Journal of Animal Science, 2021, Vol. 99, No. 12, 1–10

https://doi.org/10.1093/jas/skab330
Advance Access publication November 9, 2021
Received: 5 August 2021 and **Accepted:** 3 November 2021
Non Ruminant Nutrition

NON RUMINANT NUTRITION

Live yeast and yeast extracts with and without pharmacological levels of zinc on nursery pig growth performance and antimicrobial susceptibilities of fecal *Escherichia coli*

Jenna A. Chance,[†] Joel M. DeRouchey,[†] Raghavendra G. Amachawadi,[‡] Victor Ishengoma,[‡] Tiruvoor G. Nagaraja,[‖] Robert D. Goodband,[†,1] Jason C. Woodworth,[†] Mike D. Tokach,[†] Hilda I. Calderón,[$] Qing Kang,[$] Joseph A. Loughmiller,[¶] Brian Hotze,[¶] and Jordan T. Gebhardt[‖]

[†]Department of Animal Sciences and Industry, Kansas State University, Manhattan, KS 66506-0201, USA, [‡]Department of Clinical Sciences, College of Veterinary Medicine, Kansas State University, Manhattan, KS 66506-0201, USA, [‖]Department of Diagnostic Medicine/Pathobiology, College of Veterinary Medicine, Kansas State University, Manhattan, KS 66506-0201, USA, [$]Department of Statistics, College of Arts and Sciences, Kansas State University, Kanas State University, Manhattan, KS 66506-0201, USA, [¶]Phileo by Lesaffre, Milwaukee, WI 53214-1552, USA

[1]Corresponding author: bgoodban@oznet.ksu.edu

ORCiD numbers: 0000-0001-7268-4278 (J. C. Woodworth); 0000-0002-6144-6714 (J. T. Gebhardt).

Abstract

A total of 360 weanling barrows (Line 200 ×400, DNA, Columbus NE; initially 5.6 ± 0.03 kg) were used in a 42-d study to evaluate yeast-based pre- and probiotics (Phileo by Lesaffre, Milwaukee, WI) in diets with or without pharmacological levels of Zn on growth performance and antimicrobial resistance (AMR) patterns of fecal *Escherichia coli*. Pens were assigned to one of four dietary treatments with five pigs per pen and 18 pens per treatment. Dietary treatments were arranged in a 2 × 2 factorial with main effects of yeast-based pre- and probiotics (none vs. 0.10% ActiSaf Sc 47 HR+, 0.05% SafMannan, and 0.05% NucleoSaf from days 0 to 7, then concentrations were lowered by 50% from days 7 to 21) and pharmacological levels of Zn (110 vs. 3,000 mg/kg from days 0 to 7, and 2,000 mg/kg from days 7 to 21 with added Zn provided by ZnO). All pigs were fed a common diet from days 21 to 42 post-weaning. There were no yeast ×Zn interactions or effects from yeast additives observed on any response criteria. From days 0 to 21 and 0 to 42, pigs fed pharmacological levels of Zn had increased ($P < 0.001$) ADG and ADFI. Fecal samples were collected on days 4, 21, and 42 from the same three pigs per pen for fecal dry matter (DM) and AMR patterns of *E. coli*. On day 4, pigs fed pharmacological levels of Zn had greater fecal DM ($P = 0.043$); however, no differences were observed on day 21 or 42. *Escherichia coli* was isolated from fecal samples and the microbroth dilution method was used to determine the minimal inhibitory concentrations (MIC) of *E. coli* isolates to 14 different antimicrobials. Isolates were categorized as either susceptible, intermediate, or resistant based on Clinical and Laboratory Standards Institute (CLSI) guidelines. The addition of pharmacological levels of Zn had a tendency ($P = 0.051$) to increase the MIC values of ciprofloxacin; however, these MIC values were still well under the CLSI classified resistant breakpoint for ciprofloxacin. There was no evidence for differences ($P > 0.10$) for yeast additives or Zn for AMR of fecal *E. coli* isolates to any of the remaining antibiotics. In conclusion, pharmacological levels of Zn improved ADG, ADFI, and all isolates were

classified as susceptible to ciprofloxacin although the MIC of fecal *E. coli* tended to be increased. Thus, the short-term use of pharmacological levels of Zn did not increase antimicrobial resistance. There was no response observed from live yeast and yeast extracts for any of the growth, fecal DM, or AMR of fecal *E. coli* criteria.

