0.110
MUC2	0.42	0.34	0.36	0.34	1.12	0.467	0.241	0.520
GLP-2R	0.29	0.32	0.26	0.27	1.21	0.387	0.718	0.767
Rectum								
MCT1	0.85	1.00	1.59	1.65	1.22	< 0.001	0.477	0.634
CD147	1.16	1.12	1.15	1.45	1.16	0.147	0.265	0.136
MUC2	1.06	0.94	0.92	1.22	1.19	0.629	0.496	0.084
GLP-2R	0.59	0.80	0.77	0.89	1.26	0.238	0.146	0.599
Liver								
MCT1	0.74	0.97	0.81	0.88	1.11	0.999	0.090	0.351
CD147	1.03	1.25	1.11	1.16	1.06	0.990	0.038	0.180
GLP-2R	0.94	1.20	0.88	1.33	1.17	0.899	0.011	0.492
PEPCK1[6]	0.81	0.54	0.84	1.00	1.30	0.161	0.626	0.228

[1]DFM = direct-fed microbial.

[2]*MCT1* = monocarboxylate transporter 1.

[3]*CD147* = basigin.

[4]*MUC2* = mucin 2.

[5]*GLP-2R* = glucagon-like peptide-2 receptor.

[6]*PEPCK1* = phosphoenolpyruvate carboxykinase 1.

tissue was not affected by dietary fiber concentration or DFM addition. Pigs fed HF diets had increased ($P \leq 0.05$) *MCT1* expression in the rectum. Pigs fed DFM-containing diets had increased ($P \leq 0.05$) *CD147* and *GLP-2R* expression and tended to have increased ($P < 0.10$) *MCT1* expression in the liver.

DISCUSSION

Results of previous research indicated that weanling pig growth performance is not reduced if 7.5 and 15% DDGS is included in phase 1 and 2 diets, respectively (Spencer et al., 2007). Likewise, inclusion of 20% DDGS in diets for weanling pigs may not impact pig growth performance (Stein and Shurson, 2009), but wheat middlings included at 0, 5, 10, 15, or 20% in a corn-soybean meal diet fed to weanling pigs linearly reduced ADFI and ADG (De Jong et al., 2014). It is, therefore, likely that the inclusion of wheat middlings in the diets used in this experiment resulted in the reduction in ADG observed for the pigs fed HF diets.

The decrease in ADFI and ADG that was observed as water binding capacity increased and bulk density decreased in diets is also in agreement with previous data (Jaworski et al., 2014b). These results indicate that the increased gut fill and water binding capacity that is often associated with increased concentrations of fiber in diets may prevent weanling pigs from consuming enough feed to meet their energy requirement, but because gut fill was not determined in this experiment, this hypothesis could not be verified.

Addition of a *Bacillus*-based DFM to a corn-soybean meal diet fed to growing-finishing pigs may improve ADG (Davis et al., 2008). Addition of 8% soybean hulls or 8% peanut hulls to a corn-soybean meal diet fed to weanling pigs reduced G:F, but if a yeast culture DFM was added to those diets, G:F was maintained compared with a corn-soybean meal control diet (Kornegay et al., 1995). Thus, the observation that addition of a *Bacillus*-based DFM to LF and HF diets in this experiment improved overall G:F of pigs is in agreement with the data by Kornegay et al. (1995). However, in a review of the literature, Pollmann (1986) reported that addition of *Bacillus*-based DFM to weanling pig diets did not consistently improve growth performance, whereas a more recent review indicated that DFM addition to swine diets was beneficial in 30 of 31 research trials (Kremer, 2006). Likewise, addition of the same

3-strain bacillus-based DFM as used in the present experiment improved ADG during the initial 2 wk post-weaning and G:F during the entire nursery period as was also observed in the present experiment (Cai et al., 2015). Improved ADG compared with a negative control group was also observed if a bolus dose of DFM was provided to pigs at weaning and DFM supplemented diets were provided from weaning until d 35 post-weaning (Walsh et al., 2007). Water delivery of DFM also prevented a reduction in ADG during a salmonella challenge, whereas pigs provided water without DFM had a significant drop in ADG (Walsh et al., 2012).

