






Evaluation of digestively resistant or soluble fibers, short- and medium-chain fatty acids, trace minerals, and antibiotics in nonchallenged nursery pigs on performance, digestibility, and intestinal integrity

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Abstract

Three experiments (**EXP**) were conducted to determine the effect of feed additives on performance, intestinal integrity, gastrointestinal volatile fatty acids (**VFA**), and energy and nutrient digestion in nonchallenged nursery pigs. In EXP 1, 480 pigs (6.36-kg body weight, **BW**) were placed into 96 pens with 5 pigs/pen, and allotted to 1 of 10 dietary treatments: 1) negative control containing no feed additive (**NC**), 2) NC + 44 mg chlortetracycline and 38.5 mg tiamulin/kg diet (**CT_{sb}**), 3) NC + 5% resistant potato starch (**RS_{po}**), 4) NC + 5% soluble corn fiber (**SCF**), 5) NC + 5% sugar beet pulp (**SBP**), 6) NC + 0.30% fatty acid mix (**FAM**), 7) NC + 0.10% phytogetic blend of essential oils and flavoring compounds (**PHY**), 8) NC + 50 mg Cu and 1,600 mg zinc oxide/kg diet (**CuZn**), 9) NC + 5% resistant corn starch (**RS_{cn}**), and 10) NC + 0.05% β -glucan (**BG**) for 28 d. There was no impact of dietary treatment on BW gain or feed intake ($P \geq 0.22$). Pigs fed diets containing SCF, CT_{sb}, and RS_{po} resulted in microbial community differences compared to pigs fed the NC ($P < 0.05$). In EXP 2, 48 barrows (12.8 kg BW) were selected at the end of EXP 1 and fed the same dietary treatments they had previously received: 1) NC, 2) NC + 5% RS_{cn}, 3) NC + 5% SCF, and 4) NC + FAM for 8 d. There was no effect of feeding diets containing RS_{cn}, SCF, or FAM on in vivo intestinal permeability ($P \leq 0.21$). Ileal or colon pH, concentrations of VFA did not differ due to dietary treatment ($P \geq 0.36$), but pigs fed diets containing FAM resulted in a greater butyric acid concentration in the cecum compared to pigs fed the NC ($P \leq 0.05$). In EXP 3, 156 pigs (6.11 kg BW) were placed into 52 pens with 3 pigs/pen and allotted to 1 of 4 dietary treatments arranged in a factorial manner: 1) NC, 2) NC + 5% RS_{po}, 3) NC + 0.30% FAM, and 4) NC + 5% RS_{po} + 0.30% FAM for 24 d. Feeding pigs diets containing RS_{po} did not affect BW gain ($P = 0.91$) while pigs fed diets containing FAM grew improved BW gain ($P = 0.09$). Colonic butyric acid concentrations were greater in pigs fed diets containing RS_{po} ($P = 0.03$), while pigs fed diets containing FAM exhibited reduced total VFA concentrations ($P = 0.11$). The results indicate that supplementing diets with digestively resistant but fermentable fibers, short- and medium-chain fatty acids, or antibiotics do not have a consistent effect, positive or negative, on markers of intestinal integrity or barrier function, intestinal VFA patterns, ATTD of energy and nutrients, or on pig performance.

Lay Summary

In-feed antimicrobials have been an important technology in swine production for protecting health and supporting growth. However, with legislative restrictions on the use of most antibiotics for growth promotion, research is needed to evaluate in-feed additives in replacing this growth promoting technology. Thus, strategies to enhance energy and nutrient digestibility, intestinal function and integrity, gastrointestinal volatile fatty acid concentrations, and microbial ecology in nursery pigs are desirable targets. The results of the three experiments conducted herein do not indicate that supplementing diets with digestively resistant but fermentable fibers, short-medium-chain fatty acids, or antibiotics have a consistent positive or negative effect on markers of intestinal integrity or barrier function, VFA patterns (ileal, cecal, or colon), ATTD of energy and nutrients, or pig performance.

Key words: bone mineral content, mineral digestion, nursery pig, resistant starch, soluble corn fiber, volatile fatty acids

Abbreviations: AA, amino acid; ADFI, average daily feed intake; ADG, average daily gain; APP, Actinobacillus pleuropneumonia; ATTD, apparent total tract digestibility; BG, β -glucan; BCM, bone mineral content; BW, body weight; CTsb, chlortetracycline and tiamulin; CuZn, copper and zinc; DXA, dual-energy X-ray absorptiometry; EXP, experiment; FAM, fatty acid mix; FD4, fluorescein isothiocyanate-labeled dextran; FDR, false discovery rate; GE, gross energy; GF, gain:feed ratio; KB, Krebs-Henseleit buffer; Lys, lysine; ME, metabolizable energy; NC, negative control; OTU, operational taxonomic units; PEDV, porcine epidemic diarrhea virus; PHOS, phosphorus; PHY, phytogetic blend; PRRS, porcine reproductive and respiratory syndrome; RS_{cn}, resistant corn starch; RS_{po}, resistant potato starch; SBP, sugar beet pulp; SCF, soluble corn fiber; TER, transepithelial resistance; VFA, volatile fatty acids; VFD, veterinary feed directive

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Introduction

In-feed, nontherapeutic antibiotics have been typically utilized in swine production to improve growth and efficiency in food-producing animals (Cromwell, 2006). However, in early 2017, the Veterinary Feed Directive (VFD) took effect in the United States ensuring that antibiotics are only used at therapeutic concentrations for disease treatment, control, and prevention, rather than at growth promoting, nontherapeutic concentrations (Flynn, 2012). With the loss of subtherapeutic growth promoting antibiotic usage in the United States, many alternative dietary ingredients have been identified, including prebiotics, probiotics, short- and medium-chain fatty acids, phytochemicals, and enzymes (Cromwell, 2006; Verstegen and Williams, 2006; Windisch et al., 2008; Thacker, 2013; Liu, 2015; Ferrara et al., 2017; Liao and Nyachoti, 2017). The success of many of these alternative dietary ingredients to improve animal growth, however, has been inconsistent (Allen et al., 2013; Gabler et al., 2019). A general assumption is that many of these antibiotic alternatives modulate microbial ecology, volatile fatty acid concentrations, or pH in the digestive tract in a direction that has a positive effect on intestinal integrity, oxidative stress, nutrient utilization, or animal performance (de Lange et al., 2010; Liu et al., 2018). Because uncertainty remains as to the positive effects of various in-feed additives in young pigs, the objective was to determine the effect of various in-feed additives that may affect fore- or hind-gut functionality, including a previously used antibiotic combination and elevated levels of Cu and Zn, on pig performance, intestinal integrity, gastrointestinal microbial communities and VFA, and energy and nutrient digestion in nursery pigs.

Materials and Methods

The Iowa State University Animal Care and Use Committee approved all experimental protocols (IACUC# 7-17-8575-S; 18-155).

Animals, housing, experimental design, and diets

EXP 1

Four hundred and eighty (480) weaned pigs (average body weight (BW) 6.36 ± 0.90 kg) were obtained from a commercial swine farm and shipped to the Iowa State University Swine Nutrition Farm, Ames IA. Incoming pigs were free of Porcine Reproductive and Respiratory Syndrome (PRRS) virus, Porcine Epidemic Diarrhea Virus (PEDV), swine influenza, and *Actinobacillus pleuropneumonia* (APP), and were vaccinated for Circovirus and *Mycoplasma* at the sow farm. Upon arrival, piglets did not receive a PRRS vaccine or water-based *Escherichia coli* F18/K88 vaccine. Pigs were randomly placed into 96 pens (1.2×1.2 m) with 5 pigs/pen, with the balance of sex and BW being similar among the pens. Feeders and pigs were weighed and randomly allotted to 1 of the 10 dietary treatments and fed their respective experimental diets for 28 d. There were 10 replications for each dietary treatment except for pigs fed the BG diet for which there were 6 replications. Phase-1 diets were fed from day 0 to 14 and were formulated to contain 3,400 kcal ME/kg, 1.50% standardized ileal digestible lysine (Lys), 0.84% Ca, and 0.45% standardized total tract digestible phosphorus (PHOS; Table 1). Phase-2 diets were fed from day 15 to 28 and formulated to contain 3,400 kcal metabolizable energy (ME)/

kg, 1.35% standardized ileal digestible Lys, 0.80% Ca, and 0.40% standardized total tract digestible PHOS (Table 2). Titanium dioxide was added to all Phase-2 diets at 0.50% as an indigestible marker for calculation of apparent total tract digestibility (ATTD) of GE, N, Ca, and PHOS. In each phase, all other amino acids (AA) were formulated to meet minimum digestible AA:Lys ratios as reported in the NRC (2012). Dietary treatments consisted of: 1) negative control containing no feed additive (NC), 2) NC + subtherapeutic levels of chlortetracycline (44 mg/kg diet) and tiamulin (38.5 mg/kg diet), CT_{sb}; 3) NC + 5% resistant potato starch (RS_{po}, MSP[RS], MSP Starch Products Inc., Carberry, Manitoba); 4) NC + 5% soluble corn fiber (SCF, PROMITOR Soluble Corn Fiber, Tate and Lyle, Decatur, IL); 5) NC + 5% sugar beet pulp (SBP); 6: NC + 0.30% fatty acid mix (FAM, SANACORE EN, salts of sodium butyrate and propionate, palm fat, caprylic, capric, and lauric fatty acids, natural flavoring compounds, and a mixture of steatites and chlorite, Nutriad Inc. Hampshire, IL); 7) NC + 0.10% phytochemical blend of essential oils and natural flavoring compounds (PHY, DIGESTAROM, Biomin America Inc., Overland Park, KS); 8) NC + copper sulfate (50 mg Cu/kg diet) and zinc oxide (1,600 mg Zn/kg diet), CuZn; 9) NC + 5% resistant corn starch (RS_{cn}, HI-MAIZE 260, Ingredient Inc., Bridgewater, NJ); and 10) NC + 0.05% β -glucan (BG, Betaglucan Animal Grade, Kerry Inc., Beloit WI). A fresh fecal sample was collected from a randomly selected pig from each pen on day 2 and the same pig again on day 23, being immediately placed on ice and stored at -80 °C until extraction with Qiagen Fecal Microbiome kits (Germantown, MD). On days 25 and 26, a fresh fecal sample was collected from a randomly selected pig from each pen, dried at 75 °C, and ground for gross energy (GE), N, Ca and PHOS analysis. On day 28, pigs and feeders were weighed to calculate average daily gain (ADG), average daily feed intake (ADFI), and the gain:feed ratio (GF).

EXP 2

Thirty-two (32) barrows (average BW = 12.8 ± 1.04 kg) were selected at the end of EXP 1 and moved to individual metabolism crates (0.53×0.71 m). Pigs were fed four of the same Phase-2 dietary treatments they had previously been fed during EXP 1 which consisted of: 1) NC, 2) NC + 5% RS_{cn}, 3) NC + 5% SCF, and 4) NC + FAM (Table 2), resulting in eight replications per dietary treatment. Treatments were selected to specifically evaluate the feeding of corn-based feedstuffs (i.e., RS_{cn} and SCF) and a product containing a blend of various fatty acids (i.e., FAM). While in metabolism crates, pigs were fed twice daily, equivalent to 3.5% of their average BW (225 g at 0700 hours and 225 g at 1700 hours) with free access to water. Pigs were adapted to the crates for 2 d followed by a total collection of feces for 3 d to determine the ATTD of GE, N, Ca, and PHOS. Feces were collected twice daily during the collection period and stored at -20 °C, and at the end of the collection period, feces were dried at 70 °C for 48 h, weighed, and ground through a 2-mm screen, and a subsample from each pig was collected for subsequent analysis.