Key words: antimicrobial resistance, growth, live yeast probiotic, nursery pigs, yeast extract, zinc

Abbreviations

ADFI	average daily feed intake
ADG	average daily gain
AMR	antimicrobial resistance
BW	body weight
CFU	colony-forming unit
CP	crude protein
DFM	direct-fed microbial
DM	dry matter
G:F	gain-to-feed ratio
ME	metabolizable energy
MIC	minimal inhibitory concentration
NE	net energy
PCR	polymerase chain reaction
PWD	post-weaning diarrhea
SCFA	short-chain fatty acid
SID	standardized ileal digestible
STTD	standardized total tract digestible
ZnO	zinc oxide

Introduction

Feeding pharmacological levels (2,000 to 3,000 mg/kg) of Zn in the early nursery has been an industry-wide practice to alleviate the lag in performance and control occurrences of post-weaning diarrhea (PWD; Jacela et al., 2010). However, feeding pharmacological levels of Zn has become a concern for AMR to antibiotics of importance to human and animal medicine (Nguyen et al., 2019; Muurinen et al., 2021). Use of these minerals is restricted in some countries due to their impact on environmental buildup and their capability to create a favorable environment for gut bacteria to acquire and transmit AMR genes (Yazdankhah et al., 2014; Zhang et al., 2019).

One potential replacement strategy for pharmacological levels of added Zn in the early nursery is the use of pre- and probiotics. Prebiotics are substrates that selectively stimulate the growth of beneficial microbes in the gastrointestinal tract (Gibson et al., 2004). Feeding probiotics can alter the gut's microflora by introducing live cultures of beneficial microorganisms into the digestive tract and can aid in competitive exclusion or suppression of pathogens (Bajagai et al., 2016). This modulated microbial profile in the gut may allow for more protection against enteric diseases while subsequently improving growth performance (as reviewed by Doyle, 2001). For example, a live yeast strain of *Saccharomyces cerevisiae* and β-glucan derived from yeast cell walls have been shown to reduce the shedding of enterotoxigenic *E. coli*, shorten periods of diarrhea, and increase body weight in the early nursery period (Stuyven et al., 2009; Trckova et al., 2014). Further research suggests that dietary addition of live yeast maintains intestinal villi integrity and helps alleviate inflammation caused by enteric pathogens (Che et al., 2017). Amachawadi et al. (2018) found that probiotics may reduce the prevalence and proliferation of AMR of gastrointestinal bacteria, making pre- and probiotics an alternative of interest to high levels of Zn. Our hypothesis was that the additions of a live yeast (probiotic) and yeast extracts (prebiotics) would provide equal, if not additive, growth responses to added Zn without promoting AMR in nursery pigs. Thus, the objective of this study was to determine the effects of pharmacological levels of Zn with or without the addition of the live yeast *Saccharomyces cerevisiae* strain NCYC Sc 47 and yeast-based prebiotics derived from *S. cerevisiae* on nursery pig growth performance and AMR patterns of *E. coli* isolated from nursery pig fecal material.

Materials and Methods

General

The Kansas State University Institutional Animal Care and Use Committee approved the protocol used in this experiment. The study was conducted at the Kansas State University Segregated Early Weaning Facility in Manhattan, KS. The facility has two identical barns that are completely enclosed, environmentally controlled, and mechanically ventilated. Treatments were equally represented in each barn. Each pen contained a 4-hole, dry self-feeder and a cup waterer to provide *ad libitum* access to feed and water. Pens (1.3 ×1.3 m) had metal tri-bar floors and allowed approximately 0.33 m^2/pig.