The inconsistencies reported in the literature regarding *Bacillus*-based DFM added to weanling pig diets may be a result of differences in ingredient composition of diets or health status of pigs, but it is also possible that improvements have been made in the development and implementation of DFM. Inconsistency in a DFM response also may arise from differences in the functionality of the strains being assessed and whether single strains or a combination of strains are included in the DFM being used. It is, therefore, possible that the reason a positive response was observed in the present experiment may be that the *Bacillus*-based DFM used in this experiment was more efficient in terms of stimulating microbial enzyme synthesis compared with the DFM used in some of the previous experiments.

It has also been proposed that in situ production of enzymes by DFM's may be an additional mode of action (Ferrari et al., 1993) and *Bacillus*-based DFM may secrete enzymes that can degrade DM in swine manure (Schreier, 1993; Davis et al., 2008). Swine manure DM is mostly composed of dietary fiber due to the indigestible nature of dietary fiber fed to pigs. Therefore, we hypothesized that a *Bacillus*-based DFM added to swine diets may result in increased fermentation of dietary fiber, which may increase the amount of energy available to the pig in the form of VFA. It was expected that DFM would increase dietary fiber fermentation, resulting in a lower pH and greater VFA concentration in cecal and rectal contents.

Addition of a *Bacillus*-based DFM to growing pig diets containing multiple sources of dietary fiber increased the concentration of VFA in the feces and, therefore, enhanced fermentation and available ME, which resulted in increased ADG and final BW (Jaworski et al., 2014a). However, in the current experiment, DFM had no effect on pH or VFA concentrations in ileal, cecal, or rectal contents, but contrary to our hypothesis, concentrations of acetate, propionate, isovalerate, total short chain fatty acids, and total branched-chain fatty acids in rectal contents of pigs fed LF diets were greater compared with pigs fed HF diets. It is possible that the reason for this observation is that pigs fed the HF diets excreted more feces per d than pigs fed the LF diets, and therefore, the total quantity of VFA excreted per d may have been greater from HF-fed pigs than from LF-fed pigs despite the greater concentration of VFA in LF-fed pigs. Previous research has indicated that, indeed, pigs fed HF diets excrete more feces compared with pigs fed LF diets (Fu et al., 2004) and, therefore, this warrants further investigation. Additionally, the increased dietary fiber in HF diets may have increased rate of digesta passage, which may decrease time for microbial fermentation of dietary fiber .

Feeding of HF diets may also result in greater expression of VFA transporters, which may result in faster absorption of VFA from pigs fed HF diets compared with pigs fed LF diets, and therefore less VFA is excreted in the feces. The observation that the expression of the VFA transporter *MCT1* in the rectum of HF-fed pigs was greater compared with LF-fed pigs indicates that more VFA may have been absorbed from these pigs. Metzler-Zebeli et al. (2012) reported that *MCT1* expression in the cecum and colon of weaned pigs was positively correlated with butyrate and propionate concentration indicating that increased concentration of VFA may upregulate expression of the *MCT1* gene. However, it has been indicated that *CD147* is required for *MCT1* translocation to the plasma membrane as well as for *MCT1* transporter function (Kirk et al., 2000; Wilson et al., 2005), but in the current experiment, *CD147* expression in the rectum was not influenced by dietary fiber. Infusion of VFA into the cecum of growing pigs resulted in almost complete absorption of the VFA and less than 1% of the infused VFA were excreted in the feces (Jørgensen et al., 1997). Therefore, it appears that VFA absorption is quite efficient (Barcroft et al., 1944), and determination of the concentration of VFA in intestinal contents or feces may, therefore, not be an accurate estimate of the synthesis of VFA (Montoya et al., 2016). The VFA molar proportions in cecal and rectal contents observed in this experiment are in agreement with the VFA molar proportion (i. e., 65:25:10, acetate:propionate:butyrate) usually observed in pigs (Robertson, 2007).