Following the end of the total fecal collection period, pigs were fasted for 12 h (overnight), after which each pig received a 10-mL deionized water intragastric gavage containing 500 mg/kg BW lactulose, 50 mg/kg BW mannitol, 500 mg/kg BW sucralose (Spectrum Chemical Manufacturing Corp., Gardena, CA), and 8.4 mg/kg BW fluorescein isothiocyanate-labeled dextran (4.4 kDa; FD4, Sigma Chemical, St.

Table 1. Composition of Phase-1 experimental diets, days 0 to 14-EXP 1¹

Ingredient, %	Dietary treatment									
	NC ²	CT _{sb}	RS _{po}	RS _{cn}	SCF	SBP	FAM	PHY	CuZn	BG
Corn	45.14	44.93	40.31	40.31	40.31	39.70	44.76	45.04	44.92	45.09
Soybean meal	28.44	28.44	28.44	28.44	28.44	28.44	28.44	28.44	28.44	28.44
Dried whey	15.00	15.00	15.00	15.00	15.00	15.00	15.00	15.00	15.00	15.00
Fish meal	7.50	7.50	7.50	7.50	7.50	7.50	7.50	7.50	7.50	7.50
Soybean oil	2.12	2.12	1.58	1.58	1.58	2.62	2.10	2.12	2.12	2.12
Limestone	0.49	0.49	0.49	0.49	0.49	0.39	0.49	0.49	0.49	0.49
Sodium chloride	0.40	0.40	0.40	0.40	0.40	0.40	0.40	0.40	0.40	0.40
Vitamin mix ³	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
Trace mineral mix ⁴	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15
L-Lysine-HCL	0.27	0.27	0.27	0.27	0.27	0.26	0.27	0.27	0.27	0.27
DL-Methionine	0.16	0.16	0.16	0.16	0.16	0.16	0.16	0.16	0.16	0.16
L-Threonine	0.08	0.08	0.08	0.08	0.08	0.08	0.08	0.08	0.08	0.08
Antibiotics ⁵	–	0.22	–	–	–	–	–	–	–	–
RSpo ⁶	–	–	5.00	–	–	–	–	–	–	–
RScn ⁷	–	–	–	5.00	–	–	–	–	–	–
SCF ⁸	–	–	–	–	5.00	–	–	–	–	–
Sugar beet pulp	–	–	–	–	–	5.00	–	–	–	–
Fatty acid mix ⁹	–	–	–	–	–	–	0.30	–	–	–
Phytogen mix ¹⁰	–	–	–	–	–	–	–	0.10	–	–
CuZn ¹¹	–	–	–	–	–	–	–	–	0.22	–
β-glucan ¹²	–	–	–	–	–	–	–	–	–	0.05
TOTAL	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00
Analyzed composition										
GE, kcal/kg	3,911	3,937	3,827	3,829	3,865	3,908	3,865	3,886	3,872	3,798
CP, %	23.9	24.3	24.5	23.3	23.8	23.8	23.4	22.8	24.0	24.0
Ca, %	0.81	0.83	0.83	0.89	0.86	0.88	0.91	0.84	0.90	0.88
P, %	0.62	0.63	0.65	0.69	0.64	0.63	0.67	0.68	0.63	0.67

¹Diets were formulated to contain 3,400 kcal ME/kg, 1.50% standardized ileal digestible LYS, 0.84% Ca, and 0.45% standardized total tract digestible phosphorus.

²Abbreviations: NC, negative control; CT_{sb}, subtherapeutic level of chlortetracycline and tiamulin; RS_{po}, resistant potato starch; SCF, soluble corn fiber; SBP, sugar beet pulp; FAM, fatty acid mix; PHY, phytogen mix; CuZn, CuSO₄ plus ZnO; RS_{cn}, resistant corn starch; BG, β-glucan.

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

⁴Provided the following per kilogram of diet: Cu (as CuSO₄), 22 mg; Fe (as FeSO₄), 220 mg; I (as Ca(IO₃)₂), 0.4 mg; Mn (as MnSO₄), 52 mg; Zn (as ZnSO₄), 220 mg; and Se (Na₂SeO₃), 0.3 mg.

⁵Provided 44-mg chlorotetracycline and 38.5-mg tiamulin per kg complete diet.

⁶Resistant potato starch, MSP[RS], MSP Starch Products Inc., Carberry, Manitoba.

⁷Resistant corn starch, HI-MAIZE 260, Ingredient Inc., Bridgewater, NJ.

⁸Soluble corn fiber, PROMITOR Soluble Corn Fiber, Tate and Lyle, Decatur, IL.

⁹Fatty acid mix, SANACORE EN-contains salts of sodium butyrate and propionate, palm fat, caprylic, capric, and lauric fatty acids, natural flavoring compounds, and a mixture of steatites and chlorite, Nutriad Inc., Hampshire, IL.

¹⁰Phytogenic blend of essential oils and natural flavoring compounds, DIGESTAROM®, Biomin America Inc., Overland Park, KS.

¹¹Provided 50-mg Cu and 1,600-mg Zn per kg complete diet.

¹²β-glucan, BG; Betaglucan Animal Grade [proprietary strain of nonGMO *Saccharomyces cerevisiae* with ≥ 70% purity as measured by gluco polysaccharide (β-1,3/1,6)], Kerry Inc., Beloit, WI.

Louis, MO) as measures of in vivo intestinal permeability. Following the gavage, pigs were fed their respective experimental diet. Buckets were immediately placed under each crate for a 12 h total urine collection, each of which contained 5 mL of chlorhexidine to eliminate microbial growth. Four hours following gavage, approximately 8 mL of blood was obtained from each pig via jugular venipuncture using a 10-mL vacuum serum tube. Blood samples were allowed to clot and then centrifuged at 2,500 × g for 15 min at 4 °C and serum was harvested. Serum samples were immediately frozen and stored at –20 °C until analysis for FD4 content. After 12 h of

urine collection, urine was quantified, subsampled, and stored at –20 °C for subsequent analysis. Following urine collection all pigs were weighed and subsequently euthanized via captive bolt followed by exsanguination for ex vivo assessment of intestinal integrity and barrier function. The ileum, cecal, and colon contents were collected and frozen at –20 °C until analysis for VFA and pH.

EXP 3

One hundred and fifty-six (156) weaned pigs (average BW = 6.11 ± 0.51 kg) were obtained from a commercial

Table 2. Composition of Phase-2 experimental diets, days 15 to 28 – EXP 1 and days 1 to 8 –EXP 2¹

Ingredient, %	Dietary treatment									
	NC ²	CT _{sb}	RS _{po}	RS _{cn}	SCF	SBP	FAM	PHY	CuZn	BG
Corn	52.60	52.20	47.74	47.74	47.74	47.16	52.05	52.39	52.19	52.50
Soybean meal	27.06	27.10	27.45	27.45	27.45	27.10	27.11	27.10	27.10	27.06
Dried whey	10.00	10.00	10.00	10.00	10.00	10.00	10.00	10.00	10.00	10.00
Fish meal	5.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00
Soybean oil	2.53	2.68	1.98	1.98	1.98	3.05	2.73	2.60	2.68	2.58
Monocalcium phosphate	0.20	0.20	0.22	0.22	0.22	0.21	0.20	0.20	0.20	0.20
Limestone	0.79	0.79	0.78	0.78	0.78	0.67	0.79	0.79	0.79	0.79
Sodium chloride	0.40	0.40	0.40	0.40	0.40	0.40	0.40	0.40	0.40	0.40
Vitamin mix ³	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
Trace mineral mix ⁴	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15
L-Lysine-HCL	0.31	0.31	0.30	0.30	0.30	0.29	0.31	0.31	0.31	0.31
DL-Methionine	0.13	0.13	0.14	0.14	0.14	0.14	0.13	0.13	0.13	0.13
L-Threonine	0.08	0.08	0.09	0.09	0.09	0.08	0.08	0.08	0.08	0.08
Titanium dioxide	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50
Antibiotics ⁵	–	0.22	–	–	–	–	–	–	–	–
RSp ⁶	–	–	5.00	–	–	–	–	–	–	–
RScn ⁷	–	–	–	5.00	–	–	–	–	–	–
SCF ⁸	–	–	–	–	5.00	–	–	–	–	–
Sugar beet pulp	–	–	–	–	–	5.00	–	–	–	–
Fatty acid mix ⁹	–	–	–	–	–	–	0.30	–	–	–
Phytogen mix ¹⁰	–	–	–	–	–	–	–	0.10	–	–
CuZn ¹¹	–	–	–	–	–	–	–	–	0.22	–
β-glucan ¹²	–	–	–	–	–	–	–	–	–	0.05
TOTAL	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00
Analyzed composition										
GE, kcal/kg	3,908	3,886	3,894	3,870	3,860	3,880	3,935	3,897	3,892	3,884
CP, %	21.5	22.2	22.6	22.4	21.0	21.7	21.6	22.2	22.0	21.6
Ca, %	0.76	0.76	0.63	0.76	0.83	0.71	0.77	0.72	0.74	0.76
P, %	0.55	0.52	0.51	0.56	0.60	0.53	0.53	0.49	0.51	0.55

¹Diets were formulated to contain 3,400 kcal ME/kg, 1.35% standardized ileal digestible LYS, 0.80% Ca, and 0.40% standardized total tract digestible phosphorus.

²Abbreviations: NC, negative control; CT_{sb}, subtherapeutic level of chlortetracycline and tiamulin; RS_{po}, resistant potato starch; SCF, soluble corn fiber; SBP, sugar beet pulp; FAM, fatty acid mix; PHY, phytogen mix; CuZn, CuSO₄ plus ZnO; RS_{cn}, resistant corn starch; BG, β-glucan.

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

⁴Provided the following per kilogram of diet: Cu (as CuSO₄), 22 mg; Fe (as FeSO₄), 220 mg; I (as Ca(IO₃)₂), 0.4 mg; Mn (as MnSO₄), 52 mg; Zn (as ZnSO₄), 220 mg; and Se (Na₂SeO₃), 0.3 mg.

⁵Provided 44-mg chlortetracycline and 38.5-mg tiamulin per kg complete diet.

⁶Resistant potato starch, MSP[RS], MSP Starch Products Inc., Carberry, Manitoba.

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⁹Fatty acid mix, SANACORE EN-contains salts of sodium butyrate and propionate, palm fat, caprylic, capric, and lauric fatty acids, natural flavoring compounds, and a mixture of steatites and chlorite, Nutriad Inc., Hampshire, IL.

¹⁰Phytogenic blend of essential oils and natural flavoring compounds, DIGESTAROM, Biomin America Inc., Overland Park, KS.

¹¹Provided 50 mg Cu and 1,600 mg Zn per kg complete diet.

