

JOURNAL OF ANIMAL SCIENCE

The Premier Journal and Leading Source of New Knowledge and Perspective in Animal Science

Use of *Saccharomyces cerevisiae* fermentation product on growth performance and microbiota of weaned pigs during *Salmonella* infection

K. L. Price, H. R. Totty, H. B. Lee, M. D. Utt, G. E. Fitzner, I. Yoon, M. A. Ponder and J. Escobar

J ANIM SCI 2010, 88:3896-3908.

doi: 10.2527/jas.2009-2728 originally published online July 23, 2010

The online version of this article, along with updated information and services, is located on the World Wide Web at:

<http://www.journalofanimalscience.org/content/88/12/3896>



American Society of Animal Science

www.asas.org

Use of *Saccharomyces cerevisiae* fermentation product on growth performance and microbiota of weaned pigs during *Salmonella* infection¹

K. L. Price,*² H. R. Totty,†² H. B. Lee,* M. D. Utt,* G. E. Fitzner,‡ I. Yoon,‡
M. A. Ponder,† and J. Escobar*³

*Department of Animal and Poultry Sciences, and †Department of Food Science and Technology,
Virginia Polytechnic Institute and State University, Blacksburg 24061;
and ‡Diamond V, Cedar Rapids, IA 52405

ABSTRACT: Anaerobically fermented yeast products are a rich source of nutritional metabolites, mannanoligosaccharides, and β -glucans that may optimize gut health and immunity, which can translate into better growth performance and a reduced risk of foodborne pathogens. The objective of this study was to quantify the effects of *Saccharomyces cerevisiae* fermentation product (Diamond V Original XPC) inclusion in nursery diets on pig performance and gastrointestinal microbial ecology before, during, and after an oral challenge with *Salmonella*. Pigs ($n = 40$) were weaned at 21 d of age, blocked by BW, and assigned in a 2×2 factorial arrangement consisting of diet (control or 0.2% XPC) and inoculation (sterile broth or *Salmonella*). Pigs were fed a 3-phase nursery diet (0 to 7 d, 7 to 21 d, and 21 to 35 d) with ad libitum access to water and feed. On d 14, pigs were orally inoculated with 10^9 cfu of *Salmonella enterica* serovar Typhimurium DT104 or sterile broth. During d 17 to 20, all pigs were treated with a 5 mg/kg of BW intramuscular injection of ceftiofur-HCl. Growth performance and alterations in the gastrointestinal microbial ecology were measured during preinoculation (PRE; 0 to 14 d), sick (SCK; 14 to 21 d), and postinoculation (POST; 21 to 35 d). Body weight and ADG were measured weekly. Rectal temperature (RT)

was measured weekly during PRE and POST, and every 12 h during SCK. Diet had no effect on BW, ADG, or RT during any period ($P = 0.12$ to 0.95). Inclusion of XPC tended ($P < 0.10$) to increase *Salmonella* shedding in feces during SCK. Consumption of XPC altered the composition of the gastrointestinal microbial community, resulting in increased ($P < 0.05$) populations of *Bacteroidetes* and *Lactobacillus* after *Salmonella* infection. Pigs inoculated with *Salmonella* had decreased ADG and BW, and increased RT during SCK ($P < 0.001$). Furthermore, fecal *Salmonella* cfu (\log_{10}) was modestly correlated ($P = 0.002$) with BW ($r = -0.22$), ADFI ($r = -0.27$), ADG ($r = -0.36$), G:F ($r = -0.18$), and RT ($r = 0.52$) during SCK. After antibiotic administration, all *Salmonella*-infected pigs stopped shedding. During POST, an interaction between diet and inoculation ($P = 0.009$) on ADG indicated that pigs infected with *Salmonella* grew better when eating XPC than the control diet. The addition of XPC to the diets of weanling pigs resulted in greater compensatory BW gains after infection with *Salmonella* than in pigs fed conventional nursery diets. This increase in BW gain is likely associated with an increase in beneficial bacteria within the gastrointestinal tract.

Key words: gastrointestinal microbial ecology, pig growth performance, prebiotic feed supplement, *Salmonella*, yeast culture

©2010 American Society of Animal Science. All rights reserved.

J. Anim. Sci. 2010. 88:3896–3908
doi:10.2527/jas.2009-2728

INTRODUCTION

Anaerobically fermented yeast products may offer an alternative to antibiotic growth promoters (**AGP**) in food animal production. A commercial yeast culture [Original XPC (**XPC**); Diamond V, Cedar Rapids, IA] is a rich source of nutritional metabolites, mannanoligosaccharides, and β -glucans, and other yeast fermentation metabolites. These compounds may prevent the interaction between pathogenic bacteria and intestinal

¹This study was partially supported by Diamond V Program grant 08-1255-12.

²Indicates both authors contributed equally to this project.

³Corresponding author: escobar@vt.edu

Received December 8, 2009.

Accepted July 20, 2010.

cells, as well as strengthen the immune system (Gao et al., 2008; Shen et al., 2009). A current working hypothesis states that a healthier gut in conjunction with a robust immune system should translate into better growth performance of pigs. Currently, this is a key component for the swine industry because the use of AGP has been banned in the European Union. Additionally, several international and domestic markets are starting to demand animal-derived products from antibiotic-free animals.

With the increasing demand for safer products of animal origin, consumers are not only demanding AGP-free eggs, dairy, and meat products but also a reduced prevalence of foodborne pathogens. The improved growth performance in response to AGP was linked to the presence of environmental pathogens more than 40 yr ago (Coates et al., 1963). Thus, with the anticipated ban of AGP, many are concerned that the pathogenic load of farm animals can drastically increase. This scenario can lead to consumer apprehension toward animal products. Therefore, the quest for alternatives to the use of AGP in the animal industry is not only to prevent a potential reduction in growth performance but also to avert a possible increase in foodborne pathogens in eggs, dairy, and meat products. The objective of this study was to evaluate the effects of XPC inclusion in nursery diets on pig performance and gastrointestinal tract (GIT) microbial ecology associated with an oral challenge with *Salmonella*.

MATERIALS AND METHODS

All procedures were approved by the Virginia Tech Institutional Animal Care and Use Committee and Biosafety Committee and conducted in a Biosafety Level (BSL)-2 facility. In addition, all analytical procedures and bacterial analyses were conducted in BSL-2 laboratories.

Bacterial Strains and Culture

Feed ingredients, mixed diets, and initial fecal samples were enriched in gram-negative Hajna broth at 37°C for 24 h (BD Bioscience, Franklin Lakes, NJ) before plating onto Brilliant Green Agar (BGA, BD Bioscience) plates for qualitative determination of *Salmonella* spp. *Salmonella enterica* subspecies *enterica* serovar Typhimurium DT104 was obtained from the American Type Culture Collection (ATCC; BAA-185, Manassas, VA). This strain was isolated from a pig in Denmark and was resuscitated in 10 mL of trypticase soy broth (TSB) at 37°C for 24 h and plated onto tryptic soy agar (TSA). A single colony was rendered resistant to the antibiotics nalidixic acid (Nal, Acros Organics, Morris Plains, NJ) and novobiocin (Nov, BD Bioscience) through sequential transfer onto TSA plates of increasing concentrations until achieving a final resistance to 20 and 25 µg/mL, respectively (i.e., *S. Typhimurium* Nal^RNov^R). The choice of a Nal- and Nov-resistant strain was to greatly reduce the possibility of detecting *Salmonella* that were

not introduced in this experiment. The final strain was tested for susceptibility to 5 mg/kg of ceftiofur-HCl (Pfizer Animal Health, New York, NY) to ensure antibiotic efficacy of pig treatments. *Salmonella* Typhimurium Nal^RNov^R was cultured overnight at 37°C in TSB medium on an orbital shaker (New Brunswick Scientific, Edison, NJ) at 150 rpm and bacterial populations were estimated by spectrophotometry at 600 nm. For inocula preparation, *S. Typhimurium* Nal^RNov^R were harvested at $7,500 \times g$ for 10 min at 4°C and resuspended in sterile TSB.

The following cultures were used as positive controls to generate standard curves to determine select phyla and genera within the pig feces using quantitative real-time PCR. *Lactobacillus acidophilus* NCFM strain (ATCC 700396) was grown anaerobically in TSB at 37°C. *Bacteroides thetaiotaomicron* (ATCC 29741) was grown anaerobically in prerduced anaerobically sterilized cooked meat broth (BD Biosciences) at 37°C. *Flavobacterium* spp. was grown aerobically using R2A agar (BD Biosciences) at 25°C.

Animals, Housing, Diets, and Experimental Protocol

Pigs (Premium Genetics 1020, Smithfield, Waverly, VA) obtained from a commercial swine farm (Waverly, VA) were weaned at 21 d of age (7.02 ± 0.27 kg) and used to assess the effect of *Saccharomyces cerevisiae* fermentation product (XPC, Diamond V) inclusion in nursery diets on pig growth performance before, during, and after an oral challenge with *S. Typhimurium* Nal^RNov^R. Twenty pigs in each of 2 trials were used, for a total of 40 pigs (10 pigs per treatment). Pigs were blocked by BW and randomly assigned to treatments within block. Treatments were arranged in a 2×2 factorial arrangement of treatments consisting of diet (control or 0.2% XPC) and inoculation (sterile broth or *Salmonella*). Individual rectal swabs were collected at arrival to the BSL-2 facility to initially screen for the presence of *Salmonella*. All samples were incubated at 37°C for 24 h in gram-negative Hajna broth for enrichment, followed by plating onto BGA to screen for *Salmonella*-indicative colonies. All pigs were initially negative for *Salmonella* presence in feces.