Animals and treatment structure

A total of 360 barrows (Line 200 ×400; DNA, Columbus, NE; initial BW 5.6 ± 0.03 kg) were used in a 42-d study with five pigs per pen and 18 pens per treatment (9 pens per barn). Upon arrival to the research site, pigs were randomly assigned to pens. Pens were then assigned to one of four dietary treatments in a randomized complete block design with pens blocked by BW with each treatment represented once within each block (18 total blocks). During the study, three pigs were removed due to illness or injury.

Dietary treatments were arranged in a 2 × 2 factorial with main effects of yeast-based pre- and probiotics (none vs. 0.10% ActiSaf Sc 47 HR+, 0.05% SafMannan, and 0.05% NucleoSaf from days 0 to 7, then concentrations were lowered by 50% from days 7 to 21) and pharmacological levels of Zn (110 mg/kg vs. 3,000 mg/kg from days 0 to 7 and 2,000 mg/kg from days 7 to 21; added Zn provided by ZnO; Table 1). The live *S. cerevisiae* strain NCYC Sc 47 (ActiSaf Sc 47 HR+; Phileo by Lesaffre, Milwaukee, WI) served as the yeast-based probiotic. The yeast-based prebiotics included a yeast cell wall fraction with concentrated mannan-oligosaccharides and β-glucans from *S. cerevisiae* (SafMannan; Phileo by Lesaffre) and a yeast extract containing ≥ 6% unbound nucleotides from *S. cerevisiae* (NucleoSaf; Phileo by Lesaffre).

Diet preparation

Pigs were fed phase 1 diets from placement until day 7 and then offered phase 2 diets from days 7 to 21 (Table 1). A common

Table 1. Composition of phase 1, phase 2, and phase 3 diets (as-fed basis)[1]

Item	Phase 1	Phase 2	Phase 3
Ingredients, %			
Corn	43.98	57.10	64.70
Soybean meal, 46.5% CP	18.10	26.35	31.30
Whey powder	25.00	10.00	–
Fish meal	4.50	–	–
Enzymatically-treated soybean meal[2]	3.75	2.00	–
Soybean oil	1.50	–	–
Calcium carbonate	0.30	0.90	0.85
Monocalcium phosphate, 21% P	0.48	1.10	1.00
Sodium chloride	0.30	0.55	0.60
L-Lys-HCl	0.43	0.51	0.52
DL-Met	0.22	0.22	–
MHA[3]	–	–	0.25
L-Thr	0.18	0.21	0.22
L-Trp	0.07	0.06	0.06
L-Val	0.13	0.14	0.13
Vitamin premix[4]	0.25	0.25	–
Vitamin premix with phytase[5]	–	–	0.25
Trace mineral premix[6]	0.15	0.15	0.15
Phytase[7]	0.08	0.08	–
Zinc oxide[8]	±	±	–
Yeast additives[9]	±	±	---
Total	100	100	100
Calculated analysis			
SID amino acids, %			
Lys	1.40	1.35	1.35
Ile:Lys	56	55	55
Leu:Lys	109	111	114
Met:Lys	38	36	36
Met and Cys:Lys	57	57	57
Thr:Lys	63	63	63
Trp:Lys	20.6	20.2	20.3
Val:Lys	69	69	69
His:Lys	32	34	36
Total Lys, %	1.53	1.48	1.49
ME, kcal/kg	3,408	3,267	3,271
NE, kcal/kg	2,565	2,429	2,416
SID Lys:NE, g/Mcal	5.44	5.54	5.57
CP, %	20.9	20.5	21.2
Ca, %	0.69	0.77	0.72
P, %	0.68	0.66	0.61
STTD P, %	0.63	0.58	0.50
Zn, mg/kg	110 vs 3,000	110 vs 2,000	110