¹²β-glucan, BG; Betaglucan Animal Grade [proprietary strain of non-GMO *Saccharomyces cerevisiae* with ≥ 70% purity as measured by gluco polysaccharide (β-1,3/1,6)], Kerry Inc., Beloit, WI.

swine farm and shipped to the Iowa State University Swine Nutrition Farm, Ames IA. Incoming pigs were free of PRRS, PEDV, swine influenza, and APP and were vaccinated for circovirus and *Mycoplasma* at the sow farm. Upon arrival, piglets did not receive PRRS vaccine or water-based *E. coli* F18/K88 vaccine. Pigs were randomly placed into 52 pens (1.0 × 1.8 m) with 3 pigs/pen, with the balance of sex and BW similar among the pens. Feeders and pigs were weighed and randomly allotted to 1 of the 4 dietary treatments, with pigs fed their respective experimental diets for 24 d (Table 3).

Phase-1 diets were fed from day 0 to 12 and Phase-2 diets were fed from day 13 to 24, being formulated in a similar manner as in EXP 1. Dietary treatments were arranged in a factorial manner, consisting of 1) NC, 2) NC + 5% RS_{po}, 3) NC + 0.30% FAM, and 4) NC + 5% RS_{po} + 0.30% FAM; with treatments selected on determining potential interactive effects between a feedstuff which is suggested to affect hindgut (i.e., RS_{po}) or foregut (i.e., FAM) function. On day 24, pigs and feeders were weighed to calculate ADG, ADFI, and GF, resulting in 13 replications per dietary

Table 3. Composition of experimental diets, EXP 3

Ingredient, %	Phase 1, d 1-13 ¹			
	NC ²	RS _{po}	FAM	RS _{po} + FAM
Corn	44.29	39.00	43.99	38.70
SBM	30.84	31.66	30.84	31.66
Dried whey	15.00	15.0	15.00	15.00
Fish meal	5.00	5.00	5.00	5.00
Soybean oil	2.47	1.93	2.47	1.93
Monocalcium phosphate	0.24	0.25	0.24	0.25
CaCO ₃	0.74	0.74	0.74	0.74
Sodium chloride	0.40	0.40	0.40	0.40
Vitamin mix ³	0.25	0.25	0.25	0.25
Trace mineral mix ⁴	0.15	0.15	0.15	0.15
L-Lysine-HCL	0.34	0.33	0.34	0.33
DL-Methionine	0.18	0.19	0.18	0.19
L-Threonine	0.10	0.10	0.10	0.10
Resistant potato starch ⁵	–	5.00	–	5.00
Fatty acid mix ⁶	–	–	0.30	0.30
TOTAL	100.00	100.00	100.00	100.00

Ingredient, %	Phase 2, d 13-24 ⁷			
	NC	RS _{po}	FAM	RS _{po} + FAM
Corn	54.70	49.43	54.40	49.13
SBM	29.66	30.51	29.66	30.51
Dried whey	7.50	7.50	7.50	7.50
Fish meal	2.50	2.50	2.50	2.50
Soybean oil	2.55	2.00	2.55	2.00
Monocalcium phosphase	0.64	0.64	0.64	0.64
CaCO ₃	1.00	0.99	1.00	0.99
Sodium chloride	0.40	0.40	0.40	0.40
Vitamin mix ³	0.25	0.25	0.25	0.25
Trace mineral mix ⁴	0.15	0.15	0.15	0.15
L-Lysine-HCL	0.38	0.36	0.38	0.36
DL-Methionine	0.16	0.16	0.16	0.16
L-Threonine	0.11	0.11	0.11	0.11
Resistant potato starch ⁵	–	5.00	–	5.00
Fatty acid mix ⁶	–	–	0.30	0.30
TOTAL	100.00	100.00	100.00	100.00

¹Diets were formulated to contain 3,400 kcal ME/kg, 1.50% standardized ileal digestible LYS, 0.84% Ca, and 0.45% standardized total tract digestible phosphorus.

²Abbreviations: NC, negative control; RS_{po}, NC + resistant potato starch; FAM, NC + fatty acid mix; RS_{po} + FAM, NC + resistant potato starch + fatty acid mix.

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

⁴Provided the following per kilogram of diet: Cu (as CuSO₄), 22 mg; Fe (as FeSO₄), 220 mg; I (as Ca(IO₃)₂), 0.4 mg; Mn (as MnSO₄), 52 mg; Zn (as ZnSO₄), 220 mg; and Se (Na₂SeO₃), 0.3 mg.

⁵De Tulpen potato starch, Agrident, Farmington Hills, MI.

⁶Fatty acid mix, SANACORE EN—contains salts of sodium butyrate and propionate, palm fat, caprylic, capric, and lauric fatty acids, natural flavoring compounds, and a mixture of steatites and chlorite, Nutriad Inc. Hampshire, IL.

⁷Diets were formulated to contain 3,400 kcal ME/kg, 1.35% standardized ileal digestible LYS, 0.80% Ca, and 0.40% standardized total tract digestible phosphorus.

treatment. On day 24, one pig per pen from each of eight pens per dietary treatment was euthanized by captive bolt and exsanguinated, with cecal and colon contents collected and frozen at –20 °C until analysis for VFA and pH. In addition,

organ weights (pluck, liver, spleen, and the empty stomach, small and large intestine) were obtained to determine if dietary treatment caused any effects on a percentage of BW basis.

Apparent total tract digestibility of GE, N, Ca, and PHOS—EXP 1 and 2

Titanium was analyzed based on the method of Leone (1973), where samples were ashed in an oven and then digested with sulfuric acid and hydrogen peroxide, followed by measuring absorbance using a UV spectrophotometer against a standard curve. The GE content of feeds and feces was determined using an isoperibol bomb calorimetry (Model 6200, Parr Instruments, Moline, IL) using benzoic acid as a standard. Nitrogen content of the feed and feces was analyzed using thermocombustion (VarioMax; Elementar Analysensysteme GmbH, Hanau, Germany) while Ca and PHOS were analyzed using Method 985.01 (AOAC, 1978) and inductively coupled plasma mass spectrometry sample digestion. In brief, feed and fecal samples were dry-ashed to remove the majority of the organic matter followed by treating all samples in nitric acid to remove any remaining organic matter. Subsequently, each sample was dissolved in 1 N HCl followed by inductively coupled plasma spectrometry (Optima 5300DV, PerkinElmer, Shelton, CT) for mineral analysis.

For EXP 1, ATTD of dietary GE, N, Ca, and PHOS was accomplished using the indirect method with digestibility values determined using the difference procedure (Adeola, 2001). For EXP 2, digestibility of dietary GE, N, Ca, and PHOS was calculated based on total feed intake and fecal excretion over the 3-d collection period.

In vivo assessment of intestinal permeability—EXP 2

Lactulose, mannitol, and sucralose concentrations in urine were determined by HPLC according to the method described by Kansagra et al. (2003). Lactulose, mannitol, and sucralose were determined on a recovery basis with the lactulose:mannitol ratio used as an in vivo indicator of small intestinal permeability and the sucralose concentration used as an in vivo indicator of colon permeability (Kansagra et al., 2003). Serum FD4 analysis was also used as an in vivo indicator of nonspecific intestinal permeability (Baxter et al., 2017; Gilani et al., 2017). A fluorescent plate reader (BioTek Instruments Winooski, VT) was used to determine relative fluorescence of FD4 in the serum samples at 485 and 520 nm excitation and emission wavelengths, respectively, and a permeability coefficient was then calculated.

Ex vivo assessment of intestinal integrity and barrier function—EXP 2

Following exsanguination of pigs in EXP 2, fresh segments of ileum and colon were removed and placed on ice in Krebs-Henseleit buffer (KB) and kept under constant aeration on ice for transport to the laboratory. Prior to sample arrival, the Ussing apparatus was fully assembled with current and voltage electrodes filled with 3% noble agar that were submerged in 3 M KCl, chambers filled with KB, and system leaks were eliminated. Voltage differences between chambers were offset and fluid resistance compensation was used to account for any nontissue related resistance so that all chambers had a baseline measurement between 60 and 65 µA.

Transepithelial resistance (TER), macromolecule permeability, and active transport of glucose and glutamine were determined as described previously (Gabler et al., 2007; Moeser et al., 2012; Mani et al., 2013; Curry et al., 2017). Briefly, once tissues arrived in the laboratory, the serosal layer was removed and one jejunum section per pig was pinned onto an insert that allowed for an exposed surface area of 0.71 cm², which was then placed into chambers with mucosal and serosal membranes facing opposite chambers. Chambers were constantly aerated with a 95% O₂, 5% CO₂ gas mixture, warmed to 37 °C by circulating water bath, filled with KB, and connected by pairs of current and voltage electrodes to form an electrical circuit. Mucosal and serosal chambers contained 10 μM of D-mannitol and D-glucose, respectively, for ion balance. Sections were voltage clamped at 0 mV and allowed to stabilize for approximately 10 to 15 min. A pulse current was applied, and TER was calculated based on measured voltage and the change in short circuit current when current pulse was applied. The TER was averaged over 10 to 15 min after stabilization and measured as ohms (Ω) per cm². Active mucosal to serosal transport of glucose and glutamine was determined as described by Gabler et al. (2007) and calculated as the change in short-circuit current. Macromolecule permeability was assessed by the mucosal to serosal flux of FD4 and measured as described by Mani et al. (2013).

Determination of VFA and pH concentrations—EXP 2 and 3

Volatile fatty acid concentrations from each pig in EXP 2 and 1 pig/pen in EXP 3 were determined using the method described by Weber et al. (2010). In brief, samples were thawed and thoroughly hand-mixed with sterile stir sticks for at least 30 s. Approximately 1.0 g of digesta from each sample was placed into 15-mL polypropylene centrifuge tube and diluted with 5 mL of deionized water. Samples were mixed overnight at 1,200 rpm on a digital microplate shaker (Thermo Fisher Scientific, Waltham, MA). After mixing, samples were centrifuged at 4 °C for 23 min at 21,000 × g to separate the supernatant. Approximately 2.5 mL of clear supernatant was removed and placed into tubes and o-phosphoric acid was added to achieve a pH of 2.5. Exactly 1 mL of the pH-adjusted supernatant sample was placed into 20-mL gas chromatography vials with 0.3 g of NaCl for gas chromatography analysis (Agilent 7890A Gas Chromatograph, Agilent Technologies, Inc, Wilmington, DE). Samples were analyzed in duplicate with total VFA concentrations determined as the sum acetic, propionic, isobutyric, butyric, isovaleric, valeric, isocaproic, caproic, and heptanoic acid. To determine pH, ileal and cecal samples were not diluted while 1 g of colon contents was diluted with 1 mL of Millipore H₂O, after which pH was determined using a pH meter (Accumet AB15, Thermo Fisher Scientific, Waltham, MA) and combination electrode (Pinnacle/SI Analytics 3-in-1 combination electrode #476436, Cole-Parmer, Vernon Hills, IL).

Bone mineral content determination—EXP 2

Following exsanguination of pigs in EXP 2, the left femur from each pig was removed for determination of bone mineral content (BMC). Any soft tissue was removed after which the femurs were scanned through dual-energy X-ray absorptiometry (DXA; Hologic Discovery A, Bedford, MA) as previously described (Suster et al., 2003) with measurements made by DXA including bone mineral density and content.