Pigs were housed in individual pens and segregated in 2 identical rooms according to their assigned inocula (sterile broth or *Salmonella*) to minimize the potential for cross-contamination. Inocula conditions were tested in both rooms during the 2 trials of the study. Rooms were discretely ventilated with 100% clean air (i.e., no recirculation) and were under negative pressure at all times, and automated systems controlled the temperature and lighting (18 h light:6 h dark with lights on at 0600 h) of each individual room. Each pen contained a plastic-coated expanded metal floor, a nipple waterer, and a self-feeder. Dietary treatments were imposed immediately upon arrival, and pigs had ad libitum access to water and feed unless otherwise indicated. Pigs were

Table 1. Composition of basal diet, as-fed basis

Item	Phase 1	Phase 2	Phase 3
Ingredient, %			
Ground corn ¹	39.73	58.00	64.41
Soybean meal, 47.5% CP	20.00	28.25	30.40
Soy protein concentrate ²	4.00	—	—
Fish meal	8.00	4.60	—
Dried whey	25.00	5.00	—
Soy oil	2.00	1.80	2.00
Limestone, ground	0.28	0.60	0.80
Dicalcium phosphate	—	0.70	1.13
Salt	0.30	0.30	0.30
Vitamin premix ³	0.25	0.25	0.25
Trace mineral premix ⁴	0.15	0.15	0.15
L-Lys-HCl	0.20	0.25	0.29
L-Thr	0.07	0.10	0.20
DL-Met	0.02	—	0.07
Calculated composition			
ME, kcal/kg	3,420	3,419	3,422
CP, %	23.7	22.0	20.2
Lys, %	1.64	1.47	1.31
Met, %	0.44	0.38	0.38
Thr, %	1.05	0.95	0.95
Trp, %	0.28	0.26	0.24
Arg, %	1.40	1.38	1.30
Ca, %	0.82	0.78	0.68
Available P, %	0.47	0.37	0.28

¹XPC (Original XPC; Diamond V, Cedar Rapids, IA) was added at the expense of corn.

²Soycomil-P, ADM, Decatur, IL.

³Provided the following per kilogram of diet: vitamin A, 7,335 IU; vitamin D₃, 1,010 IU; vitamin E, 42 IU; menadione (as menadione sodium bisulfite complex), 3.3 mg; riboflavin, 6.4 mg; D-pantothenic acid, 21 mg; niacin, 31 mg; vitamin B₁₂, 27.6 µg; D-biotin, 0.3 mg; choline, 550 mg; folic acid, 1.4 mg.

⁴Provided the following per kilogram of diet: Zn, 180 mg as zinc sulfate; Fe, 180 mg as iron sulfate; Mn, 55 mg as manganese sulfate; Cu, 11 mg as copper sulfate; I, 0.5 mg as calcium iodate; and Se, 0.3 mg as sodium selenite.

fed a 3-phase nursery diet (Table 1; phase 1, 0 to 7 d; phase 2, 7 to 21 d; phase 3, 21 to 35 d postweaning). Pigs were fed control or XPC diets for 2 wk before oral inoculation with *S. Typhimurium* Nal^RNov^R and continued on their respective diets after inoculation until 35 d postweaning.

A corn-soybean meal basal diet was formulated to meet or exceed NRC (1998) recommendations for nutrients and contained no antibiotics. From the basal diet, the experimental diet was produced by displacing 0.2% of the corn with XPC. Before feeding, all feed ingredients and mixed diets were screened for the presence of *Salmonella* and were negative.

The experimental protocol was designed to simulate an enteric disease outbreak and treatment in a nursery facility after weaning. Thus, pigs were weaned, inoculated, allowed to develop clinical signs of disease, treated with antibiotics, and allowed to recover. The experiment consisted of 3 periods: preinoculation (**PRE**; 0 to 14 d), sick (**SCK**; 14 to 21 d), and postinoculation (**POST**; 21 to 35 d). Pigs and feeders were weighed every 7 d to determine ADG, ADFI, and G:F. Rectal temperatures (**RT**) were measured weekly during PRE and POST, and every 12 h during SCK. On d 14, con-

Table 2. Primer sets used during quantitative real-time PCR to determine abundance of phyla *Bacteroidetes* and *Firmicutes* and genera *Bacterioides* and *Lactobacillus* in pig feces

Phyla or genera of interest	Primer sequence	Species used to generate standard curves	Annealing temperature, °C
<i>Bacterioides</i>	Forward: AllBac296F, 5'-GAGAGGAAGGTCCCCAC-3' Reverse: AllBac412R, 5'-CGCTACTTGGCTGGTTTCAG-3'	<i>Bacterioides thetaiotaomicron</i>	60.0
<i>Bacteroidetes</i>	Forward: Bact934F, 5'-GGARCATGTGGTTTAATTCGATGAT-3' Reverse: Bact1060R, 5'-AGCTGACGACAAACCATGCAG-3'	<i>Flavobacterium</i> spp.	60.0
<i>Firmicutes</i>	Forward: Lgc353F, 5'-GCAGTGGG AAT CTT CCG-3' Reverse: 5'-EUB518R, 5'-ATTACCGCGGCTGCTGG-3'	<i>Lactobacillus acidophilus</i>	60.0
<i>Lactobacillus</i>	Forward: 5'-AGAGGTAGTAACCTGCCTTA-3' Reverse: 5'-GCGGAAACCTCCCAACA-3'	<i>L. acidophilus</i>	58.5

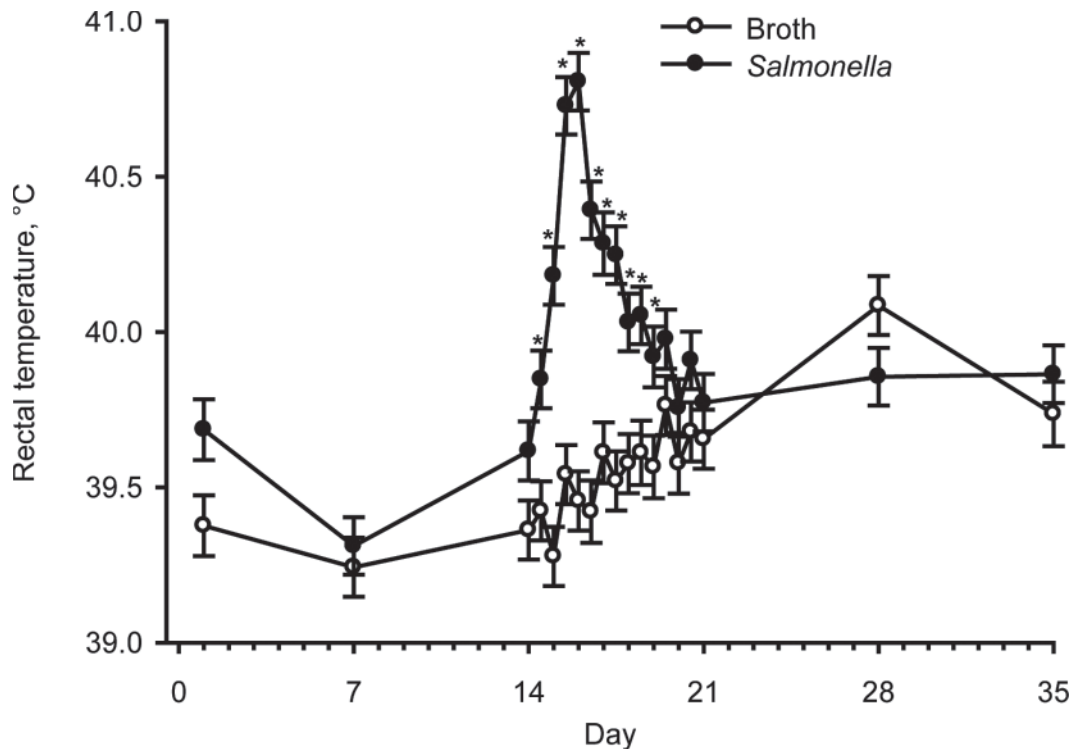


Figure 1. Effect of infection with *Salmonella* on rectal temperature. Pigs were inoculated with 5 mL containing 10^9 cfu of *Salmonella* Typhimurium resistant to the antibiotics nalidixic acid and novobiocin (*Salmonella*) on d 14 after weaning (d = 1) or received 5 mL of sterile broth (Broth). Pigs were treated daily with 5 mg/kg of BW intramuscular injection of ceftiofur-HCl on d 17 to 20. Values are means \pm SEM (n = 19 to 20). *Means differ from broth within day, $P < 0.01$.

scious pigs were given orally 5 mL of TSB containing 10^9 cfu of *S. Typhimurium* Nal^RNov^R or 5 mL of sterile TSB.

Daily rectal swabs were collected after inoculation (d 14 to 21) to determine fecal shedding of *S. Typhimurium* Nal^RNov^R. From d 17, all pigs were treated daily with 5 mg/kg of BW intramuscular (i.m.) injection of ceftiofur-HCl for 4 d. On d 35, pigs were killed with a lethal dose of 120 mg/kg of BW of sodium pentobarbital (Beuthanasia-D, Schering-Plough, Union, NJ) administered intravenously. Carcasses were disposed as regulated medical waste in accordance to university, local, state, and federal regulations.

Enumeration of *S. Typhimurium* Nal^RNov^R

Between 0800 and 1000 h, about 10 g of feces was collected from each pig daily during d 14 to 21 using a sterile fecal loop; contents were placed in a sterile filter bag and immediately processed with 90 mL of buffered peptone water (BD Biosciences) in a stomacher for 2 min to create a fecal slurry. The fecal slurry was then serially diluted and plated, in duplicate, onto BGA plates containing 20 μ g/mL of nalidixic acid and 25 μ g/mL of novobiocin. The plates were allowed to air dry and then incubated at 37°C for 24 h. Plates were then inspected for white colonies with red-pink halos, indicative of *Salmonella*. Initial presumptive positive plates were confirmed on xylose lysine tergitol 4 agar; the presence of black round colonies was indicative of *S. Typhimurium*

Nal^RNov^R. The total number of *Salmonella* colonies on each plate was quantified to determine daily shedding rates for each pig. Fecal slurries were then stored at -20°C until fecal DNA could be extracted.