[1]Phase 1 diets were fed from days 0 to 7 (approximately 5.6 to 6.1 kg) and phase 2 diets were fed from days 7 to 21 (approximately 6.1 to 11.6 kg). Both phases were manufactured at the Kansas State University Poultry Unit (Manhattan, KS). A common diet, withoutZnO or yeast probiotics, was fed during phase 3 from days 21 to 42 (approximately 11.6 to 24.0 kg). The common diet was manufactured by Hubbard Feeds (Beloit, KS).
[2]HP 300, Hamlet Protein, Findlay, OH.
[3] Methionine hydroxy analogue, Novus International, St. Charles, MO.
[4]Provided per kg of premix: 1,653,465 IU vitamin A; 661,386 IU vitamin D; 17,637 IU vitamin E; 1,322 mg vitamin K; 13.2 mg vitamin B12; 19,841 mg niacin; 11,023 mg pantothenic acid; 3,307 mg riboflavin.
[5] Ronozyme HiPhos GT 2700 (DSM Nutritional Products, Parsippany, NJ) provided 1,250 FTU/kg and an expected STTD P release of 0.12%. Provided per kg of premix: 1,653,465 IU vitamin A; 661,386 IU vitamin D; 17,637 IU vitamin E; 1,322 mg vitamin K; 13.2 mg vitamin B12; 19,841 mg niacin; 11,023 mg pantothenic acid; 3,307 mg riboflavin.
[6]Provided per kg of premix: 73 g Zn from Zn sulfate; 73 g Fe from iron sulfate; 22 g Mn from manganese oxide; 11 g Cu from copper sulfate; 0.2 g I from calcium iodate; 0.2 g Se from sodium selenite.
[7]Ronozyme HiPhos 2700 (DSM Nutritional Products, Parsippany, NJ) provided 2,027 FTU/kg and an estimated release of 0.14% STTD P in phases 1 and 2.
[8] ZnO was fed to supply 3,000 mg/kg of Zn for the duration of phase 1 and 2,000 mg/kg of Zn for the duration of phase 2.
[9]Yeast pre- and probiotics included 0.10% ActiSaf Sc 47 HR+, 0.05% SafMannan, and 0.05% NucleoSaf in phase 1 diets and then concentrations were lowered by 50% in phase 2 diets (Phileo by Lesaffre, Milwaukee, WI).

phase 3 diet, without yeast additives or pharmacological levels of ZnO, was fed to all pigs from days 21 to 42. Phase 1 diets were formulated to a 1.40% standardized ileal digestible (SID) Lys and phase 2 and 3 diets were formulated to a 1.35% SID Lys. All other nutrients were formulated to meet or exceed National Research Council (NRC, 2012) requirement estimates. Phase 1 and 2 diets were manufactured at the Kansas State University Poultry Unit (Manhattan, KS) and the common phase 3 diet was manufactured at a commercial feed mill (Hubbard Feeds; Beloit, KS). Diets in all three phases were fed in meal form. Pens of pigs were weighed and feed disappearance recorded weekly during the course of this study to determine average daily gain (ADG), average daily feed intake (ADFI), and gain-to-feed ratio (G:F).

Chemical analysis

Phase 1 and 2 diet samples were collected at manufacturing and phase 3 diets were collected from every fourth 23-kg bag using a feed probe to collect a representative sample for each respective diet and phase. Complete diet samples were stored at –20 °C until they were homogenized, subsampled, and submitted for analysis. Duplicate composite samples per dietary treatment were analyzed (Ward Laboratories; Kearney, NE) for dry matter (method 935.29; AOAC International, 2019), crude protein (method 990.03; AOAC International, 2019), and zinc (Campbell and Plank, 1991). Separate composite samples per dietary treatment were analyzed for active live yeast (Analabs; Fulton, IL; method 997.02; AOAC International, 1998) for phases 1 and 2 (Table 2).

Fecal collection

Fecal samples were collected on days 4, 21, and 42 of the experiment for antimicrobial susceptibility and resistance profiles of *E. coli* and fecal dry matter (DM) analysis. Fecal samples were collected directly from the rectum of the same three randomly selected pigs from each pen and pooled by pen to form one composite sample. Fecal samples were collected using a sterile, single-use cotton tipped applicator (Fisher Healthcare, Pittsburgh, PA) and were stored in a clean, single-use zipper storage bag. Samples were immediately transported on ice to the Kansas State University College of Veterinary Medicine for bacterial isolation and antimicrobial susceptibility testing of *E. coli*. The remaining contents, after samples were collected for *E. coli* isolation, were stored at –20 °C until subsequent fecal dry matter analysis. Fecal samples were pooled by pen, within day of collection, and dried at 55 °C in a forced air oven for 48 h.