Microbial ecology analysis—EXP 1

16S rRNA gene amplicons of the V4 region were generated from DNA extracted from the feces as described in Trachsel et al. (2019), in accordance with the protocol described by Kozich et al. (2013). Amplicons were sequenced on an Illumina Miseq (La Jolla, CA) using the V2 reagent kit (2 × 250 bp read lengths). Operational taxonomic units (OTUs) were generated with mothur (Schloss et al., 2009) and classified using the SILVA v132 taxonomy. Global singletons were removed and samples with fewer than 2,000 total reads were omitted. For the calculation of alpha- and beta-diversity metrics sample counts were rarefied to 2000 per sample. The R (R Core Team, 2021) package vegan (Oksanen et al., 2022) was used to calculate Bray-Curtis dissimilarities between samples and perform PERMANOVA tests. DESeq2 (Love et al., 2014) was used for detection of differentially abundant OTUs between the NC group and all other treatments, as well as linear relationships between OTU abundance weight gain. OTUs with fewer than 10 counts globally were removed and the unrarefied OTU tables were used as inputs. Phyloseq (McMurdie and Holmes, 2013) and tidyverse (Wickham et al., 2019) packages were used for data processing and visualization.

Statistical analysis

Data were analyzed as a completely randomized design with individual pen (EXP 1 and 3) or pig (EXP 2) as the experimental unit using Proc MIXED of SAS version 9.4 (SAS Institute Inc., NC, USA). Means were reported and separated using LSMEANS, and if the overall model was found to be significant ($P \leq 0.10$), a pair-wise comparison was used to compare individual dietary treatments to the NC only, not among each other treatment. For EXP 1 and 2, treatments were independent of each other while in EXP 3, treatments were analyzed as a factorial design. In EXP 1, a global PERMANOVA test was used to assess the impact of time and treatment on bacterial community similarity. Differences in bacterial community structure between all other treatments and the NC group were assessed using pairwise PERMANOVA tests and the resulting P values were corrected by the false discovery rate (FDR). Testing for differentially abundant OTUs relative to the NC group and for associations between OTU abundances and weight gain used Wald tests with parametric fits and FDR-corrected P values as implemented in the DESeq2 package.

Results

Pig performance and total tract energy and nutrient digestibility—EXP 1

No significant effects were observed for a dietary treatment impact on ADG or ADFI ($P \geq 0.22$). GF was lower for pigs fed diets containing SBP ($P \leq 0.10$) or CT_{sb}, RS_{po}, SCF, PHY, and RS_{cn} ($P \leq 0.05$) compared to pigs fed the NC (Table 4). Apparent total tract digestibility of GE was lower in pigs fed diets containing CT_{sb} and BG ($P \leq 0.10$) and in pigs fed diets containing RS_{po}, SCF, SBP, FAM, and PHY ($P \leq 0.05$) compared to pigs fed the NC (Table 5). Pigs fed diets containing RS_{po} and PHY exhibited reduced ATTD of N, Ca, and PHOS ($P \leq 0.05$) compared to pigs fed the NC. Pigs fed diets containing SCF had reduced ATTD of N ($P \leq 0.05$) and pigs fed diets containing FAM had reduced ATTD of N and PHOS ($P \leq 0.05$) compared to pigs fed the NC (Table 5).

Table 4. Performance of pigs fed different dietary feed additives, EXP 1¹

Criterion	Dietary treatment ²										Statistics ³	
	NC ²	CT _{sb}	RS _{po}	RS _{cn}	SCF	SBP	FAM	PHY	CuZn	BG	SEM	Pv
ADG, g	0.279	0.262	0.263	0.284	0.271	0.278	0.275	0.262	0.301	0.266	0.011	0.23
ADFI, g	0.397	0.393	0.403	0.426	0.430	0.412	0.395	0.400	0.441	0.389	0.015	0.22
GF	0.703 ^{ax}	0.666 ^b	0.654 ^b	0.668 ^b	0.631 ^b	0.676 ^y	0.696	0.658 ^b	0.682	0.688	0.011	0.01

¹There were 10 replications for each dietary treatment except for pigs fed the BG diet for which there were 6 replications. The trial lasted 28 d with an initial and final BW of 6.36 and 14.05 kg, respectively.

²Abbreviations: NC, negative control; CT_{sb}, NC + 44 mg chlortetracycline and 38.5 mg tiamulin/kg diet; RS_{po}, NC + 5% resistant potato starch; RS_{cn}, NC + 5% resistant corn starch; SCF, NC + 5% soluble corn fiber; SBP, NC + 5% sugar beet pulp; FAM, NC + 0.3% fatty acid mix; PHY, NC + 0.10% phytoen mix; CuZn, NC + 50 mg Cu plus 1,600 mg Zn/kg feed; BG, NC + 0.05% β-glucan.

³Model standard error of the mean (SEM) and model *P*-value (Pv). Differences denoted by 'ab' represent differences ($P \leq 0.05$) while differences denoted by 'xy' represent differences ($0.10 \geq P \geq 0.05$) between pigs fed a specific treatment compared to pigs fed the NC.

Table 5. Apparent total tract digestibility of energy and nutrients in pigs fed different dietary feed additives, EXP 1¹

ATTD	Dietary treatment ²										Statistics ³	
	NC ²	CT _{sb}	RS _{po}	RS _{cn}	SCF	SBP	FAM	PHY	CuZn	BG	SEM	Pv
GE	88.6 ^{ax}	87.7 ^y	87.6 ^b	88.4	86.6 ^b	87.5 ^b	87.1 ^b	87.4 ^b	88.0	87.7 ^y	0.37	0.01
N	86.2 ^a	85.3	84.7 ^b	85.8	82.5 ^b	85.2	83.7 ^b	84.6 ^b	85.7	85.3	0.49	0.01
Ca	74.4 ^a	73.8	67.2 ^b	72.9	73.9	74.9	73.2	70.7 ^b	74.5	72.9	1.01	0.01
PHOS	61.9 ^a	59.2	57.1 ^b	62.7	62.8	63.9	57.6 ^b	55.9 ^b	59.9	59.2	1.20	0.01

¹There were 10 replications for each dietary treatment except for pigs fed the BG diet for which there were 6 replications. The trial lasted 28 days with an initial and final BW of 6.36 and 14.05 kg, respectively. Apparent total tract digestibility (ATTD) based on pooled fecal sample from each pen of pigs obtained on days 25 and 26.

²Abbreviations: NC, negative control; CT_{sb}, NC + 44 mg chlortetracycline and 38.5 mg tiamulin/kg diet; RS_{po}, NC + 5% resistant potato starch; RS_{cn}, NC + 5% resistant corn starch; SCF, NC + 5% soluble corn fiber; SBP, NC + 5% sugar beet pulp; FAM, NC + 0.3% fatty acid mix; PHY, NC + 0.10% phytoen mix; CuZn, NC + 50 mg Cu plus 1,600 mg ZnO/kg feed; BG, NC + 0.05% β-glucan; PHOS, phosphorus.

³Model standard error of the mean (SEM) and model *P*-value (Pv). Differences denoted by 'ab' represent differences ($P \leq 0.05$) while differences denoted by 'xy' represent differences ($0.10 \geq P \geq 0.05$) between pigs fed a specific treatment compared to pigs fed the NC.

Dietary impacts on microbial community—EXP 1

In general, the effect of dietary additives on the overall microbial community structure in feces was smaller ($F = 1.5$) than the effect of time, representing the maturation of microbial communities in the gastrointestinal tract during the post-weaning period ($F = 54.6$). No significant treatment effect was observed in overall community structure at the beginning of the experiment ($F = 1.1$, $P = 0.11$). However, on day 24, a treatment effect on overall community structure was detected ($F = 1.5$, $P = 0.001$; [Figure 1](#)). Pairwise PERMANOVA tests comparing each treatment to the controls revealed that not all treatments had significant impacts on the community structure of the fecal microbiome where it was observed that only pigs fed diets containing SCF, CT_{sb}, and RS_{po} resulted in community differences compared to pigs fed the NC on day 23 ($P < 0.05$; [Figure 2](#)).

Considering all dietary treatments, 224 OTUs belonging to 46 taxonomic genera were differentially abundant relative to pigs fed the NC on day 23 ([Figure 3](#), [Supplementary Table S1](#)). Because gram-negative bacteria belonging to Proteobacterial groups are of interest from a food safety perspective and are often indicator species for conditions in the gastrointestinal tract, the OTUs that belong to these taxa are highlighted in [Figure 4](#). Pigs fed the SCF and CT_{sb} diets were associated with the most significantly different microbes in the Proteobacterial taxonomic groups. Feeding the SCF diet was associated with a reduction in five OTUs belonging to the highlighted

Proteobacterial taxa compared to pigs fed the NC, while the diet containing CT_{sb} was associated with a decrease in three OTUs and an increase in one OTU compared to pigs fed the NC.

The relationship between OTU and pig performance was also evaluated. When evaluating this relationship on a global basis, ignoring any dietary treatment groupings, 47 OTUs showed significant linear relationships with ADG, where it was observed that five OTUs exhibited a positive relationship to ADG while 42 OTUs exhibited a negative relationship with ADG ([Figure 5](#)). Positive log₂FoldChange values for this test indicate that animals with higher relative abundances of these OTUs also gained more weight in the nursery period, while negative log₂FoldChange values indicate that animals with higher abundances of these OTUs gained less weight in the nursery period. OTUs identified in this manner represent bacterial taxa that could be targeted for reduction to improve nursery performance or those that could be encouraged or administered to enhance performance in nursery pigs.

To investigate the possibility that certain pigs may perform better on a specific diet due to differences in their microbiota, bacterial OTUs were identified which had significant linear relationship with nursery performance within a diet (i.e., in each diet, did certain OTUs exhibit a linear relationship with nursery performance; [Supplementary Table S2](#)). This identification was narrowed to only include OTUs that

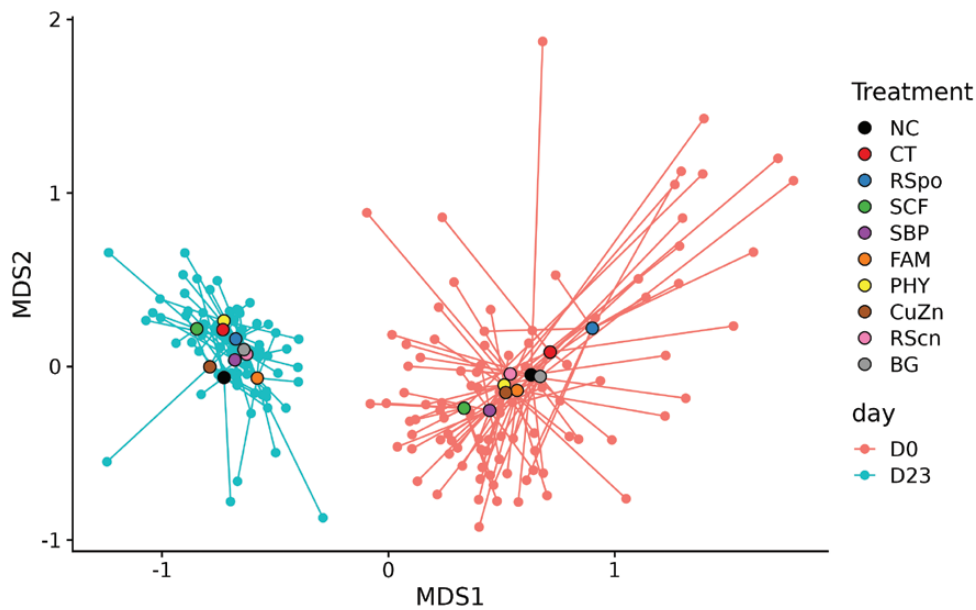


Figure 1. NMDS ordination constructed from Bray-Curtis dissimilarities depicting fecal bacterial community similarity on days 0 and 23 of pigs fed different in-feed additives in experiment 1. Individual communities are shown with the solid points colored according to the day of the study with individual communities joined to their treatment group centroid with line segments. Treatment group centroids are shown in the outlined colored circles. Abbreviations: NC, negative control; CT, subtherapeutic level of chlortetracycline and tiamulin; RSpO, resistant potato starch; SCF, soluble corn fiber; SBP, sugar beet pulp; FAM, fatty acid mix; PHY, phytogen mix; CuZn, CuSO₄ plus ZnO; RScn, resistant corn starch; BG, β-glucan; NMDS, nonmetric multidimensional scaling.