The observation that the G:F, expressed as kg/Mcal NE, was not different between pigs fed LF or HF diets indicates that weanling pigs are as efficient converting dietary NE from HF diets to BW gain as they are converting NE from LF diets. However, the increased bulk associated with HF diets appears to be the major barrier that prevents weanling pigs from consuming the same quantities of HF diets as they do of LF diets as indicated by the reduced ADFI for pigs fed the HF diets although it is also possible that the HF diets had reduced palatability compared with the LF diets (Seabolt et al., 2010; Kim et al., 2012).

The improved G:F, expressed as kg/Mcal NE, that was observed for pigs fed the DFM containing diets

indicate that the DFM may have contributed to improved fermentation of the fiber in the HF diets, which may have increased the amount of energy the pigs received from the diets. It is possible that addition of DFM to diets fed to weanling pigs may also reduce maintenance energy requirement of the pig by reduced immune stimulation, reduced endogenous secretions of nutrients, or improved gastrointestinal integrity. However, the observation that plasma concentration of TNF-α was not different between pigs fed diets without and with DFM indicates that the pro-inflammatory immune cell regulation at the systemic level was not affected by DFM addition. However, changes in pro-inflammatory immune responses are also possible at the mucosal level (Elsasser et al., 2008), but this was not experimentally verified in the current experiment. Therefore, it cannot be ruled out that pig pro-inflammatory immune cell regulation may be affected by DFM addition to the diets.

The increase in the expression of *GLP-2R* in the liver of pigs fed DFM-containing diets indicates that the concentration of *GLP-2* in the liver may also have been increased as has previously been suggested (Connor et al., 2015). Glucagon-like peptide-2 increased expression of maltase-glucoamylase and sucrose-isomaltase digestive enzymes (Petersen et al., 2001, 2002), decreased gastric emptying, gastric acid secretion, and gut motility (Wøjdemann et al., 1999; Guan et al., 2012), and increased intestinal cell proliferation and reduced apoptosis in weanling pigs (Burrin et al., 2005; 2007). It may, therefore, be speculated that the greater *GLP-2R* expression in the liver was a result of increased *GLP-2* being synthesized, and therefore, the improved G:F of pigs fed the DFM diets may partly be a result of a reduced maintenance energy requirement due to reduced gastric emptying, reduced gastric acid secretions, and less gut motility. However, this hypothesis needs to be experimentally verified.

Conclusion

Inclusion of 10% wheat middlings and 7.5% DDGS in phase 1 diets and 10% wheat middlings and 15% DDGS in phase 2 diets fed to weanling pigs, reduced phase 1 ADFI and overall ADG and ADFI, resulting in a decreased BW of pigs on d 43 post-weaning. However, G:F was unaffected by dietary fiber concentration, indicating that weanling pigs are as efficient in converting dietary NE from HF diets into BW gain as they are of converting NE from LF diets. Addition of a 3 strain *Bacillus*-based DFM to LF or HF diets improved overall G:F, but had no effect on VFA concentration in cecal or rectal contents. However, because a balance study was not conducted to determine the total quantity of fecal excretion, and therefore total VFA excretion in feces, it was not possible to determine if the *Bacillus*-based DFM influenced total synthesis of VFA. The increased energy utilization that was observed for diets that contained the *Bacillus*-based DFM may have been a result of increased synthesis of *GLP-2* because the relative expression of *GLP-2R* in the liver increased in pigs fed diets fortified with DFM.

LITERATURE CITED

AOAC International. 2007. Official Methods of Analysis of AOAC Int. 18th ed. Rev. 2. W. Hortwitz and G. W. Latimer Jr., editors. AOAC Int., Gaithersburg, MD.

Barcroft, J., R. A. McAnally, and A. T. Phillipson. 1944. Absorption of VFA from the alimentary tract of the sheep and other animals. J. Exp. Biol. 20:120–132.