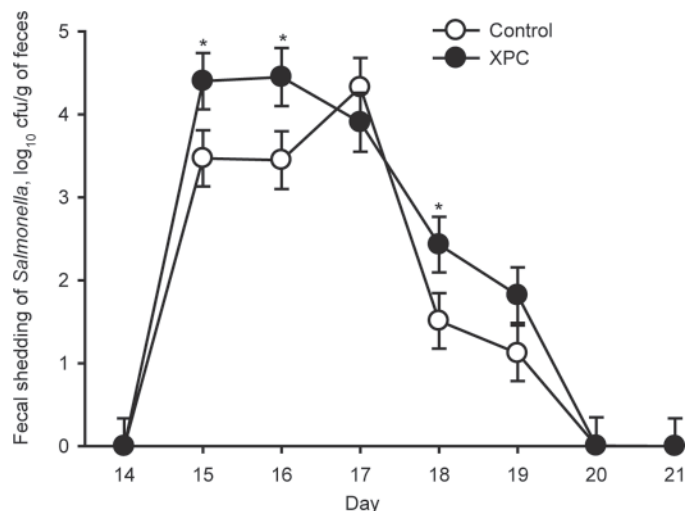


Figure 2. Effect of infection with *Salmonella* and consumption of XPC (Original XPC, Diamond V, Cedar Rapids, IA) on fecal shedding of *Salmonella*. From weaning (d = 1), pigs had ad libitum access to a nursery diet with (XPC) or without (control) 0.2% XPC inclusion. Pigs were inoculated with 5 mL containing 10^9 cfu of *Salmonella* Typhimurium resistant to the antibiotics nalidixic acid and novobiocin (*Salmonella*) on d 14 after weaning or received 5 mL of sterile broth. Pigs were treated daily with 5 mg/kg of BW intramuscular injection of ceftiofur-HCl on d 17 to 20. Values are means \pm SEM (n = 9 to 10). *Means differ from control within day, $P < 0.05$.

Table 3. Least squares means for growth performance and rectal temperature of pigs consuming postweaning diets without (control) or with (XPC¹) *Saccharomyces cerevisiae* fermentation product and orally gavaged with sterile broth or *Salmonella*²

Item	Broth		<i>Salmonella</i> ²		SEM	<i>P</i> -value		
	Control	0.2% XPC	Control	0.2% XPC		Inoc. ³	Diet	Inoc. × diet
Preinoculation ⁴								
ADG, kg	0.087	0.104	—	—	0.014	—	0.42	—
ADFI, kg	0.394	0.295	—	—	0.037	—	0.060	—
G:F	0.393	0.589	—	—	0.242	—	0.57	—
BW, kg	7.48	7.60	—	—	0.13	—	0.49	—
Rectal temperature, °C	39.34	39.45	—	—	0.05	—	0.15	—
Sick ⁵								
ADG, kg	0.478	0.485	0.285	0.272	0.042	0.001	0.95	0.82
ADFI, kg	0.939	0.826	0.668	0.641	0.045	0.001	0.12	0.34
G:F	0.554	0.590	0.233	0.529	0.106	0.073	0.12	0.22
BW, kg	9.79	10.04	8.66	8.94	0.19	0.001	0.16	0.92
Rectal temperature, °C	39.49	39.60	40.15	40.15	0.04	0.001	0.19	0.20
Postsick ⁶								
ADG, kg	0.603	0.476	0.559	0.592	0.030	0.23	0.12	0.009
ADFI, kg	1.078	1.108	0.879	0.938	0.047	0.001	0.34	0.76
G:F	0.599	0.466	0.715	0.734	0.058	0.001	0.33	0.19
BW, kg	15.88	15.78	13.97	14.26	0.38	0.001	0.80	0.62
Rectal temperature, °C	39.73	39.85	39.92	39.73	0.07	0.60	0.55	0.020
Overall								
ADG, kg	0.441	0.422	0.316	0.323	0.028	0.001	0.82	0.63
ADFI, kg	0.898	0.816	0.657	0.651	0.031	0.001	0.16	0.23
G:F	0.577	0.545	0.344	0.614	0.091	0.37	0.19	0.099
BW, kg	10.77	10.86	9.65	9.94	0.19	0.001	0.31	0.61
Rectal temperature, °C	39.49	39.60	40.01	39.98	0.03	0.001	0.23	0.067

¹XPC: Original XPC (Diamond V, Cedar Rapids, IA).

²*Salmonella* Typhimurium resistant to the antibiotics nalidixic acid (Nal^R) and novobiocin (Nov^R).

³Inoc., inoculation with sterile broth (Broth) or *S. Typhimurium* Nal^RNov^R.

⁴From weaning to before pigs were orally gavaged with sterile broth or 10⁹ cfu *S. Typhimurium* Nal^RNov^R on d 14 postweaning.

⁵d 14 to 21 postweaning. All pigs were treated with 5 mg/kg of BW intramuscular injection of ceftiofur-HCl on d 17 to 20.

⁶d 21 to 35 postweaning.

Intestinal Morphology

Upon euthanasia, intestinal samples were collected for morphology. A sample from the duodenum, jejunum, and ileum (2 to 3 cm in length) was placed in 15-mL plastic conical tubes containing 10 mL of phosphate-buffered formalin (Fisher Scientific, Fairlawn, NJ). Tissue sections of duodenum (about 20 cm caudal of gastroduodenal junction), jejunum (about 50% of intestinal length), and ileum (about 20 cm cranial of ileocecal junction) were sent to a commercial histology laboratory (HISTO-Scientific Research Laboratories, Mt. Jackson, VA) for microscope slide preparation and staining (Zhao et al., 2007). Three random cuts (5 μ m each) from each tissue section were mounted on microscope slides and stained with Alcian blue and Periodic acid-Schiff to identify goblet cells (Dunsford et al., 1990). One evaluator per intestinal section was used to obtain morphological data. Evaluators randomly reviewed slides without knowledge of treatments. For each segment sample, 4 different readings per cut in each of 3 cuts per microscope slide (i.e., 12 readings per tissue section) were collected. The following endpoints were measured: villus height (μ m), villus width (μ m), crypt depth (μ m), number of goblet cells in the villus

perimeter, and number of goblet cells in each crypt. Villus perimeter (VP) was calculated as follows: VP = ht \times 2 + w, where ht is villus height and w is villus width. A modified cylinder area equation was used to calculate villus area (VA) as follows: VA = [$\pi \times (w \div 2)^2$] + ($\pi \times w$) \times ht, where w is width of villus and ht is the height of villus. Data from the 3 tissue cuts per tissue section were averaged to create a single value for each of the described endpoints.

Community Profiling

Product Amplification. Fecal DNA was extracted (UltraClean Fecal DNA kit, Mo Bio Laboratories, Carlsbad, CA) per the manufacturer's instructions. The 16S rRNA gene was amplified from the total fecal microflora DNA (50 ng/ μ L) to generate a 566-bp fragment using the primers 341-f (5'-CCT ACG GGA GGC AGC AG-3') and 907r (5'-CCG TCA ATT CMT TTG AGT TT-3'). The forward primer was modified to add a 40-nucleotide guanine/cytosine clamp at the 5' end (5'-CGC CCG CCG CGC GCG GCG GGC GGG GCG GGG GCA CGG GGG G-3'). Each 25- μ L reaction contained 1.5 mM of MgCl₂, 50 mM of KCl, 0.2 mM of each dinucleotide, 1% of dimethylsulfoxide, 25

Table 4. Least squares means for small intestinal morphology of pigs consuming postweaning diets without (control) or with (XPC¹) *Saccharomyces cerevisiae* fermentation product and orally gavaged with sterile broth or *Salmonella*²

Item	Broth		<i>Salmonella</i> ²			<i>P</i> -value		
	Control	0.2% XPC	Control	0.2% XPC	SEM	Inoc. ³	Diet	Inoc. × diet
Duodenum								
Villus height, μm	539.0	616.7	609.6	539.1	23.3	0.88	0.88	0.003
Crypt depth, μm	374.6	355.9	369.5	356.7	15.1	0.89	0.30	0.85
Villus/crypt ratio	1.47	1.76	1.68	1.63	0.10	0.71	0.26	0.098
Villus width, μm	148.0	140.6	134.1	139.7	4.5	0.11	0.84	0.16
No. of goblet cells/villus	24.9	29.5	24.3	22.9	2.4	0.14	0.52	0.23
Villus goblet, No./μm	0.021	0.020	0.017	0.018	0.001	0.096	0.95	0.63
No. of goblet cells/crypt	20.3	20.6	18.7	17.3	1.2	0.044	0.64	0.46
Crypt goblet, No./μm	0.055	0.058	0.051	0.049	0.003	0.065	0.95	0.44
Villus area, mm ²	0.267	0.288	0.286	0.251	0.015	0.57	0.63	0.062
Jejunum								
Villus height, μm	478.9	456.7	471.6	499.0	16.5	0.30	0.88	0.14
Crypt depth, μm	304.8	300.8	294.1	321.9	10.3	0.62	0.26	0.13
Villus/crypt ratio	1.59	1.58	1.61	1.57	0.08	0.98	0.80	0.88
Villus width, μm	136.4	130.5	125.8	132.0	2.8	0.12	0.96	0.040
No. of goblet cells/villus	20.3	21.6	21.6	19.0	2.2	0.77	0.78	0.39
Villus goblet, No./μm	0.019	0.020	0.018	0.018	0.001	0.26	0.53	0.61
No. of goblet cells/crypt	21.6	23.3	22.9	24.6	1.4	0.35	0.21	1.00
Crypt goblet, No./μm	0.075	0.078	0.072	0.072	0.004	0.32	0.68	0.80
Villus area, mm ²	0.219	0.200	0.203	0.221	0.009	0.79	0.96	0.056
Ileum								
Villus height, μm	421.6	421.2	466.4	426.1	17.5	0.16	0.24	0.25
Crypt depth, μm	305.0	277.8	295.3	286.0	9.4	0.94	0.062	0.35
Villus/crypt ratio	1.43	1.55	1.58	1.47	0.07	0.66	1.00	0.12
Villus width, μm	140.9	136.3	132.7	128.9	2.9	0.012	0.16	0.89
No. of goblet cells/villus	24.1	22.7	26.6	21.5	2.0	0.75	0.11	0.35
Villus goblet, No./μm	0.025	0.023	0.024	0.022	0.002	0.76	0.23	0.81
No. of goblet cells/crypt	23.1	19.9	21.8	22.0	1.4	0.79	0.28	0.23
Crypt goblet, No./μm	0.076	0.071	0.077	0.075	0.004	0.53	0.36	0.81
Villus area, mm ²	0.198	0.196	0.208	0.185	0.007	0.98	0.10	0.16