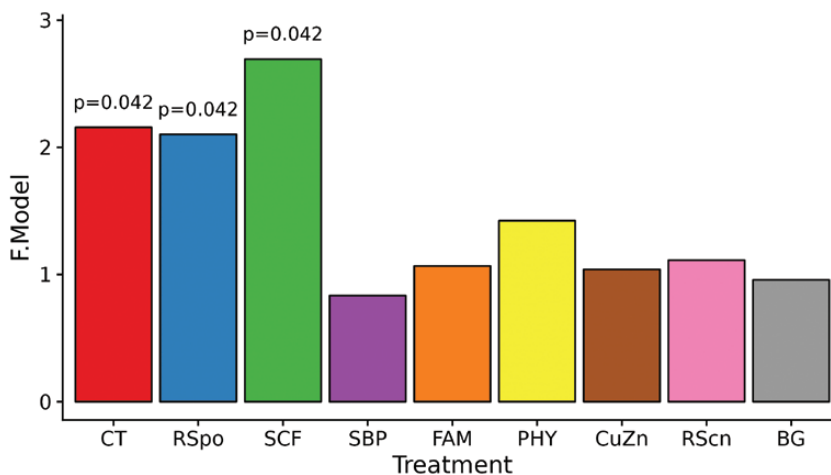


Figure 2. Differences in fecal community structure relative to pigs fed the NC diet as determined by 16S rRNA gene amplicons on day 23 of pigs fed different in-feed additives in experiment 1. Differences were calculated using pairwise PERMANOVA tests and Bray-Curtis dissimilarities, comparing each treatment to the pigs fed the NC diet. The y-axis displays the PERMANOVA pseudo F statistic (total intergroup dissimilarity divided by total intragroup dissimilarity) with greater pseudo F values indicating greater differences between that treatment and the NC. *P* values were corrected by the FDR method for all comparisons performed and are displayed when *P* < 0.05. Abbreviations: NC, negative control; CT, subtherapeutic level of chlortetracycline and tiamulin; RSpO, resistant potato starch; SCF, soluble corn fiber; SBP, sugar beet pulp; FAM, fatty acid mix; PHY, phytogen mix; CuZn, CuSO₄ plus ZnO; RScn, resistant corn starch; BG, β-glucan; FDR, false discovery rate.

were both enriched in a diet relative to pigs fed the NC as well as exhibiting a linear relationship with nursery performance in their same diet. Only three OTUs met these criteria using this method of evaluation (Figure 6). Two OTUs were significantly enriched in a treatment relative to pigs fed the NC, having a significant negative relationship with nursery performance as represented by one OTU from the *Treponema_2* genus in pigs fed the FAM diet and one OTU

from the *Prevotellaceae* family in pigs fed the RS_{cn} diet. In contrast, one OTU was significantly enriched in pigs fed the RS_{po} or RS_{cn} diets relative to pigs fed the NC and had a positive linear relationship with nursery performance. The OTU associated with increased gain within the two resistant starch-containing diets belonged to the genus *Bifidobacteria*, whose members are associated with starch degradation and intestinal health.

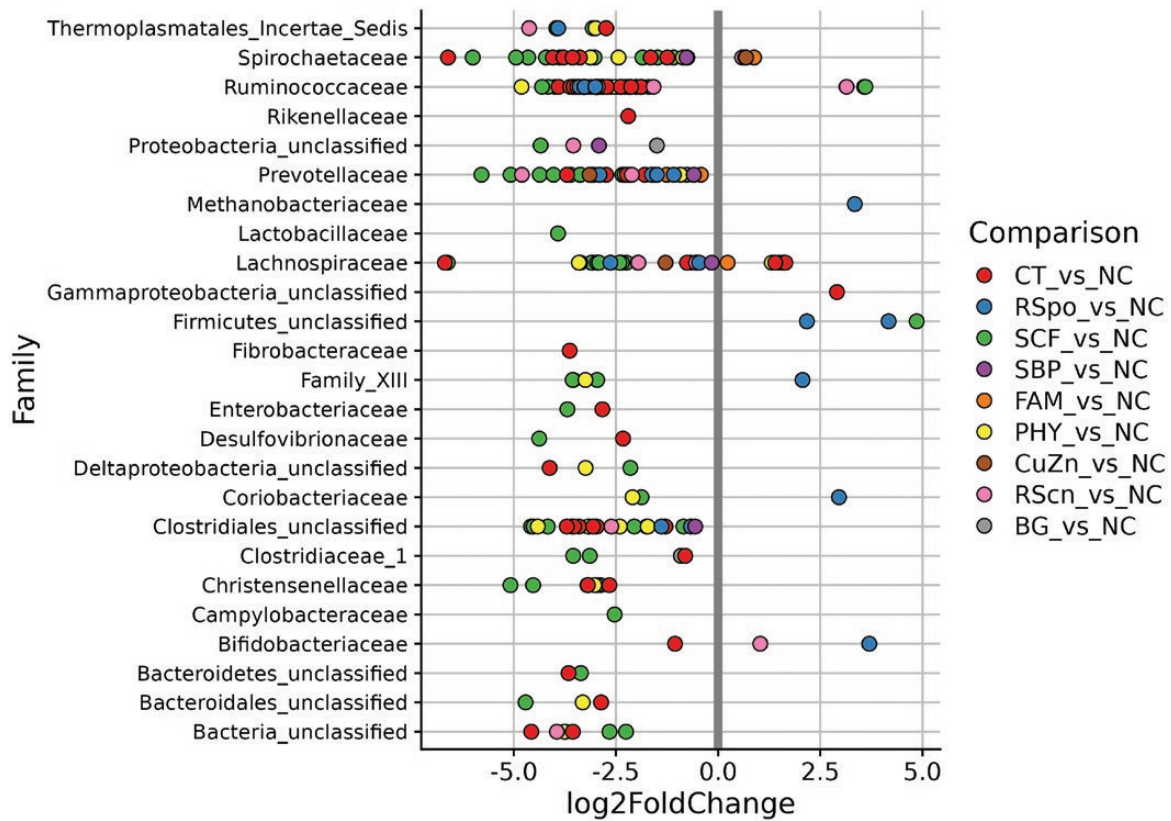


Figure 3. Differentially OTUs between pigs fed each treatment and pigs fed the negative control diet on day 23 of pigs fed different in-feed additives in experiment 1. Significance was calculated using the R package DESeq2 using Wald tests with parametric fits and FDR-corrected P -values. Prior to testing, OTUs with fewer than 10 counts globally were removed, and the resulting unrarefied counts were used as the input for DESeq2. Only OTUs with FDR corrected P -values < 0.05 are shown. Positive log2FoldChange values indicate enrichment of that OTU in the indicated group relative to the NC group, negative log2FoldChange indicates a reduction in that OTU relative to the NC group. Abbreviations: NC, negative control; CT, subtherapeutic level of chlortetracycline and tiamulin; RSpO, resistant potato starch; SCF, soluble corn fiber; SBP, sugar beet pulp; FAM, fatty acid mix; PHY, phytogen mix; CuZn, CuSO₄ plus ZnO; RScn, resistant corn starch; BG, β -glucan; FDR, false discovery rate; OTUs, operational taxonomic units.

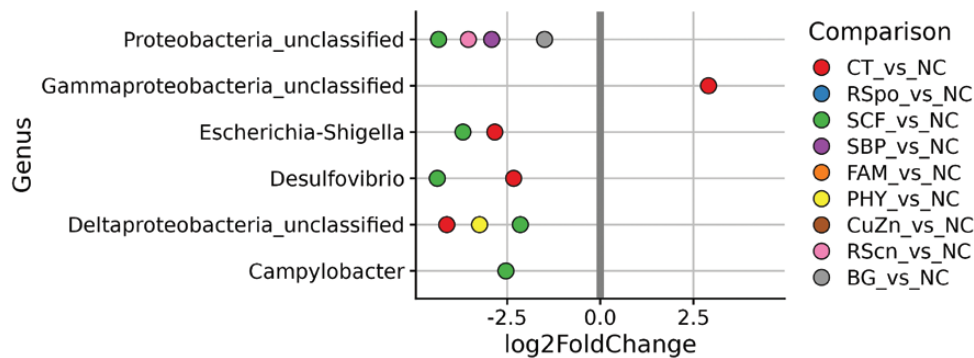


Figure 4. A subset of differentially abundant operational taxonomic units (OTUs) belonging to gram-negative genera of pigs fed different in-feed additives in experiment 1. Only OTUs with FDR corrected P -values < 0.05 are shown. Positive log2FoldChange values indicate enrichment of OTU in the indicated treatment relative to the negative control group. Negative log2FoldChange indicates a reduction in that OTU relative to pigs fed the negative control. Abbreviations: NC, negative control; CT, subtherapeutic level of chlortetracycline and tiamulin; RSpO, resistant potato starch; SCF, soluble corn fiber; SBP, sugar beet pulp; FAM, fatty acid mix; PHY, phytogen mix; CuZn, CuSO₄ plus ZnO; RScn, resistant corn starch; BG, β -glucan; FDR, false discovery rate; OTUs, operational taxonomic units.

Intestinal integrity and barrier function—EXP 2

In EXP 2, there was no effect of feeding diets containing RS_{en}, SCF, or FAM on in vivo measures of intestinal permeability ($P \leq 0.21$) when measured by urinary L:M ratio, urinary sacralose excretion, or serum FD4 concentrations (Table 6).

When examining ex vivo markers of intestinal integrity, pigs fed diets containing SCF tended to have increased ileal TER compared to pigs fed the NC ($P \leq 0.10$), but no other dietary treatment effects were noted on ileal or colonic barrier function, ($P \geq 0.44$, Table 7).