Bindelle, J., P. Leterme, and A. Buldgen. 2008. Nutritional and environmental consequences of dietary fibre in pig nutrition: a review. Biotechnol. Agron. Soc. Environ. 12:69-80.

Burrin, D. G., B. Stoll, X. Guan, L. Cui, X. Chang, and D. Hadsell. 2007. GLP-2 rapidly activates divergent intracellular signaling pathways involved in intestinal cell survival and proliferation in neonatal piglets. Am. J. Physiol. Endocrinol. Metab. 292:E281–E291

Burrin, D. G., B. Stoll, X. Guan, L. Cui, X. Chang, and J. J. Holst. 2005. Glucagon-like peptide 2 dose-dependently activates intestinal cell survival and proliferation in neonatal piglets. Endocrinology 146:22–32. doi:10.1210/en.2004-1119

Cai, L., S. Indrakumar, E. Kiarie, and I. H. Kim. 2015. Effects of a multi-strain Bacillus species-based direct-fed microbial on growth performance, nutrient digestibility, blood profile, and gut health in nursery pigs fed corn-soybean meal-based diets. J. Anim. Sci. 93:4336–4342. doi:10.2527/jas.2015-9056

Cervantes-Pahm, S. K., Y. Liu, and H. H. Stein. 2014. Comparative digestibility of energy and nutrients and fermentability of dietary fiber in eight cereal grains fed to pigs. J. Sci. Food Agric. 94:841-849. doi:10.1002/jsfa.6316

Connor, E. E., C. M. Evock-Clover, M. P. Walker, T. H. Elsasser, and S. Kahl. 2015. Comparative Gut Physiology Symposium: Comparative physiology of glucagon-like peptide-2: Implications and applications for production and health of ruminants. J. Anim. Sci. 93:492–502. doi:10.2527/jas.2014-8577

Cromwell, G. L., T. R. Cline, J. D. Crenshaw, T. D. Crenshaw, R. A. Easter, R. C. Ewan, C. R. Hamilton, G. M. Hill, A. J. Lewis, D. C. Mahan, J. L. Nelssen, J. E. Pettigrew, T. L. Veum, and J. T. Yen. 2000. Variability among sources and laboratories in analyses of wheat middlings. J. Anim. Sci. 78:2652–2658. doi:10.2527/2000.78102652x

Cromwell, G. L. 2013. Feed additives in swine diets. In: L. I. Chiba, editor, Sustainable Swine Nutrition. 1st ed. John Wiley & Sons, Ames, IA. p. 302–304.

Davis, M. E., T. Parrott, D. C. Brown, B. Z. de Rodas, Z. B. Johnson, C. V. Maxwell, and T. Rehberger. 2008. Effect of a *Bacillus*-based direct-fed microbial feed supplement on growth performance and pen cleaning characteristics of growing-finishing pigs. J. Anim. Sci. 86:1459–1467. doi:10.2527/jas.2007-0603

De Jong, J. A., J. M. DeRouchey, M. D. Tokach, S. S. Dritz, and R. D. Goodband. 2014. Effects of dietary wheat middlings, corn dried distillers grains with solubles, and net energy formulation on nursery pig performance. J. Anim. Sci. 92:3471–3481. doi:10.2527/jas.2013-7350

de Lange, C. F. M., J. Pluske, J. Gong, and C. M. Nyachoti. 2010. Strategic use of feed ingredients and feed additives to stimulate gut health and development in young pigs. Livest. Sci. 134:124–134. doi:10.1016/j.livsci.2010.06.117

de Lange, C. F. M., W. C. Sauer, R. Mosenthin, and W. B. Souffrant. 1989. The effect of feeding different protein-free diets on the recovery and amino acid composition of endogenous protein collected from the distal ileum and feces in pigs. J. Anim. Sci. 67:746–754. doi:10.2527/jas1989.673746x

Eigel, W. N. 1989. Ability of probiotics to protect weanling pigs against challenge with enterotoxigenic *E. coli.* In: Proc. Chr. Hansen Biosystems Tech. Conf. San Antonio, TX. p. 10-19.