¹XPC: Original XPC (Diamond V, Cedar Rapids, IA).²*Salmonella* Typhimurium resistant to the antibiotics nalidixic acid (Nal^R) and novobiocin (Nov^R).³Inoc., inoculation with sterile broth (Broth) or *S. Typhimurium* Nal^RNov^R.

mM of Tris-HCl (pH 8), 1 U/ μL of HotStart-IT Fidelity Taq DNA polymerase (USB, Cleveland, OH), 0.5 μM of each primer, and 50 ng of DNA. The PCR protocol consisted of 94°C for 5 min, followed by 19 cycles of 94°C for 1 min, amplification at 64°C for 1 min (decreasing 1°C every second cycle), and elongation at 72°C for 3 min; followed by 9 additional cycles of denaturation at 94°C for 1 min, amplification at 55°C for 1 min, and elongation at 72°C for 3 min; and, finally, 1 cycle of 94°C for 1 min, amplification at 55°C for 1 min, and a final elongation step at 72°C for 10 min. The size and intensity of PCR products were electrophoretically confirmed using 0.9% agarose gels (Fisher-Scientific, Atlanta, GA).

Denaturing Gradient Gel Electrophoresis Conditions. The PCR products were run on a 8% polyacrylamide gel in a 30 to 60% denaturant gradient of urea and formamide [100% denaturant corresponds to 7 M urea plus 40% (vol/vol) of deionized formamide; DCode Universal Detection System, Bio-Rad, Hercules, CA]. Twenty-two microliters of PCR products was separated at constant voltage of 85 V and temperature

of 60°C for 17 h. The DNA bands were visualized by staining with ethidium bromide (5 $\mu\text{g}/\text{mL}$) and photographed (Molecular Imager GelDoc XR, Bio-Rad). Two different gels were analyzed for each sample, and species richness was determined by the number of bands present within a sample.

Quantification of Select Gastrointestinal Members. Real-time PCR was done on fecal DNA extracted from pigs in both diets on d 14, 15, 17, 18, and 35 (i.e., d 0, 1, 3, 4, and 21 postinfection) to examine the abundance of total bacteria, specific phyla (*Bacteroidetes* and *Firmicutes*), and genera (*Bacteroides* and *Lactobacillus*). Standard curves were produced from DNA isolated using the Puregene DNA purification kit (GENTRA Systems, Minneapolis, MN) per the manufacturer's instructions. Serial dilutions of the DNA were made to create 10-fold serial dilutions from 100 to 0.1 ng/ μL . Standard curves using real-time PCR amplification were prepared with specific primers (Table 2): 100, 10, 1, and 0.1 ng. Each 25- μL reaction contained a respective amount of DNA template, 12.5 μL of HotSart-IT SYBR Green qPCR Master Mix 2 ×

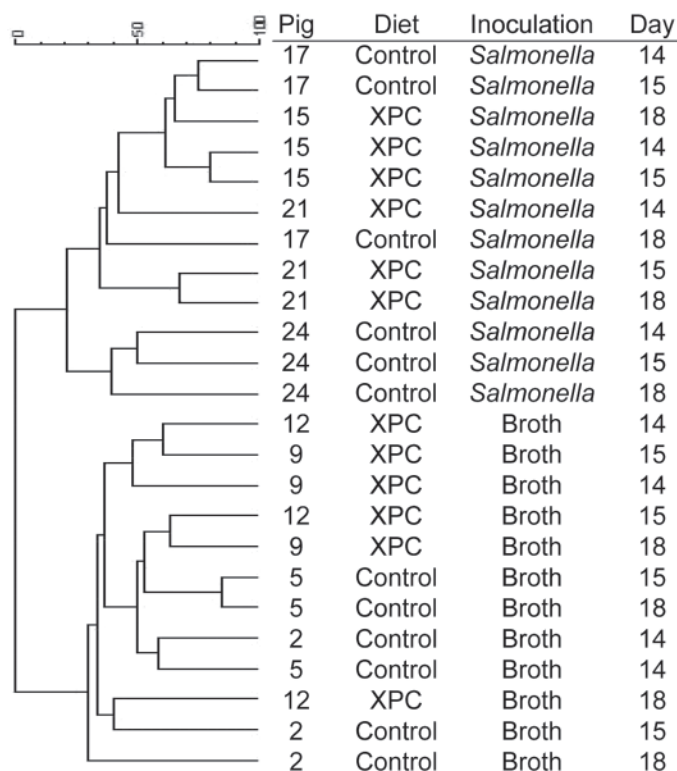


Figure 3. Effect of infection with *Salmonella* and consumption of XPC (Original XPC, Diamond V, Cedar Rapids, IA) on fecal species richness determined by denaturing gradient gel electrophoresis. From weaning (d = 1) pigs had ad libitum access to a nursery diet with (XPC) or without (control) 0.2% XPC inclusion. Pigs were inoculated with 5 mL containing 10^9 cfu *Salmonella* Typhimurium resistant to the antibiotics nalidixic acid and novobiocin (*Salmonella*) on d 14 after weaning or received 5 mL of sterile broth (Broth).

(USB), which contains 5 mM $MgCl_2$, and 0.4 mM of nucleotides, 10 nM of fluorescein as passive reference dye (USB), and 0.5 μM of forward and reverse primers (Table 2). The PCR conditions were denaturation at 95°C for 2 min, followed by 40 cycles of denaturation at 95°C for 30 s, annealing for 30 s at appropriate temperature (Table 2), and elongation at 72°C for 1 min. Each concentration in the standard curve was done in triplicate using separate plates. Melting curve analysis of the PCR products was conducted after each assay to confirm that the fluorescence signal was originated from specific PCR product. Amplification was carried out (iQ Optical system Real Time PCR detection system; Bio-Rad).

Statistical Analysis

Growth performance and RT data were analyzed with the PROC MIXED procedure (SAS Inst. Inc., Cary, NC) as a complete randomized block design in a 2 × 2 factorial with repeated measurements, and replicate was used as a random effect (Kaps and Lamberson, 2004). The model included ADG, ADFI, BW, and RT across the 3 periods (PRE, SCK, and POST). Fecal analysis of *Salmonella* shedding, number of copies of bacterial species (both \log_{10}) per gram of feces was also

determined with PROC MIXED with replicate as a random effect and using repeated measures. Treatment effects were assessed by least squares means obtained using the Tukey adjustment. The denaturing gradient gel electrophoresis (DGGE) bands were visualized (Quantity One-1D analysis software, Bio-Rad), and the DGGE profiles were clustered based on similarity using the unweighted pair group method with mathematical averages (UPGMA; Dice coefficient of similarity) using GelCompar II (Applied Maths, Austin, TX) and reported as dendrograms. Significance was declared at $P \leq 0.05$ and tendency at $P < 0.10$.

RESULTS

RT and *Salmonella* Shedding

All pigs were negative for *Salmonella* presence in feces before inoculation. Furthermore, pigs that received sterile broth never shed *Salmonella* or developed a febrile response. Inclusion of XPC in the diet had no effect on RT ($P = 0.57$). Experimental infection with *Salmonella* resulted in a marked increase ($P < 0.001$) in RT during SCK (Figure 1). A linear reduction in RT of *Salmonella*-infected pigs was observed from d 17 (start of i.m. ceftiofur-HCl) to the end of antibiotic treatment on d 21. Inclusion of XPC in the diet tended ($P < 0.10$) to increase *Salmonella* shedding in feces (Figure 2).

Growth Performance

Inclusion of XPC had no effect on ADG, G:F ratio, BW, or RT during the PRE period (Table 3). During this period, however, pigs consuming XPC tended ($P = 0.06$) to reduce ADFI, which resulted in numerical improvement (50%) in G:F. Inoculation with *Salmonella* drastically reduced ($P < 0.001$) ADG, ADFI, and BW of pigs compared with noninoculated pigs. The G:F tended ($P = 0.07$) to be less in *Salmonella*-infected pigs mainly because of a numeric reduction in pigs consuming the control diet. During the recovery period (i.e., POST), there was a diet × inoculation interaction ($P = 0.02$), indicating that infected pigs consuming XPC gained more BW and had decreased RT compared with infected pigs consuming the control diet. Overall, interaction trends ($P < 0.10$) were detected for G:F and RT. Growth performance was modestly correlated ($P = 0.002$) with fecal shedding of *Salmonella* during SCK: BW ($r = -0.22$), ADFI ($r = -0.27$), ADG ($r = -0.36$), and G:F ($r = -0.18$).