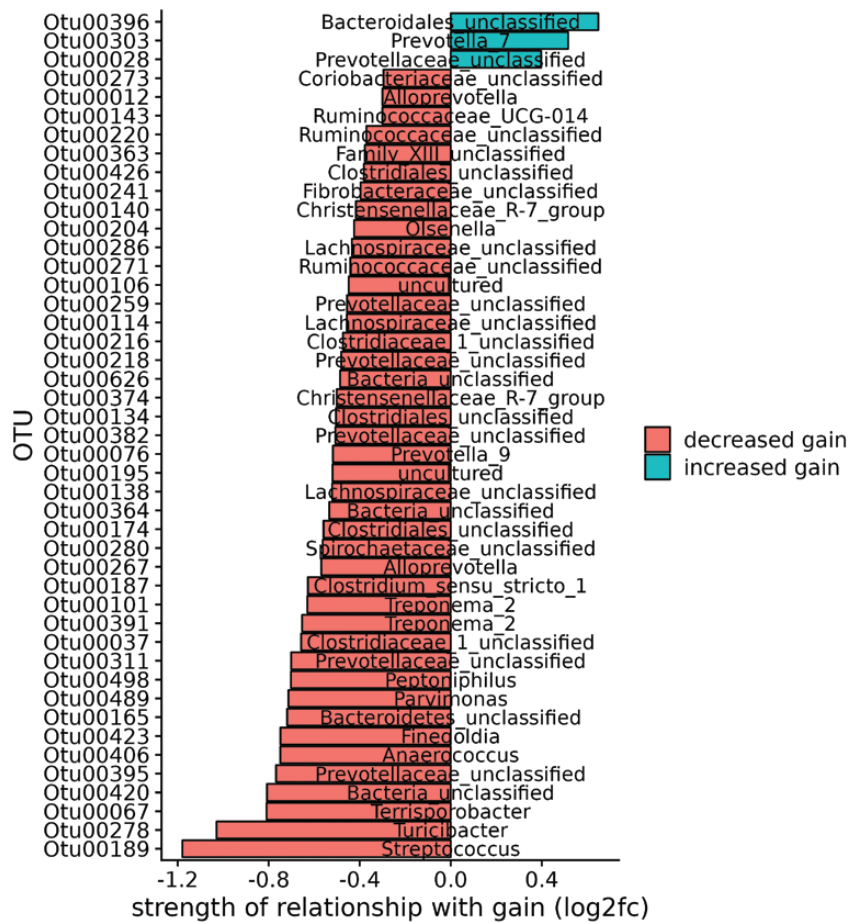


Figure 5. Operational taxonomic units (OTUs) with a significant linear relationship between their abundance and nursery performance of pigs fed different in-feed additives in experiment 1. Negative log₂FoldChange values indicate increased abundance of the OTU was associated with decreased performance over the nursery period while positive log₂FoldChange values indicate that increased abundance of that OTU was associated with improved nursery performance. Linear relationships between each OTU nursery performance were calculated with DeSeq2 using weight gain as a continuous covariate. *P*-values were corrected according to the false discovery rate (FDR) method, only OTUs with FDR *P*-value < 0.05 are shown.

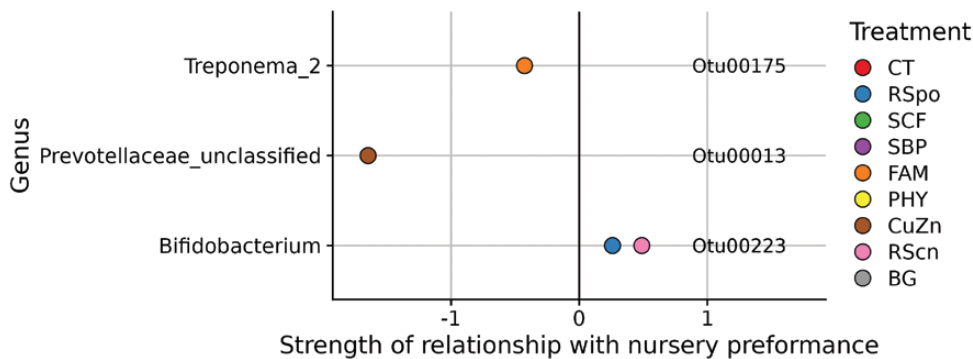


Figure 6. OTUs significantly enriched in a treatment group and significant linear relationship with nursery performance of pigs fed different in-feed additives in experiment 1. Within each treatment group in isolation, linear relationships between OTU abundance and nursery performance were calculated using gain as a continuous covariate, only OTUs with significant relationships were retained (FDR corrected *P*-values < 0.05). This list was then further filtered to only include OTUs enriched in a specific treatment group relative to the NC group. Abbreviations: NC, negative control; CT, subtherapeutic level of chlortetracycline and tiamulin; RSpO, resistant potato starch; SCF, soluble corn fiber; SBP, sugar beet pulp; FAM, fatty acid mix; PHY, phytogen mix; CuZn, CuSO₄ plus ZnO; RScn, resistant corn starch; BG, β-glucan; FDR, false discovery rate; OTUs, operational taxonomic units.

Ileal, cecal, and colon contents—EXP 2

Ileal pH, concentrations of acetic, propionic, and butyric acid, or total VFA did not differ due to dietary treatment (*P* ≥ 0.36; Table 8). There was a treatment effect on cecal

butyric acid concentration, as pigs fed diets containing FAM resulted in a greater butyric acid concentration in the cecum compared to pigs fed the NC (*P* ≤ 0.05). No other treatment effects on cecal butyric acid concentration were observed.

Compared to pigs fed the NC, no effect of dietary treatment was noted on cecal pH, acetic and propionic acid, or total VFA ($P \geq 0.12$). Colon pH, concentrations of acetic, propionic, and butyric acid, or total VFA did not differ due to dietary treatment compared to pigs fed the NC ($P \geq 0.30$, Table 8).

Apparent total tract digestibility—EXP 2

There were no observed differences in ATTD of GE, N, Ca, or PHOS due to feeding RS_{cn}, SCF, or FAM ($P \geq 0.16$, Table 9). In addition, there was no effect of dietary treatment on BMC ($P = 0.70$), averaging 602 mg/kg of BW (Table 9).

Pig performance, organ weights, and VFA—EXP 3

There was no interactive effect between diets containing RS_{po} and FAM on pig performance, organ weights, or on cecal or colon pH and VFA concentrations ($P \geq 0.16$, Tables 10, 11, and 12, respectively). No effect of dietary RS_{po} or FAM was noted on ADFI ($P \geq 0.42$). Feeding pigs diets containing RS_{po} did not affect ADG ($P = 0.91$), but pigs fed diets containing RS_{po} tended to result in a reduction in GF compared to pigs fed diets not containing RS_{po} (0.557 versus 0.590, respectively; $P = 0.11$, Table 10). Pigs fed diets containing FAM

grew faster than pigs fed diets not containing FAM (0.185 versus 0.164, respectively; $P = 0.09$) and were more efficient (0.594 versus 0.552, respectively; $P = 0.05$) compared to pigs not consuming FAM (Table 10). There was no effect of dietary RS_{po} or FAM on relative organ weights ($P \geq 0.32$, Table 11).

Butyric acid concentrations in the colon were greater in pigs fed diets containing RS_{po} compared to pigs not fed RS_{po} (24.1 versus 19.6 mM/L, respectively; $P = 0.03$), but feeding RS_{po} had no other notable effects on cecal and colon pH or VFA concentrations ($P \geq 0.28$, Table 12). Pigs fed diets containing FAM exhibited reduced cecal concentrations of acetic acid (44.1 mM/L; $P = 0.11$), propionic (24.2 mM/L; $P = 0.09$), and total VFA (92.1 mM/L; $P = 0.11$) compared to pigs fed diets not containing FAM (68.6, 35.6, and 134.4 mM/L; respectively, Table 12).

Discussion

The use of subtherapeutic antibiotics for the promotion of growth in farm animals dates back to the late 1940s (Dibner and Richards, 2005; Aminov, 2010; Brown et al., 2017) with the FDA approving the use of penicillin and chlortetracycline as animal feed additives in 1951. However, with the implementation of the VFD in 2017, the use of medically important antibiotics used in feed or water for food animal species require a VFD, or prescription, for use, largely due to antimicrobial resistance concerns. As a result, producers and allied industry are seeking out alternative compounds or feedstuffs having antibiotic-like growth promoting properties (Liu et al., 2018; Kurt et al., 2019). Based on a broad review of antibiotic alternatives in 2019 (Gabler et al., 2019), the current studies selected various in-feed additives or feedstuffs of interest by the swine industry and those which have shown 'some' positive effects on intestinal integrity, gastrointestinal volatile fatty acid concentrations, or mineral digestion, and more importantly, on parameters of pig performance. For better comparisons and efficacy of action, the studies conducted herein included a negative control which was a diet formulated without any potential growth promoting compounds, a diet containing a combination of growth promoting

Table 6. In vivo measures of intestinal permeability of pigs fed nonantibiotic alternatives, EXP 2¹

Criterion	Dietary treatment ²				Statistics ⁵	
	NC	RS _{cn}	SCF	FAM	SEM	P-value
L:M ³	0.15	0.11	0.10	0.10	0.02	0.24
Sucralose ³ , %	1.82	1.60	1.45	1.31	0.17	0.21
FD4 ⁴ , ng/ml	15.48	9.67	13.75	11.95	3.00	0.57

¹There were seven to eight observations per dietary treatment, with an average body weight of 13.40 kg.

²Abbreviations: NC, negative control; RS_{cn}, 5% resistant corn starch; SCF, 5% soluble corn fiber; FAM, 0.3% fatty acid mix.

³Urinary lactulose to mannitol ratio (L:M) or sucralose concentration.

⁴Serum fluorescein isothiocyanate-dextran (FD4) with an average molecular weight of 4,000 g/M.

⁵Model standard error of the mean (SEM) and P value.

Table 7. Ex vivo transepithelial resistance (TER) and permeability coefficient of fluorescein isothiocyanate-dextran (PaPP) in ileum and colon and ex vivo active transport of glucose and glutamine in ileum of pigs fed resistant corn starch, soluble corn fiber, and a fatty acid mix, EXP 2¹

Criterion	Dietary treatment ²				Statistics ⁵	
	NC	RS _{cn}	SCF	FAM	SEM	P-value
Ileum						
TER, $\Omega \times \text{cm}^3$	39.8 ^y	39.0	46.4 ^x	39.2	1.9	0.04
Glucose, μA^3	125.6	117.1	99.8	84.7	18.9	0.44
Glutamine, μA^3	20.9	14.1	18.7	15.1	5.0	0.75
PaPP ⁴	31.8	39.8	45.2	50.6	9.4	0.52
Colon						
TER, $\Omega \times \text{cm}^2$	64.8	69.8	67.7	67.0	5.2	0.93
PaPP	45.8	36.9	76.1	39.1	26.0	0.68

¹There were six to eight observations per dietary treatment, with an average body weight of 13.40 kg.

²Abbreviations: NC, negative control; RS_{cn}, 5% resistant corn starch; SCF, 5% soluble corn fiber; FAM, 0.3% fatty acid mix.

³Active absorption calculated by subtracting μA before substrate (glucose or glutamine) from μA after substrate addition.

⁴PaPP = permeability coefficient of fluorescein isothiocyanate-dextran (average molecular wt, 4,000 g/M) permeability.