Elsasser, T. H., T. J. Caperna, C-J. Li, S. Kahl, and J. L. Sartin. 2008. Critical control points in the impact of the proinflammatory immune response on growth and metabolism. J. Anim. Sci. 86(E. Suppl.):E105-E125. doi:10.2527/jas.2007-0634

Erwin, E. A., G. J. Marco, and E. M. Emery. 1961. Volatile fatty acid analyses of blood and rumen fluid by gas chromatography. J. Dairy Sci. 44:1768–1771. doi:10.3168/jds.S0022-0302(61)89956-6

FAO/WHO (Food and Agriculture Organization/World Health Organization). 2001. Health and nutritional properties of probiotics in food including powder milk with live lactic acid bacteria. Report of a Joint FAO/WHO Expert Consultation on Evaluation of Health and Nutritional Properties of Probiotics in Food Including Powder Milk with Live Lactic Acid Bacteria. Córdoba, Argentina. p. 1-33.

Ferrari, E., A. Jarnagin, and B. Schmidt. 1993. Commercial production of extracellular Enzymes. In: A. Sonenshein, J. Hoch, and R. Losick, editors, Bacillus subtilis and other gram-positive bacteria. ASM Press, Washington, DC. p. 917–937. doi:10.1128/9781555818388.ch62

Fu, S. X., M. Johnston, R. W. Fent, D. C. Kendall, J. L. Usry, R. D. Boyd, and G. L. Allee. 2004. Effect of corn distiller's dried grains with solubles (DDGS) on growth, carcass characteristics and fecal volume in growing-finishing pigs. J. Anim. Sci. 82(Suppl. 2):80 (Abstr.).

Guan, X., H. E. Karpen, J. Stephens, J. T. Bukowski, S. Niu, G. Zhang, B. Stoll, M. J. Finegold, J. J. Holst, D. L. Hadsell, B. L. Nichols, and D. G. Burrin. 2006. GLP-2 receptor localizes to enteric neurons and endocrine cells expressing vasoactive peptides and mediates increased blood flow. Gastroenterology. 130:150–164. doi:10.1053/j.gastro.2005.11.005

Guan, X., X. Shi, X. Li, B. Chang, Y. Wang, D. Li, and L. Chan. 2012. GLP-2 receptor in POMC neurons suppresses feeding behavior and gastric motility. Am. J. Physiol. Endocrinol. Metab. 303:E853–E864. doi:10.1152/ajpendo.00245.2012

Jaworski, N. W., A. Owusu-Asiedu, A. A. Awati, and H. H. Stein. 2014a. Effect of *Bacillus* spp. direct-fed microbials on fecal VFA concentrations, growth performance, and carcass characteristics of growing-finishing pigs. J. Anim. Sci. 92(Suppl. 2):56 (Abstr.).

Jaworski, N. W., J. Shoulders, J. C. González-Vega, and H. H. Stein. 2014b. Effects of using copra meal, palm kernel expellers, or palm kernel meal in diets for weanling pigs. Prof. Anim. Sci. 30:243–251.

Jørgensen, H., T. Larsen, X. Q. Zhao, and B. O. Eggum. 1997. The energy value of short-chain fatty acids infused into the cecum of pigs. Br. J. Nutr. 77:745–756. doi:10.1079/BJN19970072

Kenny, M., H. Smidt, E. Mengheri, and B. Miller. 2011. Probiotics–do they have a role in the pig industry? Animal 5:462–470. doi:10.1017/S175173111000193X

Kim, B. G., Y. Zhang, and H. H. Stein. 2012. Sulfur concentration in diets containing corn, soybean meal, and distillers dried grains with solubles does not affect feed preference or growth performance of weanling or growing-finishing pigs. J. Anim. Sci. 90:272–281. doi:10.2527/jas.2010-3777

Kirk, P., M. C. Wilson, C. Heddle, M. H. Brown, A. N. Barclay, and A. P. Halestrap. 2000. CD147 is tightly associated with lactate transporters MCT1 and MCT4 and facilitates their cell surface expression. EMBO J. 19:3896–3904. doi:10.1093/emboj/19.15.3896