Intestinal Morphology

These measurements were taken 2 wk after pigs stopped shedding in feces and had clinically recovered from *Salmonella* infection. Pigs receiving the *Salmonella* inoculation had a reduced ($P < 0.05$) number of goblet cells present in the duodenal crypt (Table 4). A diet × inoculation interaction ($P < 0.01$) for villi

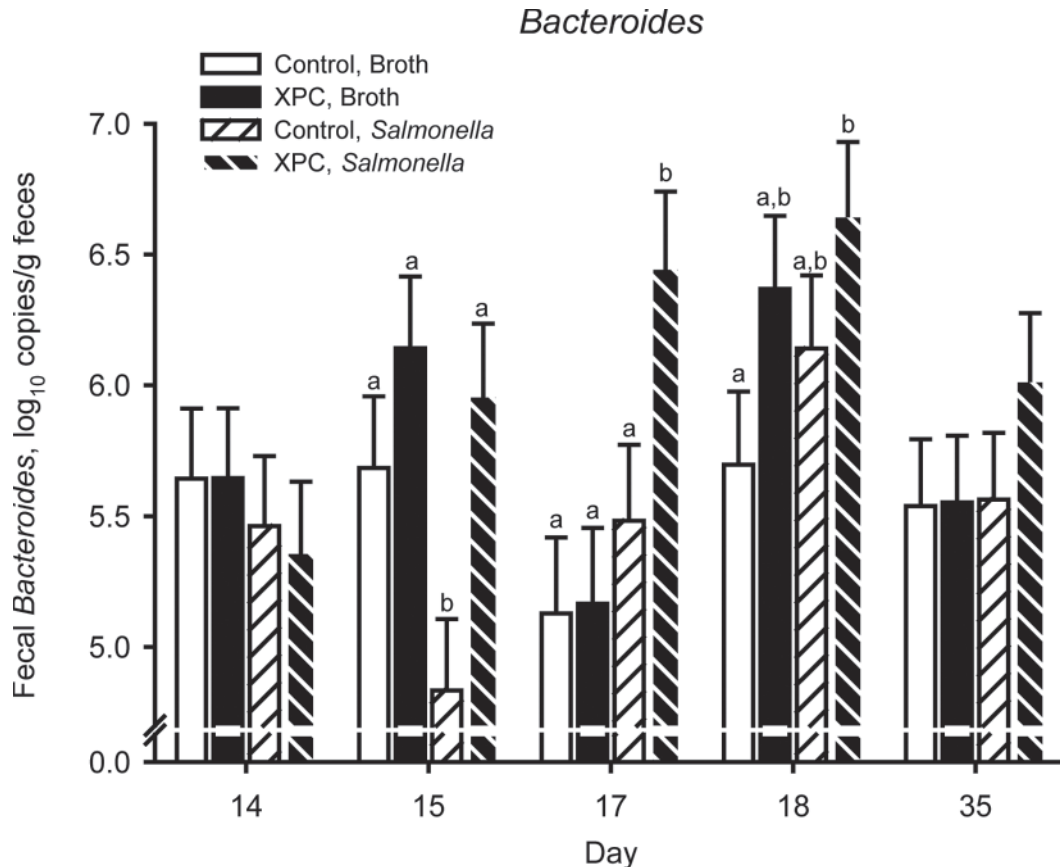


Figure 4. Effect of infection with *Salmonella* and consumption of XPC (Original XPC, Diamond V, Cedar Rapids, IA) on the number of *Bacteroides* copies (log₁₀) determined by real-time-PCR from fecal samples. From weaning (d = 1) pigs had ad libitum access to a nursery diet with (XPC) or without (control) 0.2% XPC inclusion. Pigs were inoculated with 5 mL containing 10⁹ cfu of *Salmonella* Typhimurium resistant to the antibiotics nalidixic acid and novobiocin (*Salmonella*) on d 14 after weaning or received 5 mL of sterile broth (Broth). Values are means \pm SEM (n = 9 to 10). ^{a,b}Means without a common letter within day differ, $P < 0.05$.

height indicates that XPC increased villus height in noninfected pigs but had the opposite effect on *Salmonella*-challenged pigs. Diet \times inoculation interaction tendencies ($P < 0.10$) for duodenal villus/crypt ratio and villus area indicated improved intestinal morphology in healthy pigs consuming XPC compared with controls. In the jejunum, however, a diet \times inoculation interaction ($P = 0.04$) indicated a greater villus width in *Salmonella*-infected pigs consuming XPC compared with noninfected pigs receiving dietary XPC. A tendency ($P < 0.06$) for a similar beneficial effect of XPC was quantified for villus area in the jejunum. In the ileum, the main site of *Salmonella* infection, XPC tended to reduce ($P = 0.07$) crypt depth. Inoculation of pigs with *Salmonella* resulted in a significant reduction ($P = 0.02$) in villus width in the ileum.

Denaturing Gradient Gel Electrophoresis

The overall total species richness, determined by the number of DGGE bands, was not different between the pigs fed a control diet and those fed XPC. However, shifts in the community composition and apparent abundance, as indicated by band position and intensity, were observed. Several members, indicated by band pattern, are present within both diets, representing sta-

ble community members. The number of bands in the DGGE profiles varied between 16 and 20 bands for fecal samples (results not shown). The similarity indices between individual animals consuming the same diet ranged from 65 to 70%. Infection of the pigs with *Salmonella* resulted in a shift in composition of the fecal community in samples 1 d postinfection. The DGGE profiles of feces from infected vs. noninfected pigs clustered individually, where infection status indicates approximately 30 to 40% similarity (Figure 3). In pigs challenged with *Salmonella* Nal^RNov^R, the groupings of the individual pigs were more similar, with d 14 and 15 being clustered together, yet distinctly different from d 18, with the exception of pig 21 (Figure 3). Marked shifts in the species richness of all pigs were seen at d 18, which corresponded to the day after administration of the antibiotic, ceftiofur-HCl.

Bacterial Populations

Over the entire trial, inclusion of XPC in the diet increased ($P < 0.001$) the number of copies in feces of *Bacteroides* by 2.6-fold (Figure 4) and *Lactobacillus* spp. by 3.5-fold (Figure 5), and reduced *Firmicutes* by 50% (Figure 6). In addition, a diet \times inoculation ($P < 0.001$) interaction resulted in a 2.8-fold increase ($P <$

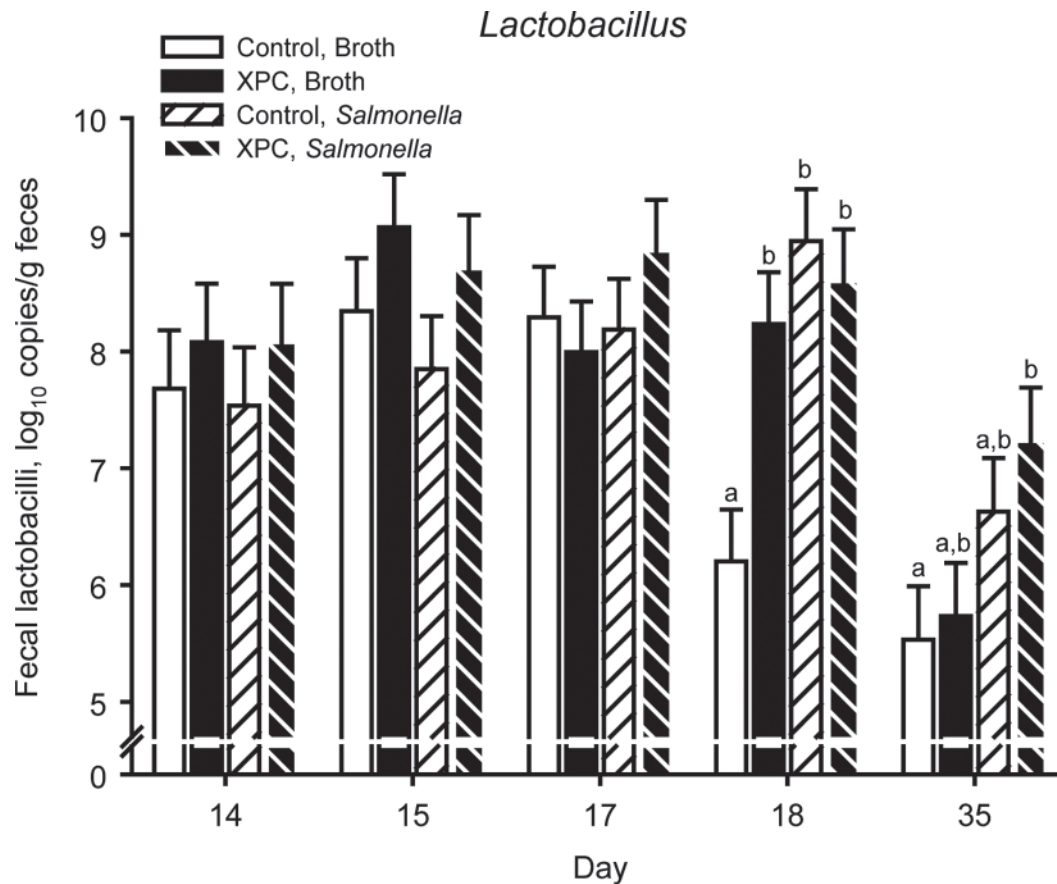


Figure 5. Effect of infection with *Salmonella* and consumption of XPC (Original XPC, Diamond V, Cedar Rapids, IA) on the number of *Lactobacillus* copies (log₁₀) determined by real-time-PCR from fecal samples. From weaning (d = 1) pigs had ad libitum access to a nursery diet with (XPC) or without (control) 0.2% XPC inclusion. Pigs were inoculated with 5 mL containing 10⁹ cfu of *Salmonella* Typhimurium resistant to the antibiotics nalidixic acid and novobiocin (*Salmonella*) on d 14 after weaning or received 5 mL of sterile broth (Broth). Values are means \pm SEM (n = 9 to 10). ^{a,b}Means without a common letter within day differ, $P < 0.05$.