E. coli isolation and identification

Approximately, 1 g of pooled fecal sample was suspended in 9 mL of phosphate-buffered saline and vortexed for a minute.

Table 2. Diet analysis (as-fed basis), %[1]

	No yeast additives		Yeast additives[2]	
	Low Zn	High Zn	Low Zn	High Zn
Phase 1 diets				
DM, %	91.8	91.9	91.9	91.9
CP, %	20.5	19.9	20.2	20.1
Ca, %	1.23	1.23	1.20	1.20
P, %	0.77	0.75	0.77	0.76
Zn, mg/kg	263	3,230	245	3,204
Live yeast, CFU/g	200	500	7,100,000	9,700,000
Phase 2 diets				
DM, %	90.2	90.1	89.7	89.8
CP, %	18.9	19.0	19.1	19.6
Ca, %	1.38	1.41	1.38	1.33
P, %	0.73	0.72	0.74	0.72
Zn, mg/kg	234	2,435	257	2,233
Live yeast, CFU/g	300	700	10,500,000	5,900,000
Phase 3 common diet				
DM, %	88.4	–	–	–
CP, %	20.7	–	–	–
Ca, %	0.95	–	–	–
P, %	0.61	–	–	–
Zn, mg/kg	199	–	–	–

[1] Complete diet samples were obtained from each treatment during manufacturing and homogenized to form a composite sample. Samples were submitted to Ward Laboratories (Kearney, NE) to analyze DM, CP, Ca, P, and Zn. Phase 1 and 2 diets were also sent to Analabs (Fulton, IL) to analyze active live yeast.
[2]Yeast pre- and probiotics included ActiSaf SC 47 HR+ at 0.1%, SafMannan at 0.05% and NucleoSaf at 0.05% in phase 1 diets and then concentrations were lowered by 50% in phase 2 diets (Phileo by Lesaffre,Milwaukee, WI).

Fifty microliters of the fecal suspension were spread-plated onto a MacConkey agar plate (Becton Dickinson, Sparks, MD) for the isolation of *E. coli*. Two lactose-fermenting colonies were picked from each plate, individually streaked onto a blood agar plate (Remel, Lenexa, KS) and incubated at 37 °C for 24 h. Spot indole test was done and indole-positive isolates were stored in cryo-protect beads (Cryocare, Key Scientific Products, Round Rock, TX) at –80 °C. Species confirmation of *E. coli* was by polymerase chain reaction (PCR) for *uidA* and *clpB* genes.

Antimicrobial susceptibility testing of *E. coli* isolates

Antimicrobial susceptibility testing was accomplished on one *E. coli* isolate per fecal sample recovered on days 4, 21, and 42. The microbroth dilution method as outlined by the Clinical and Laboratory Standards Institute (CLSI, 2018) was used to determine the minimal inhibitory concentrations (MIC) of 14 antibiotics. The antimicrobials tested included amoxicillin/clavulanic acid, ampicillin, azithromycin, cefoxitin, ceftiofur, ceftriaxone, chloramphenicol, ciprofloxacin, gentamicin, nalidixic acid, streptomycin, sulfisoxazole, tetracycline, and trimethoprim/sulfamethoxazole. Each isolate, stored in cryo-protect beads, was streaked onto a blood agar plate and incubated at 37 °C for 24 h. Individual colonies were suspended in demineralized water (Trek Diagnostic Systems, Cleveland, OH) and turbidity was adjusted to 0.5 McFarland turbidity standard. Then, 10 µL of the bacterial inoculum was added to Mueller–Hinton broth (11 ml) and vortexed to mix. A Sensititre automated inoculation delivery system (Trek Diagnostics Systems) was used to dispense 100 µL of the culture into National Antimicrobial Resistance Monitoring System (NARMS) panel plates designed for Gram-negative (CMV3AGNF, Trek Diagnostic Systems) bacteria. *Escherichia coli* ATCC 25922 (American Type Culture Collection, Manassas, VA) strain was included as quality control. Plates were incubated at 37 °C for 18 h and bacterial growth was assessed using Sensititre ARIS and Vizion systems (Trek Diagnostic Systems). Clinical and Laboratory Standards Institute (CLSI, 2018; Table 3) guidelines were used to classify each isolate as susceptible, intermediate, or resistant according to the breakpoints established for each antimicrobial.