⁵Model standard error of the mean and P value. Differences denoted by 'ab' represent differences ($P \leq 0.05$) and 'xyz' represent differences ($0.10 \geq P \geq 0.05$) between pigs fed a specific treatment compared to pigs fed the NC.

antibiotics to be reflective of preVFD diet formulation, and a diet containing elevated concentrations of both Cu and Zn (50 and 1,600 mg/kg diet, respectively). While the level of Cu is below levels use in the industry or suggested as an optimal level in the literature (Jongbloed et al., 2011; Ma et al., 2015), the level of Zn used in the current study (1,600 mg Zn/kg diet) is reflective in industry and literature reviews (Hill et al., 2001; Sales, 2013). The in-feed antibiotic combination of chlortetracycline and tiamulin (44 and 39.6 mg/kg diet, respectively) was selected because it was a commonly used growth promoting antibiotic combination used in the swine industry prior to the 2017 VFD. The use of elevated levels of Cu or Zn in the form of CuSO_4 and ZnO ,

respectively, was selected because they have been established to improve pig performance, particularly in the weaned pig (Hahn and Baker, 1993; Cromwell et al., 1998; Carlson et al., 1999; Hollis et al., 2005; Perez et al., 2011; Shelton et al., 2011). However, like in-feed antibiotics, elevated levels of minerals are now facing legislative scrutiny in Europe (EFSA, 2014, 2016). Consequently, diet formulations containing ingredients that can modulate intestinal integrity, animal growth, and immune function are potential substitutes to antibiotic and pharmacological mineral growth promoters.

Digestively resistant, but highly fermentable carbohydrates (resistant corn and potato starch, soluble corn fiber,

Table 8. pH and VFA (mM/L wet digesta) of ileal, cecal, and colon contents of pigs fed resistant corn starch, soluble corn fiber, and a fatty acid mix, EXP 2¹

Criterion	Dietary treatment ²				Statistics ³	
	NC	RS _{cn}	SCF	FAM	SEM	P-value
Ileum						
pH	7.6	7.8	7.6	7.2	0.3	0.36
Acetic	11.9	9.3	12.3	13.1	2.9	0.83
Propionic	0.1	0.2	0.1	0.1	0.1	0.70
Butyric	0.9	0.8	0.8	0.5	0.3	0.76
Total ⁴	12.9	10.4	13.1	13.7	3.2	0.89
Cecum						
pH	5.7	5.9	5.9	5.7	0.1	0.12
Acetic	114.1	92.2	107.4	109.7	9.7	0.42
Propionic	46.1	38.2	43.4	51.0	3.7	0.12
Butyric	18.1 ^b	13.9	15.7	24.8 ^a	1.6	0.01
Total	182.9	147.3	170.5	192.5	12.4	0.08
Colon						
pH	6.4	6.4	6.4	6.5	0.1	0.70
Acetic	174.4	177.6	145.3	115.0	25.5	0.30
Propionic	27.9	32.5	27.3	28.6	3.1	0.63
Butyric	17.9	20.6	17.9	18.8	1.4	0.46
Total	232.5	242.6	201.1	177.1	26.7	0.30

¹There were eight observations per dietary treatment, with an average body weight of 13.40 kg.

²Abbreviations: NC, negative control; RS_{cn}, 5% resistant corn starch; SCF, 5% soluble corn fiber, FAM, 0.3% fatty acid mix containing salts of sodium butyrate and propionate, palm fat, caprylic, capric, and lauric fatty acids, natural flavoring compounds, and a mixture of steatites and chlorite.

³Model standard error of the mean and P value. Differences denoted by 'ab' represent differences ($P \leq 0.05$) and 'xyz' represent differences ($0.10 \geq P \geq 0.05$) between pigs fed a specific treatment compared to pigs fed the NC.

⁴Total volatile fatty acids represents the sum of acetic, propionic, butyric, isobutyric, isovaleric, valeric, isocaproic, caproic, and heptanoic acids.

Table 9. Apparent total tract digestibility of energy and nutrients in pigs fed resistant corn starch, soluble corn fiber, and fatty acid mix, EXP 2¹

ATTD	Dietary treatment ²				Statistics ³	
	NC	RS _{cn}	SCF	FAM	SEM	P-value
GE	92.1	92.2	91.4	91.6	1.03	0.94
N	91.3	90.8	90.4	90.8	0.26	0.16
Ca	83.8	85.3	84.5	84.3	1.47	0.91
PHOS	77.8	79.9	76.5	78.2	2.01	0.74
BMC ⁴ , mg/kg BW	583	619	614	593	25	0.70

¹There were eight observations per dietary treatment, with an average body weight of 13.40 kg.

²Abbreviations: NC, negative control; RS_{cn}, 5% resistant corn starch; SCF, 5% soluble corn fiber, FAM, 0.3% fatty acid mix.

³Model standard error of the mean and P value. Differences denoted by 'ab' represent differences ($P \leq 0.05$) and 'xyz' represent differences ($0.10 \geq P \geq 0.05$) between pigs fed a specific treatment compared to pigs fed the NC.

⁴Bone mineral content of the femur.

Table 10. Effects of resistant potato starch with or without a fatty acid mix on growth performance in nursery pigs, EXP 3¹

Criterion	Dietary treatment				SEM	P-value		
	NC ²	RS _{po}	FAM	RS _{po} + FAM		RS _{po} × FAM	RS _{po}	FAM
ADG, g	0.160	0.169	0.191	0.180	0.012	0.43	0.91	0.09
ADFI, g	0.290	0.308	0.305	0.312	0.016	0.70	0.42	0.54
GF	0.556	0.547	0.624	0.567	0.021	0.26	0.11	0.04

¹ Trial lasted 24 d, with a final BW of 10.34 kg with 13 replications per dietary treatment. Initial BW (average 6.11 kg) used as a covariate for performance data.

² NC, negative control; RPS, NC + resistant potato starch; FAM, NC + fatty acid mix; RPS-FAM, NC = resistant potato starch + fatty acid mix.

Table 11. Effects of resistant potato starch with or without a fatty acid mix on relative organ weights in nursery pigs, EXP 3¹

Criterion	Dietary treatment				SEM	P-value		
	NC ²	RS _{po}	FAM	RS _{po} + FAM		RS _{po} × FAM	RS _{po}	FAM
Pluck	2.30	2.16	2.24	2.19	0.07	0.50	0.17	0.80
Liver	3.28	3.46	3.22	3.41	0.15	0.97	0.25	0.73
Spleen	0.18	0.20	0.18	0.21	0.02	0.93	0.15	0.97
Stomach	0.90	0.93	1.05	0.86	0.08	0.16	0.33	0.62
Small intestine	7.33	7.68	7.09	7.14	0.39	0.71	0.61	0.33
Large intestine	2.44	3.01	2.84	2.95	0.25	0.37	0.19	0.51

¹ Samples harvested from 1 pig per pen and 8 pens per dietary treatment on 24 d with an average final BW of 10.66 kg.

² NC, negative control; RS_{po}, NC + resistant potato starch; FAM, NC + fatty acid mix; RPS-FAM, NC = resistant potato starch + fatty acid mix.

Table 12. pH and VFA (mM/L wet digesta) of cecal and colon contents of nursery pigs fed resistant potato starch with or without a fatty acid mix, EXP 3¹

Criterion	Dietary treatment				SEM	P-value		
	NC ²	RS _{po}	FAM	RS _{po} + FAM		RS _{po} × FAM	RS _{po}	FAM
Cecum								
pH	6.1	6.2	6.0	6.5	0.2	0.37	0.28	0.56
Acetic	68.4	68.7	51.8	36.8	14.5	0.61	0.62	0.11
Propionic	36.9	34.4	29.9	18.4	6.5	0.50	0.30	0.09
Butyric	23.7	25.7	22.0	15.1	4.1	0.30	0.56	0.15
Total	134.3	134.6	109.2	74.9	25.1	0.50	0.51	0.11
Colon								
pH	6.5	6.6	6.4	6.3	0.2	0.69	0.74	0.30
Acetic	94.2	88.5	88.9	92.7	7.0	0.50	0.89	0.94
Propionic	26.4	25.0	25.1	24.4	1.6	0.84	0.52	0.59
Butyric	17.9	25.4	21.3	22.8	1.9	0.14	0.03	0.81
Total	149.9	152.8	147.9	152.1	9.9	0.95	0.72	0.89

¹ Samples harvested from 1 pig per pen and 8 pens per dietary on 24 d with an average final BW of 10.66 kg.

² NC, negative control; RS_{po}, NC + resistant potato starch; FAM, NC + fatty acid mix; RPS-FAM, NC = resistant potato starch + fatty acid mix.

β-glucans, and sugar beet pulp) were selected for use in the current experiment because these compounds are suggested to selectively stimulate the growth and/or activity of a number of resident bacterial species in the gastrointestinal tract in a manner that is beneficial to the host (Dritz et al., 1995; Hiss and Sauerwein, 2003; Gibson et al., 2004; Bauer et al., 2006; Hahn et al., 2006; Li et al., 2006; Haenen et al., 2013; Keenan et al., 2015; Umu et al., 2015; Newman et al., 2016; Faba et al., 2020). Further, a short and medium chain

FAM and a phytogetic blend of essential oils was selected because these compounds have been shown to improve gain and efficiency in pigs as well as modulate gastrointestinal microbiota (Dierick et al., 2002a, b; Hong et al., 2012; Hanczakowska et al., 2013; Bedford and Gong, 2018; Soler et al., 2018).

Dietary additives may mediate their impact on the animal host through compositional and functional shifts in intestinal microbial membership. Herein, the tested dietary additives

had small or nonsignificant effects on the overall fecal community composition of the weaned pigs in EXP 1. Certain prebiotic dietary additives often have detectable impacts on the microbial community structure in the gastrointestinal tract of swine, particularly resistant starch additives (Umu et al., 2015; Sun et al., 2016; Metzler-Zebeli et al., 2019; Trachsel et al., 2019; Zhang et al., 2020). However, only the SCF and RS_{po} exhibited significant differences from the NC group, and these differences were modest and did not translate into improved growth performance. The lack of consistent response to in-feed prebiotic additives, such as SCF and RS_{po} may be related to differing microbiotas in the gastrointestinal tract of pigs across different experiments, as prebiotic additives require the presence of specific bacterial taxa to ferment the compound. Interindividual variation in microbial communities in the gastrointestinal tract is high and previous work suggests the initial communities, or bacterial membership, may have different abilities to produce host-benefits from prebiotic diets (Baxter et al., 2019).

Some of the differences in abundance of bacterial OTUs between the experimental diets and the NC group in EXP 1 were in line with previous observations or otherwise notable. In agreement with a previous report (Trachsel et al., 2019), the resistant starch containing diets, RS_{cn} and RS_{po}, exhibited enrichments of OTUs classified to the genus *Bifidobacteria*, suggesting this genera is important for a response to resistant starch in swine. Interestingly, the SCF group exhibited reductions in several gram-negative taxa, including OTUs classified as *Escherichia-Shigella* and *Campylobacter*. These taxonomic groups contain important foodborne pathogens and are also used as indicator species to infer ecological conditions within the gastrointestinal tract (Rizzatti et al., 2017; Zeng et al., 2017). A decreased abundance in the indicator groups suggests a healthier microbiota, though it is only one measure in a complex system. In a companion investigation to this study, pigs were inoculated with *Salmonella enterica* serovar I 4,[5],12:i:- following 28 d on various dietary treatments and monitored *Salmonella* fecal shedding while continuing in-feed additives for 21 d. Pigs administered an RS_{po} diet exhibited a reduction in *Salmonella* fecal shedding and increased concentrations of short chain fatty acids in the cecum compared to swine on the control diet. Furthermore, the RS_{po}-fed pigs that shed quantitatively lower levels of *Salmonella* tended to have greater cecal concentrations of short chain fatty acids compared to swine on the same diet that shed higher quantities of *Salmonella* (Trachsel et al., 2022). Collectively, the microbial changes associated with some resistant starch additives suggests they may produce benefits for the host and gastrointestinal community without large impacts on nursery performance under normal conditions but may provide benefit during intestinal infection or when other stressors are present.