0.01) in *Bacteroidetes* copies in *Salmonella*-infected pigs consuming XPC (Figure 7). During SCK, consumption of XPC increased ($P < 0.01$) the populations of *Bacteroides*, *Bacteroidetes*, and *Lactobacillus*, which are all considered beneficial bacteria. During POST, a diet \times inoculation tended ($P = 0.07$) to increase total bacterial (Figure 8) copies in the feces of *Salmonella*-infected pigs consuming XPC compared with infected animals eating the control diet.

DISCUSSION

Prebiotic compounds offer an attractive alternative to the use of AGP. Growth promotion associated with prebiotics is believed to result from enhanced energy gained by the fermentation of these compounds within the lower GIT, allowing the host animal to generate muscle mass and effectively producing a desirable market weight (Branner and Roth-Maier, 2006). Other health benefits, such as stimulation of intestinal motility, mineral absorption, elimination of ammonium, direct stimulation of the immune system, and the inhibition of toxin binding, are associated with host/prebiotic synergy (Macfarlane et al., 2008). However, the greatest protection against pathogenic bacterial infections are

achieved by stimulating GIT bacteria to produce short-chain fatty acids that are inhibitory to some pathogens and increase in quantity, therefore reducing attachment sites for pathogens on the GIT mucosa (Niba et al., 2009).

Prebiotics act by stimulating diverse communities of microorganisms to colonize the GIT. Culture-independent analyses reveal that inclusion of prebiotic compounds considerably alters the abundance of certain members of the fecal microbial flora but do not change the overall species richness (Santos et al., 2006; Mountzouris et al., 2006). Prebiotic compounds such as galactooligosaccharides have been previously shown to increase the composition of beneficial bacteria, such as *Bifidobacterium* and *Lactobacillus*, in the colon of humans and mice (Tzortzis et al., 2005). Few studies have characterized changes to microbial communities in swine fed prebiotic diets. In this study, we showed that the inclusion of a commercial dietary supplement, XPC, containing nutritional metabolites, mannanoligosaccharides, and β -glucans produced during the anaerobic fermentation of *Saccharomyces cerevisiae* results in an increased number of copies for *Bacteroides* and *Lactobacillus* present in the feces of pigs compared with controls. Increased amounts of β -glucans have been

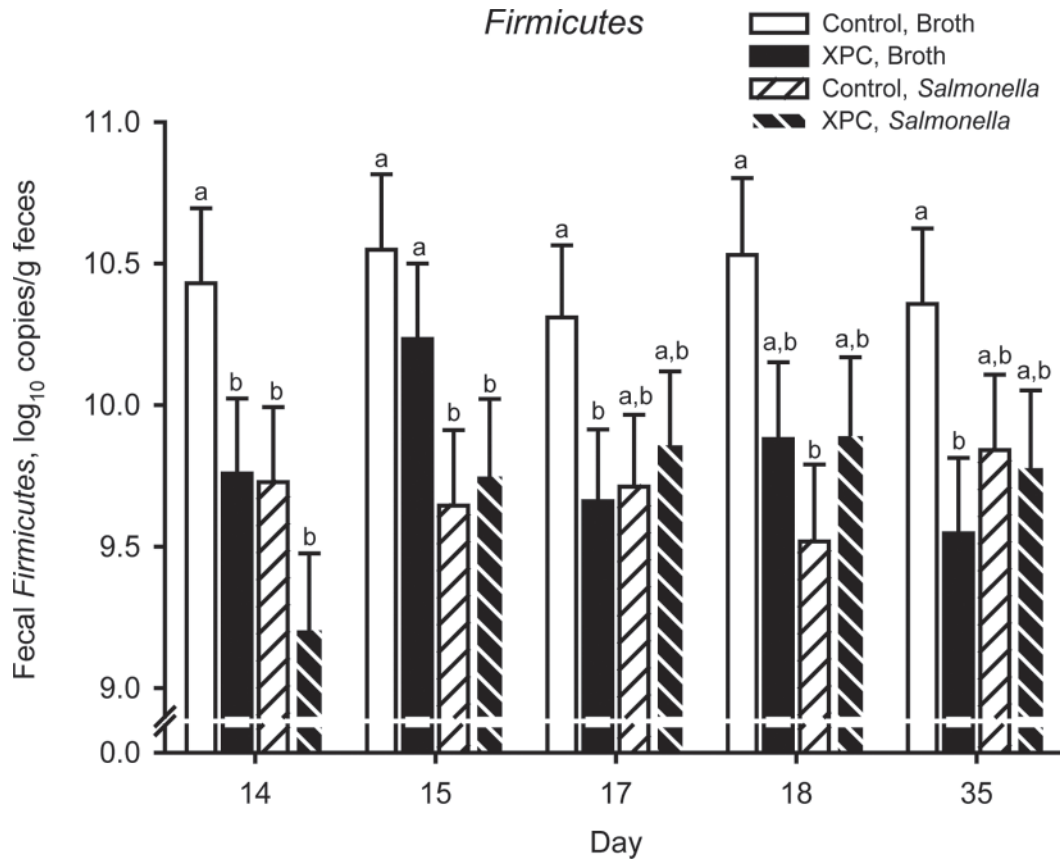


Figure 6. Effect of infection with *Salmonella* and consumption of XPC (Original XPC, Diamond V, Cedar Rapids, IA) on the number of *Firmicutes* copies (log₁₀) determined by real-time-PCR from fecal samples. From weaning (d = 1) pigs had ad libitum access to a nursery diet with (XPC) or without (control) 0.2% XPC inclusion. Pigs were inoculated with 5 mL containing 10⁹ cfu *Salmonella* Typhimurium resistant to the antibiotics nalidixic acid and novobiocin (*Salmonella*) on d 14 after weaning or received 5 mL of sterile broth (Broth). Values are means ± SEM (n = 9 to 10). ^{a,b}Means without a common letter within day differ, *P* < 0.05.

shown to increase digesta retention time in the small intestine, affecting the digestibility of other nutrients, particularly protein and starch (Leterme et al., 2000). Inclusion of mixed-linked β -glucans in the diet of rats (Snart et al., 2006) and pigs (Pieper et al., 2008) corresponded with increased populations of *Lactobacillus* (Jonsson and Hemmingsson, 1991), which agrees with the findings of this study.

Diverse communities of microorganisms colonize the swine GIT. Fecal samples commonly contain about 10⁹ cfu/g of culturable bacteria (Moore et al., 1987), whereas molecular tests based on amplification of the 16S rDNA report about 10¹¹ copies/g of feces (Guo et al., 2008b) that reside primarily within the small and large intestines (Dowd et al., 2008). Thirteen major phylogenetic lineages of bacteria are present within the swine GIT; however, the majority of these bacteria belong to just 2 lineages: *Firmicutes* and *Bacteroidetes* (Leser et al., 2002). The majority of swine fecal microbiota (70%) is dominated by members of the *Firmicutes* (including *Clostridium* spp., *Lactobacillus* spp., and *Streptococcus* spp.), whereas *Bacteroidetes* averaged about 9% of the total microbiota (Dowd et al., 2008; Guo et al., 2008b). The ability to stimulate certain members of these populations is associated with increased BW gain. In mice, increased numbers of *Firmicutes* have been positively

associated with increased obesity (Ley et al., 2005). In contrast, increased fecal populations of *Bacteroides* and *Bacteroidetes* have been negatively correlated with obesity in humans and mice (Ley et al., 2005; Turnbaugh et al., 2009) and with backfat thickness in pigs (Guo et al., 2008a). By stimulating select members of this microbiota, it may be possible to improve the recovery of sick animals, while maintaining adequate lean growth. The increase in beneficial *Bacteroides* and *Lactobacillus* spp., with an overall reduction in *Firmicutes* members, may be responsible for the improved growth performance of *Salmonella*-infected pigs consuming XPC compared with those consuming the control diet.