König, B., S. Fischer, S. Schlotte, G. Wen, K. Eder, and G. I. Stangl. 2010. Monocarboxylate transporter 1 and CD147 are up-regulated by natural and synthetic peroxisome proliferator-activated receptor α agonists in livers of rodents and pigs. Mol. Nutr. Food Res. 54:1248–1256. doi:10.1002/mnfr.200900432

Kornegay, E. T., D. Rhein-Welker, M. D. Lindemann, and C. M. Wood. 1995. Performance and nutrient digestibility in weanling pigs as influenced by yeast culture additions to starter diets containing dried whey or one of two fiber sources. J. Anim. Sci. 73:1381-1389. doi:10.2527/1995.7351381x

Kremer, B. 2006. DFM products provide consistent outcomes: producers look for consistently performing feed additives, and research has found that certain direct-fed microbial products can fit this description. Feedstuffs. 24 April 2006: 78: p. 14-15.

Littell, R. C., P. R. Henry, and C. B. Ammerman. 1998. Statistical analysis of repeated measures data using SAS procedures. J. Anim. Sci. 76:1216-1231. doi:10.2527/1998.7641216x

Leonard, S. G., T. Sweeney, B. Bahar, B. P. Lynch, and J. V. O'Doherty. 2011. Effects of dietary seaweed extract supplementation in sows and post-weaned pigs on performance, intestinal morphology, intestinal microflora and immune status. Br. J. Nutr. 106:688–699. doi:10.1017/S0007114511000997

Metzler-Zebeli, B. U., M. G. Gänzle, R. Mosenthin, and R. T. Zijlstra. 2012. Oat b-glucan and dietary calcium and phosphorus differentially modify intestinal expression of proinflammatory cytokines and monocarboxylate transporter 1 and cecal morphology in weaned pigs. J. Nutr. 142:668–674. doi:10.3945/jn.111.153007

Montoya, C. A., S. M. Rutherfurd, and P. J. Moughan. 2016. Kiwifruit fibre level influences the predicted production and absorption of SCFA in the hindgut of growing pigs using a combined *in vivo-in vitro* digestion methodology. Br. J. Nutr. 115:1317–1324. doi:10.1017/S0007114515002883

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

Petersen, Y. M., D. G. Burrin, and P. T. Sangild. 2001. GLP-2 has differential effects on small intestine growth and function in fetal and neonatal pigs. Am. J. Physiol. Regul. Integr. Comp. Physiol. 281:R1986–R1993.

Petersen, Y. M., J. Elnif, M. Schmidt, and P. T. Sangild. 2002. Glucagon-like peptide 2 enhances maltase-glucoamylase and sucrase-isomaltase gene expression and activity in parenterally fed premature neonatal piglets. Pediatr. Res. 52:498–503. doi:10.1203/00006450-200210000-00007

Pollmann, D. S. 1986. Probiotics in pig diets. In: W. Haresign and D. J. A. Cole, editors, Recent Advances in Animal Nutrition. 1st ed. Butterworths, London, UK. p. 193-205.

Qu, H., S. S. Donkin, and K. M. Ajuwon. 2015. Heat stress enhances adipogenic differentiation of subcutaneous fat depot-derived porcine stromovascular cells. J. Anim. Sci. 93:3832–3842. doi:10.2527/jas.2015-9074

Robertson, M. D. 2007. Metabolic cross talk between the colon and the periphery: Implications for insulin sensitivity. Proc. Nutr. Soc. 66:351–361. doi:10.1017/S0029665107005617

Robertson, J. A., F. D. de Monredon, P. Dysseler, F. Guillon, R. Amado, and J. Thibault. 2000. Hydration properties of dietary fibre and resistant starch: A European collaborative study. Lebensm. Wiss. Technol. 33:72–79. doi:10.1006/fstl.1999.0595