Changes in intestinal morphological structure and function as well as mucosal inflammation that occur at weaning are associated with poor performance (Pluske et al., 1997; Hu et al., 2013). Therefore, it is at this stage of development in postweaned pigs when antibiotics and antibiotic alternatives likely have their largest effect. Further, weaning stress is associated with increased intestinal permeability and reduced performance (Pie et al., 2004; Lalles and David, 2011; Moeser et al., 2017). To assess if diet may mitigate weaning stress induced changes in intestinal permeability, in vivo and ex vivo markers of intestinal permeability were examined. The ratio of lactulose to mannitol in urine is a commonly used in vivo

indicator of small intestinal paracellular permeability, with the disaccharide (lactulose) assumed to pass through via the paracellular route under conditions of compromised barrier function, and the monosaccharide (mannitol) acting as a measure of absorptive surface (Wijten et al., 2011). In the current study, however, there were no differences in urinary lactulose to mannitol ratio or serum FD4 between treatment groups for pigs in EXP 2, indicating that the treatments evaluated (i.e., RS_{cn}, SFC, and FAM) did not impact intestinal integrity. While the lack of a dietary effect on the lactulose to mannitol ratio (or other parameters measured in EXP 2) may be a function of these pigs being 36 d postweaning, the lactulose to mannitol ratio observed was similar to that observed previously by this laboratory (Lindblom et al., 2018; Overholt et al., 2018; Kerr et al., 2020). An ex vivo measurement of intestinal integrity and function was also utilized in this experiment (EXP 2), where the integrity of both the ileum and colon was assessed using modified Ussing chambers to measure TER, which indicates permeability status across tight junction pores, macromolecule permeability (an indicator paracellular transport), as well as glucose and glutamine transport. Except for one TER comparison in the ileum (pigs fed SCF versus pigs fed the NC), the ex vivo measures of intestinal integrity and function were similar to the aforementioned results from the in vivo assay, where there were no treatment effects on TER, macromolecule permeability, and active glucose and glutamine transport. No published data were found to compare the current data with, as previous studies in pigs fed resistant potato starch (Bhardari et al., 2009; Krause et al., 2010; Heo et al., 2014; Trachsel et al., 2019), retrograde tapioca starch (Haenen et al., 2013), an enzymatically modified waxy corn starch (Neuman et al., 2016), an unspecified resistant starch (Umu et al., 2015), or a product that contained coated short- and medium-chain fatty acids plus essential oils (Soler et al., 2018) did not measure these parameters.

In terms of intestinal health, an increase in VFA and a reduction in pH is often considered positive (Keenan et al., 2015; Umu et al., 2015). Herein, it was expected that feeding the digestively resistant but fermentable fibers, a fatty acid mix, or a phytogetic compound mixture would result in changes in intestinal VFA and pH concentrations (Topping and Clifton, 2001; Slavin, 2013; Giuberti et al., 2015; Bedford and Gong, 2018; Long et al., 2018). In EXP 2, there was no effect of feeding RS_{cn}, SFC, or FAM to nursery pigs on the pH of ileal, cecal, and colon contents (except for a singular increase in the butyric acid concentration in the cecum of pigs fed diets containing FAM). In contrast, in EXP 3, pigs fed diets containing the FAM had decreased cecal concentrations of acetic and propionic acid. These inconsistent results are similar to Bhardari et al. (2009) who reported no effect of feeding 6-kg-BW pigs diets containing 7% or 14% resistant potato starch on ileal or colon VFA or pH concentrations and Xu et al. (2018) who fed 9-kg-BW pigs an organic acid or essential oil, and diet combinations thereof. In contrast, Heo et al. (2014) fed 7-kg-BW pigs either 0.5% or 1.0% resistant potato starch and reported increased VFA and decreased ileal and cecal pH. As for the addition of short- and medium-chain fatty acids, the data is likewise inconclusive. Long et al. (2018) fed 9-kg-BW pigs two different organic acid blends and reported increased fecal VFA concentrations, while Zentek et al. (2013) reported variable effects with either separate or the combination of organic and medium chain fatty acids on intestinal VFA and pH. As expected (Bergman, 1990), a higher concentration of

VFA was noted in the cecum as well as the large colon compared to the ileum regardless of treatment (EXP 2).

Digestively resistant fibers, short- and medium-chain fatty acids, Cu and Zn, and antibiotics have been shown to affect GE and N digestibility and retention (Roth and Kirchgessner, 1993; Gaskins et al., 2002; Liu et al., 2008; Hong et al., 2012; Souza da Silva et al., 2014; Giuberti et al., 2015). This may be due to the shifting of N excretion from the urine to the feces (Zervas and Zijlstra, 2002) or due to where products are digested versus fermented (Fouhse and Zijlstra, 2018). While this shifting of N excretion from urine to feces might explain the reduction in ATTD of GE and N in EXP 1 for pigs fed diets containing RS_{po} , SCF, FAM, and PHY, this negative effect in digestibility was not consistent as pigs fed diets containing RS_{cn} and BG in EXP 1 did not exhibit a reduction in ATTD of GE or N; and pigs fed diets containing RS_{cn} , SCF, and FAM in EXP 2 had no impact on ATTD of GE or N.

The potential impact of dietary treatment, especially the digestively resistant but fermentable fibers or the FAM and PHY treatments, on mineral digestibility was also of interest based on a review of human- and rodent-based literature suggesting increased mineral digestibility due to resistant fibers reaching the large intestine causing a reduction in pH and subsequent increase in Ca digestion (Cashman, 2003; Scholz-Ahrens et al., 2007; Whisner et al. 2014, 2016; Jake-man et al., 2016; Wallace et al., 2017). While ATTD of Ca and PHOS was reduced in pigs fed diets containing RS_{po} and PHY in EXP 1, no effect on ATTD of Ca or PHOS was noted in EXP 2 when pigs were fed diets containing RS_{cn} , SCF, or FAM. In addition, BMC of the femur was not affected in pigs fed diets containing RS_{cn} , SCF, or FAM in EXP 2. The lack of a change in ATTD of Ca and PHOS is supported by the lack of dietary treatment effects on intestinal pH or VFA concentrations. While no change in ATTD of Ca or PHOS was unexpected based on the literature cited above, no effect on ATTD of Ca or PHOS was reported by Heo et al. (2014) in pigs fed 0.5% or 1.0% resistant potato starch. It has been reported, however, that feeding an organic acid mix (Long et al., 2018) or an organic acid mix and an essential oil (Xu et al., 2018) may result in increased ATTD of Ca or PHOS. The lack of any dietary effect on BMC was not surprising, given that dietary treatment had no effect on Ca or PHOS digestibility, and the fact that the diets were formulated to be adequate in Ca or PHOS according to the NRC (2012).

The lack of changes in pig performance (ADG or ADFI) in EXP 1 were not unexpected given the summarization of the literature (Jongbloed et al., 2011; Sales, 2013; Gabler et al., 2019), inconsistent changes or lack of changes in ATTD of energy and minerals, no effects noted on intestinal permeability and function, and little to no effects on intestinal pH or VFA concentrations. The general decrease in GF due to dietary treatment in EXP 1 is perplexing and cannot be explained, although in general it agrees with a general decrease in ATTD of GE and N. In EXP 2, pigs fed diets containing the FAM did result in improved ADG and GF, which while not noted in EXP 1, may be worthy of future research endeavors. It is worth noting that no positive response to feeding antibiotics (i.e., CT_{sb}) was observed in EXP 1, and this lack of a positive antibiotic effect, and in theory for any other in-feed additives evaluated, may be due to a high health status of the pigs being tested, the cleanliness of the research location, and no overly

apparent disease challenge in the facilities in which these experiments were conducted.

While no overt disease was detectable in these facilities, some commensal bacteria (or opportunistic pathogens) may still have had influences on pig performance. The *Streptococcus* genus contains taxa known to be pig pathogens capable of causing septicemia, meningitis, and other complications (Goyette-Desjardins et al., 2014). In EXP 1, an OTU classified to the genus *Streptococcus* exhibited the strongest negative relationship between its abundance and nursery performance. However, no experimental treatment group was associated with a difference in abundance of this *Streptococcus* OTU relative to the NC group. These results suggest that the relationship between the abundance of *Streptococcus*-like organisms and nursery performance in the absence of clinical symptoms should be further investigated.

The effects of most of the additives tested are mediated through the gastrointestinal microbiota, meaning their effects are dependent on the communities of microbes already existing in the gastrointestinal tract and vertically or horizontally transferred to the piglets from the sow or environment following farrowing, respectively. A possibility for the lack of a response phenotype in the experimental diets compared to the NC group could be the lack of resident microbes and/or communities in gastrointestinal tract being required to take full advantage of the dietary additives. For example, in humans fed resistant potato starch, an increase in fecal butyrate was dependent on the study participant having an increased abundance of putative primary starch degrading taxa classified as *Ruminococcus bromii* (Baxter et al., 2019). In EXP 1, within each diet, we detected OTUs whose abundances had positive associations with nursery performance, and the identified taxonomic groups could be investigated for their ability to enhance the effects of different dietary additives. In addition, the introduction of bacterial taxa not already present could further impact responses to in-feed additives. Collectively, microbial membership or administration of a probiotic may need to be considered when feeding specific dietary additives to a herd, and while positive changes in microbiota may be noted with dietary treatment application, it may not result in measurable changes in performance. However, the changes may result in increased disease resilience upon pathogen encounter or stressful events.

To examine how diets alter body composition and carcass weights, there was interest in exploring if dietary treatment had an impact on gastrointestinal tract weights, where it was observed in EXP 3 that pigs fed diets containing RS_{po} or FAM or the combination thereof had no effect on relative organ weights. This is in contrast to Martinez-Puiz et al. (2003), Bolhuis et al. (2007), and Souza da Silva et al. (2014) who reported that empty weights of the colon and total gastrointestinal tract were greater in pigs fed diets containing resistant starch compared to pigs fed diets not containing resistant starch but is supported by the few changes that were noted in fecal or colon VFA concentrations.

In summary, the results indicate that supplementing diets with digestively resistant but fermentable fibers, short- and medium-chain fatty acids, or antibiotics do not have a consistent, positive or negative, effect on markers of intestinal integrity or barrier function, intestinal VFA patterns, intestinal pH, ATTD of energy, N, Ca and PHOS, or on pig performance.

Supplementary Data

Supplementary data are available at *Journal of Animal Science* online.

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Conflict of Interest Statement

S.M.C. is employed by DSM Nutritional Products AG and W.P.S. is employed by Zinpro Corporation. All other authors have no conflicts of interest.

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