Increased abundance of efficient fermenters such as *Lactobacillus* could result in increased growth performance in the postillness period. Isolates of *Lactobacillus* have been able to inhibit the invasion of tissue culture cells by the enteric pathogens *Salmonella enterica* and *Escherichia coli* O157:H7 (Casey et al., 2004; Silva et al., 2004). Cultures of *Bifidobacter lactis* and *Lactobacillus rhamnosus* decrease the adherence of *Salmonella*, *Escherichia coli*, and *Clostridium* to intestinal mucosa (Collado et al., 2007). Weanling pigs supplemented with a mixture of probiotic bacteria (3 species of *Lactobacillus* and *Pediococcus* spp.) and subsequently challenged with *Salmonella* Typhimurium showed re-

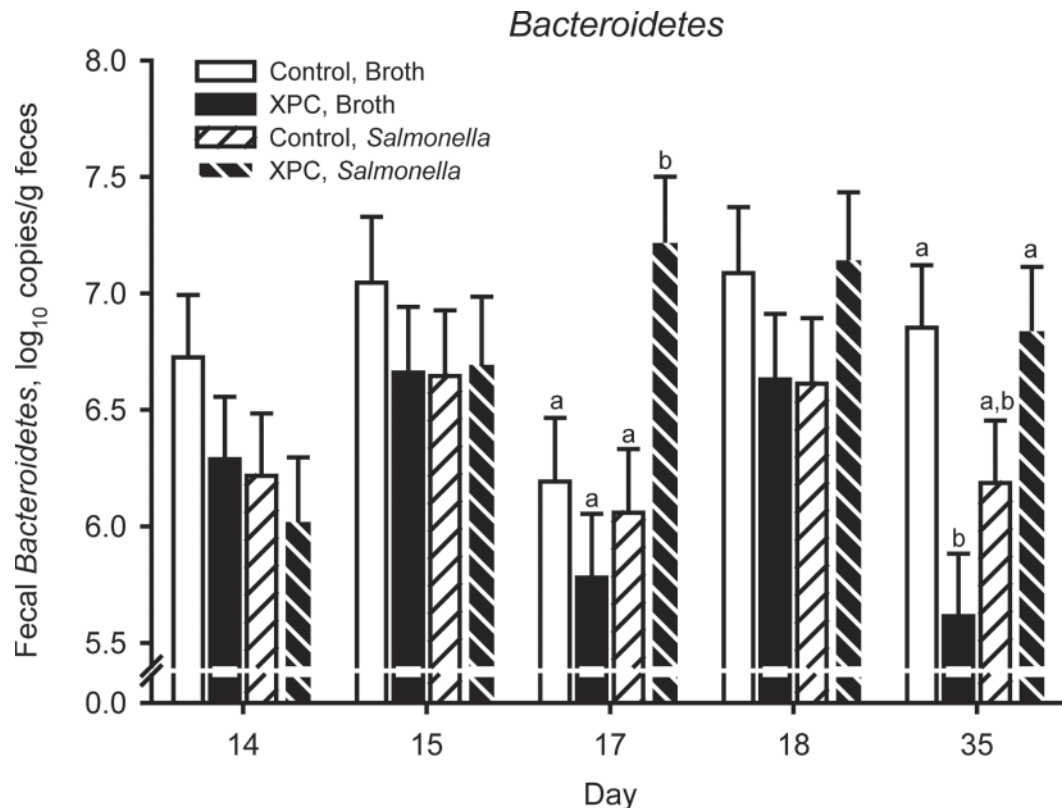


Figure 7. Effect of infection with *Salmonella* and consumption of XPC (Original XPC, Diamond V, Cedar Rapids, IA) on the number of *Bacteroidetes* copies (log₁₀) determined by real-time-PCR from fecal samples. From weaning (d = 1) pigs had ad libitum access to a nursery diet with (XPC) or without (control) 0.2% XPC inclusion. Pigs were inoculated with 5 mL containing 10⁹ cfu of *Salmonella* Typhimurium resistant to the antibiotics nalidixic acid and novobiocin (*Salmonella*) on d 14 after weaning or received 5 mL of sterile broth (Broth). Values are means \pm SEM (n = 9 to 10). ^{a,b}Means without a common letter within day differ, $P < 0.05$.

duced incidence and duration of diarrhea and shedding of *Salmonella* (Casey et al., 2007). It is possible that XPC itself possesses antimicrobial activity. The prebiotic compound used in the present study consists of metabolites of *Saccharomyces cerevisiae* fermentation, and the soluble metabolites were associated with growth inhibition of *Candida tropicalis* and *Escherichia coli* in vitro, likely because of competition for nutrients (Jensen et al., 2008). Soluble compounds may also increase the composition of other members of the microbial community not considered in this study. Microbial communities of fecal samples seem to be affected by the presence of *Salmonella*, as indicated by DGGE analysis. Differences in similarity indexes may be due to alterations in the members of other phylogenetic groups that were not considered in this study. Enteric salmonellosis has been reported to alter the microbial ecology, specifically increasing the numbers of *Clostridia* in the murine GIT preceding the onset of diarrhea, indicating the involvement of pathogen-commensal interactions or host responses or both unrelated to diarrhea (Barman et al., 2008).

Enhanced intestinal morphology has been associated with greater BW gain in healthy and sick pigs (Pluske et al., 1996; Zijlstra et al., 1997). In this study, inclusion of XPC increased jejunal villi width and area in the jejunum of *Salmonella*-infected pigs compared

with infected controls. Thus, a greater digestive and absorptive intestinal capacity may have contributed to the enhanced growth performance during the recovery phase of *Salmonella*-inoculated pigs consuming XPC compared with infected controls. Changes in bacterial populations and intestinal morphology could be acting concomitantly to enhance the recovery of pigs experimentally infected with *Salmonella*. The tendency to increase *Salmonella* shedding when consuming XPC can be interpreted to indicate a rapid elimination of the pathogen from the GIT, which may result in reduced infection rates and enhanced clearance of the pathogen (Bovee-Oudenhoven et al., 2003). Rectal temperature results clearly indicate that noninfected pigs indeed were kept free of *Salmonella* and any other pathogen that would have triggered a systemic inflammatory response. The febrile response of *Salmonella*-infected pigs is a clear indication that animals were clinically sick and had developed a systemic immune response. Pigs infected with *Salmonella* in the present study were febrile for 5.5 d after inoculation (i.e., 2.5 d after i.m. treatment with ceftiofur-HCl) compared with 1 to 3 d in a comparable study (Burkey et al., 2004). This comparison indicates that the strain of *Salmonella* Typhimurium used in the present study seems to be more pathogenic to pigs. This observation is important to consider because pigs did not recover naturally from

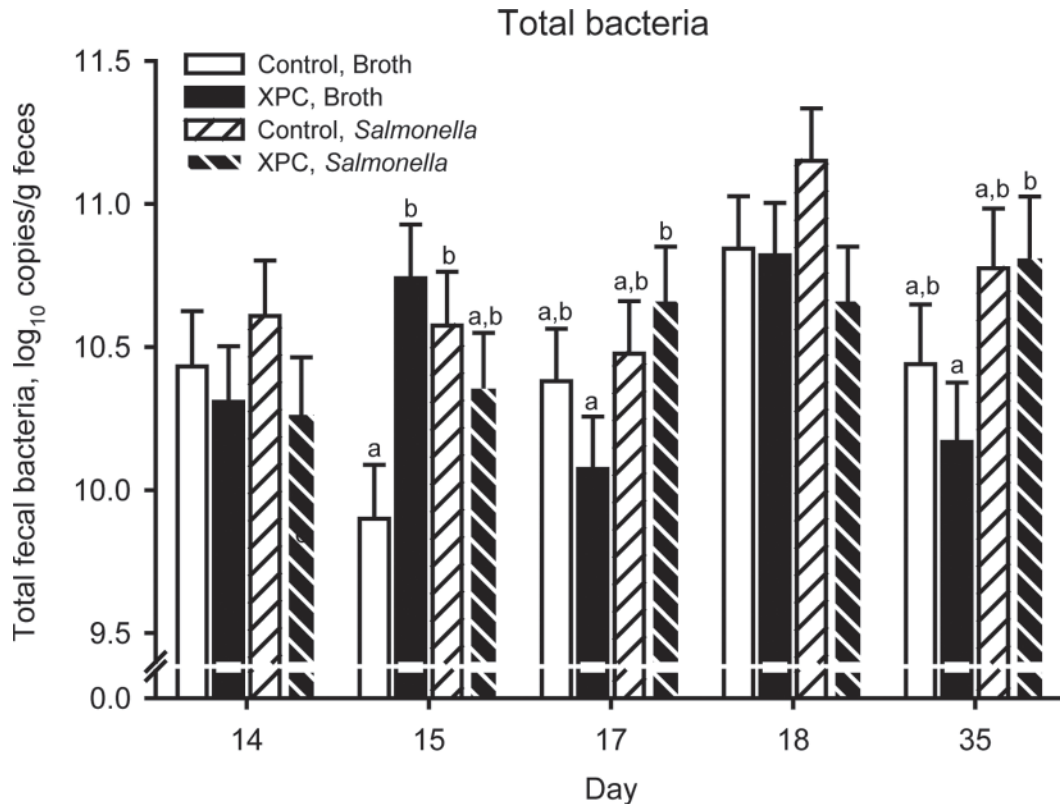


Figure 8. Effect of infection with *Salmonella* and consumption of XPC (Original XPC, Diamond V, Cedar Rapids, IA) on the number of total bacterial copies (log₁₀) determined by real-time-PCR from fecal samples. From weaning (d = 1), pigs had ad libitum access to a nursery diet with (XPC) or without (control) 0.2% XPC inclusion. Pigs were inoculated with 5 mL containing 10⁹ cfu of *Salmonella* Typhimurium resistant to the antibiotics nalidixic acid and novobiocin (*Salmonella*) on d 14 after weaning or received 5 mL of sterile broth (Broth). Values are means ± SEM (n = 9 to 10). ^{a,b}Means without a common letter within day differ, *P* < 0.05.

the disease and, instead, received treatment with an antibiotic to which *Salmonella* was specifically tested to be sensitive. Yet, it took 2.5 d of i.m. antibiotic treatment to return RT to PRE temperatures and 3 d to eliminate *Salmonella* shedding in feces. Taking into account the beneficial changes in bacterial populations induced by XPC intake during SCK, we hypothesized that inclusion of XPC may have a stronger effect on growth performance in animals suffering from longer infections than the one used in this study. Under the commercial production environment, inclusion of XPC in diets for pigs may enhance the growth performance of pigs because they are more likely to naturally clear infections or have chronic exposure to pathogens. Finally, the prebiotic effects of XPC may enhance the recovery of animals after an infection by altering GIT morphology and by maintaining or enhancing the populations of beneficial bacteria within the GIT, which may in turn contribute to improve the lean growth of pigs.