Schreier, H. J. 1993. Biosynthesis of glutamine and glutamate and the assimilation of ammonia. In: A. L. Sonenshein, J. A. Hoch, and R. Losick, editors, *Bacillus subtilis* and Other Gram-Positive Bacteria. Am. Soc. Microbiol, Washington, DC. p. 281–298. doi:10.1128/9781555818388.ch20

Seabolt, B. S., E. van Heugten, S. W. Kim, K. D. Ange-van Heugten, and E. Roura. 2010. Feed preferences and performance of nursery pigs fed diets containing various inclusion levels and qualities of distillers coproducts and flavor. J. Anim. Sci. 88:3725–3738. doi:10.2527/jas.2009-2640

Shulman, G. I., and K. F. Petersen. 2012. Metabolism. Updated. In: W. F. Boron and E. L. Boulpaep, editors, Medical Physiology: A Cellular and Molecular Approach. 2nd ed. Saunders Elsevier Inc., Philadelphia, PA. p. 1218–1220.

Smith, H. W., and S. Halls. 1968. The production of oedema disease and diarrhoea in weaned pigs by the oral administration of *Escherichia coli*: Factors that influence the course of the experimental disease. J. Med. Microb. 1:45–59. doi:10.1099/00222615-1-1-45

Spencer, J. D., G. I. Petersen, A. M. Gaines, and N. R. Augsburger. 2007. Evaluation of different strategies for supplementing distillers dried grains with solubles (DDGS) to nursery pig diets. J. Anim. Sci. 85(Suppl. 2):96–97 (Abstr.).

Stavric, S., and E. T. Kornegay. 1995. Microbial probiotics for pigs and poulty. In: R. J. Wallace and A. Chesson, editors., Biotechnology in Animal Feeds and Feeding. 1st ed. VCH Verlagsgesellscharft, Weinheim, Germany. p. 205-231. DOI: 10.1002/9783527615353.ch10

Stein, H. H., and G. C. Shurson. 2009. Board-Invited Review: The use and application of distillers dried grains with solubles (DDGS) in swine diets. J. Anim. Sci. 87:1292-1303. doi:10.2527/jas.2008-1290

Vigors, S., T. Sweeney, C. J. O'Shea, J. A. Browne, and J. V. O'Doherty. 2014. Improvements in growth performance, bone mineral status and nutrient digestibility in pigs following the dietary inclusion of phytase are accompanied by modifications in intestinal nutrient transporter gene expression. Br. J. Nutr. 112:688–697. doi:10.1017/S0007114514001494

Walsh, M. C., M. H. Rostagno, G. E. Gardiner, A. L. Sutton, B. T. Richert, and J. S. Radcliffe. 2012. Controlling Salmonella infection in weanling pigs through water delivery of direct-fed microbials or organic acids. Part I: Effects on growth performance, microbial populations, and immune status. J. Anim. Sci. 90:261–271. doi:10.2527/jas.2010-3598

Walsh, M. C., K. L. Saddoris, D. M. Sholly, R. B. Hinson, A. L. Sutton, T. J. Applegate, B. T. Richert, and J. S. Radcliffe. 2007. The effects of direct fed microbials delivered through the feed and/or in a bolus at weaning on growth performance and gut health. Livest. Sci. 108:254–257. doi:10.1016/j.livsci.2007.01.051

Wilson, M. C., D. Meredith, J. E. Fox, C. Manoharan, A. J. Davies, and A. P. Halestrap. 2005. Basigin (CD147) is the target for organomercurial inhibition of monocarboxylate transporter isoforms 1 and 4: The ancillary protein for the insensitive MCT2 is EMBIGIN (gp70). J. Biol. Chem. 280:27213–27221. doi:10.1074/jbc.M411950200

Wøjdemann, M., A. Wettergren, B. Hartmann, L. Hilsted, and J. J. Holst. 1999. Inhibition of sham feeding-stimulated human gastric acid secretion by glucagon-like peptide-2. J. Clin. Endocrinol. Metab. 84:2513–2517. doi:10.1210/jcem.84.7.5840