LITERATURE CITED

- Barman, M., D. Unold, K. Shifley, E. Amir, K. Hung, N. Bos, and N. Salzman. 2008. Enteric salmonellosis disrupts the microbial ecology of the murine gastrointestinal tract. *Infect. Immun.* 76:907–915.
- Bovee-Oudenhoven, I. M., S. J. ten Bruggencate, M. L. Lettink-Wissink, and R. van der Meer. 2003. Dietary fructo-oligosaccharides and lactulose inhibit intestinal colonisation but stimulate translocation of salmonella in rats. *Gut* 52:1572–1578.
- Branner, G. R., and D. A. Roth-Maier. 2006. Influence of pre-, pro-, and synbiotics on the intestinal availability of different B-vitamins. *Arch. Anim. Nutr.* 60:191–204.
- Burkey, T. E., S. S. Dritz, J. C. Nietfeld, B. J. Johnson, and J. E. Minton. 2004. Effect of dietary mannanoligosaccharide and sodium chlorate on the growth performance, acute-phase response, and bacterial shedding of weaned pigs challenged with *Salmonella enterica* serotype Typhimurium. *J. Anim. Sci.* 82:397–404.
- Casey, P. G., G. D. Casey, G. E. Gardiner, M. Tangney, C. Stanton, R. P. Ross, C. Hill, and G. F. Fitzgerald. 2004. Isolation and characterization of anti-*Salmonella* lactic acid bacteria from the porcine gastrointestinal tract. *Lett. Appl. Microbiol.* 39:431–438.
- Casey, P. G., G. E. Gardiner, G. Casey, B. Bradshaw, P. G. Lawlor, P. B. Lynch, F. C. Leonard, C. Stanton, R. P. Ross, G. F. Fitzgerald, and C. Hill. 2007. A five-strain probiotic combination reduces pathogen shedding and alleviates disease signs in pigs challenged with *Salmonella enterica* serovar Typhimurium. *Appl. Environ. Microbiol.* 73:1858–1863.
- Coates, M. E., R. Fuller, G. F. Harrison, M. Lev, and S. F. Suffolk. 1963. A comparison of the growth of chicks in the Gustafsson germ-free apparatus and in a conventional environment, with and without dietary supplements of penicillin. *Br. J. Nutr.* 17:141–150.
- Collado, M. C., L. Grzeskowiak, and S. Salminen. 2007. Probiotic strains and their combination inhibit in vitro adhesion of pathogens to pig intestinal mucosa. *Curr. Microbiol.* 55:260–265.
- Dowd, S. E., Y. Sun, R. D. Wolcott, A. Domingo, and J. A. Carroll. 2008. Bacterial tag-encoded FLX amplicon pyrosequencing (bTEFAP) for microbiome studies: Bacterial diversity in the

- ileum of newly weaned *Salmonella*-infected pigs. Foodborne Pathog. Dis. 5:459–472.
- Dunsford, B. R., W. E. Haensly, and D. A. Knabe. 1990. Neutral and acidic goblet cell concentrations in the small intestine of the unweaned pig. Biol. Neonate 57:194–199.
- Gao, J., H. J. Zhang, S. H. Yu, S. G. Wu, I. Yoon, J. Quigley, Y. P. Gao, and G. H. Qi. 2008. Effects of yeast culture in broiler diets on performance and immunomodulatory functions. Poult. Sci. 87:1377–1384.
- Guo, X., X. Xia, R. Tang, and K. Wang. 2008a. Real-time PCR quantification of the predominant bacterial divisions in the distal gut of Meishan and Landrace pigs. Anaerobe 14:224–228.
- Guo, X., X. Xia, R. Tang, J. Zhou, H. Zhao, and K. Wang. 2008b. Development of a real-time PCR method for *Firmicutes* and *Bacteroidetes* in faeces and its application to quantify intestinal population of obese and lean pigs. Lett. Appl. Microbiol. 47:367–373.
- Jensen, G. S., K. M. Patterson, and I. Yoon. 2008. Nutritional yeast culture has specific anti-microbial properties without affecting healthy flora. Preliminary results. J. Anim. Feed Sci. 17:247–252.
- Jonsson, E., and S. Hemmingsson. 1991. Establishment in the piglet gut of lactobacilli capable of degrading mixed-linked beta-glucans. J. Appl. Bacteriol. 70:512–516.
- Kaps, M., and W. R. Lamberson. 2004. Biostatistics for Animal Science. CABI Publ., Cambridge, MA.
- Leser, T. D., J. Z. Amenuvor, T. K. Jensen, R. H. Lindecrone, M. Boye, and K. Moller. 2002. Culture-independent analysis of gut bacteria: The pig gastrointestinal tract microbiota revisited. Appl. Environ. Microbiol. 68:673–690.
- Leterme, P., W. B. Souffrant, and A. Thewis. 2000. Effect of barley fibres and barley intake on the ileal endogenous nitrogen losses in piglets. J. Cereal Sci. 31:229–239.
- Ley, R. E., F. Backhed, P. Turnbaugh, C. A. Lozupone, R. D. Knight, and J. I. Gordon. 2005. Obesity alters gut microbial ecology. Proc. Natl. Acad. Sci. USA 102:11070–11075.
- Macfarlane, G. T., H. Steed, and S. Macfarlane. 2008. Bacterial metabolism and health-related effects of galacto-oligosaccharides and other prebiotics. J. Appl. Microbiol. 104:305–344.
- Moore, W. E., L. V. Moore, E. P. Cato, T. D. Wilkins, and E. T. Kornegay. 1987. Effect of high-fiber and high-oil diets on the fecal flora of swine. Appl. Environ. Microbiol. 53:1638–1644.
- Mountzouris, K. C., C. Balaskas, F. Fava, K. M. Tuohy, G. R. Gibson, and K. Fegeros. 2006. Profiling of composition and metabolic activities of the colonic microflora of growing pigs fed diets supplemented with prebiotic oligosaccharides. Anaerobe 12:178–185.
- Niba, A. T., J. D. Beal, A. C. Kudi, and P. H. Brooks. 2009. Bacterial fermentation in the gastrointestinal tract of non-ruminants: Influence of fermented feeds and fermentable carbohydrates. Trop. Anim. Health Prod. 41:1393–1407.
- NRC. 1998. Nutrient Requirements of Swine. 10th rev. ed. Natl. Acad. Press, Washington, DC.
- Pieper, R., R. Jha, B. Rossnagel, A. G. Van Kessel, W. B. Souffrant, and P. Leterme. 2008. Effect of barley and oat cultivars with different carbohydrate compositions on the intestinal bacterial communities in weaned piglets. FEMS Microbiol. Ecol. 66:556–566.
- Pluske, J. R., M. J. Thompson, C. S. Atwood, P. H. Bird, I. H. Williams, and P. E. Hartmann. 1996. Maintenance of villus height and crypt depth, and enhancement of disaccharide digestion and monosaccharide absorption, in piglets fed on cows' whole milk after weaning. Br. J. Nutr. 76:409–422.
- Santos, A., M. San Mauro, and D. M. Diaz. 2006. Prebiotics and their long-term influence on the microbial populations of the mouse bowel. Food Microbiol. 23:498–503.
- Shen, Y. B., X. S. Piao, S. W. Kim, L. Wang, P. Liu, I. Yoon, and Y. G. Zhen. 2009. Effects of yeast culture supplementation on growth performance, intestinal health, and immune response of nursery pigs. J. Anim. Sci. 87:2614–2624.
- Silva, A. M., F. H. Barbosa, R. Duarte, L. Q. Vieira, R. M. Arantes, and J. R. Nicoli. 2004. Effect of *Bifidobacterium longum* ingestion on experimental salmonellosis in mice. J. Appl. Microbiol. 97:29–37.
- Snart, J., R. Bibiloni, T. Grayson, C. Lay, H. Zhang, G. E. Allison, J. K. Laverdiere, F. Temelli, T. Vasanathan, R. Bell, and G. W. Tannock. 2006. Supplementation of the diet with high-viscosity beta-glucan results in enrichment for lactobacilli in the rat cecum. Appl. Environ. Microbiol. 72:1925–1931.
- Turnbaugh, P. J., M. Hamady, T. Yatsunenkov, B. L. Cantarel, A. Duncan, R. E. Ley, M. L. Sogin, W. J. Jones, B. A. Roe, J. P. Affourtit, M. Egholm, B. Henrissat, A. C. Heath, R. Knight, and J. I. Gordon. 2009. A core gut microbiome in obese and lean twins. Nature 457:480–484.
- Tzortzis, G., A. K. Goulas, J. M. Gee, and G. R. Gibson. 2005. A novel galactooligosaccharide mixture increases the bifidobacterial population numbers in a continuous in vitro fermentation system and in the proximal colonic contents of pigs in vivo. J. Nutr. 135:1726–1731.
- Zhao, J., A. F. Harper, M. J. Estienne, K. E. Webb Jr., A. P. McElroy, and D. M. Denbow. 2007. Growth performance and intestinal morphology responses in early weaned pigs to supplementation of antibiotic-free diets with an organic copper complex and spray-dried plasma protein in sanitary and nonsanitary environments. J. Anim. Sci. 85:1302–1310.
- Zijlstra, R. T., S. M. Donovan, J. Odle, H. B. Gelberg, B. W. Petschow, and H. R. Gaskins. 1997. Protein-energy malnutrition delays small-intestinal recovery in neonatal pigs infected with rotavirus. J. Nutr. 127:1118–1127.

References

This article cites 32 articles, 13 of which you can access for free at:
<http://www.journalofanimalscience.org/content/88/12/3896#BIBL>

Citations

This article has been cited by 7 HighWire-hosted articles:
<http://www.journalofanimalscience.org/content/88/12/3896#otherarticles>