Statistical analysis

Growth, fecal dry matter, and economics

Growth performance and fecal dry matter data were analyzed using the *nlme* package of R (Version 4.0.0, R Foundation for Statistical Computing, Vienna, Austria) as a randomized complete block design with body weight (BW) and barn serving as the blocking factor and pen as the experimental unit. The main effects of yeast-derived pre- and probiotics and pharmacological levels of zinc, as well as their interactions, were tested. Blocking factor was included within the statistical model as a random intercept. Fecal DM was analyzed using repeated measures analysis considering the multiple measures taken on the same experimental unit over the study. Differences between treatments were considered significant at $P \leq 0.05$ and marginally significant at $0.05 < P \leq 0.10$.

Antimicrobial susceptibility

For each of the 14 antimicrobials, MIC data were summarized with appropriate descriptive statistics by treatment group at each sampling day. Because all isolates were resistant, MICs of tetracycline were excluded from the statistical analysis. The MIC data of the remaining antimicrobials were analyzed using the linear mixed model. To better achieve model assumptions, data underwent natural log transformation before statistical modeling. Statistical analysis was performed using the MIXED procedure of SAS (version 9.4; Cary, NC) with option DDFM=KR in the MODEL statement. Fixed effects of the model included Zn, yeast, sampling time, and their second- and third-order interactions. Random effects included block and pen. Treatment effect was assessed via back-transformed least squares means, i.e., geometric means of the MIC values. The variance-covariance structure of pen was taken as either compound symmetry, first-order autoregressive or unstructured according to the model fitting criteria.

Results

Growth performance

There were no interactions observed between the dietary addition of pharmacological levels of Zn and yeast-based pre- and probiotics ($P > 0.05$; Table 4). In phases 1 (days 0 to 7) and 2 (days 7 to 21), pigs fed pharmacological levels of Zn had increased ($P < 0.05$) ADG, ADFI, and heavier days 7 and 21 BW compared to pigs fed the diet containing basal trace mineral amounts of Zn (110 mg/kg). Pigs that were fed diets containing ZnO had improved ($P < 0.001$) G:F in phase 1 while the dietary addition of live yeast and yeast extracts had a tendency ($P = 0.077$) for improved G:F in phase 1, but the addition of live yeast and yeast extracts had no other effects on any further growth performance criteria in this study.

For the experimental period (days 0 to 21), pigs fed pharmacological levels of Zn had increased ($P < 0.001$) ADG and

Table 3. Resistance breakpoints and evaluated concentrations for antimicrobials of National Antimicrobial Resistance Monitoring System Gram-negative bacteria panel (CMV3AGNF; WHO, 2018)[1]

Antimicrobial	WHO classification[2]	Susceptible breakpoints, µg/mL	Intermediate breakpoints, µg/mL	Resistant breakpoint, µg/mL
Amoxicillin:clavulanic acid 2:1 ratio	Critically important	≤ 8/4	16/8	≥ 32/16
Ampicillin	Critically important	≤ 8	16	≥ 32
Azithromycin	Critically important	≤ 16	N/A[3]	≥ 32
Cefoxitin	Highly important	≤ 8	16	≥ 32
Ceftiofur	Critically important	≤ 2	4	≥ 8
Ceftriaxone	Critically important	≤ 1	2	≥ 4
Chloramphenicol	Highly important	≤ 8	16	≥ 32
Ciprofloxacin	Critically important	≤ 0.06	≥ 0.12	≥ 0.12
Gentamicin	Critically important	≤ 4	8	≥ 16
Nalidixic acid	Critically important	≤ 16	N/A	≥ 32
Streptomycin	Critically important	≤ 16	N/A	≥ 32
Sulfisoxazole	Highly important	≤ 256	N/A	≥ 512
Tetracycline	Highly important	≤ 4	8	≥ 16
Trimethoprim/ sulfamethoxazole 1:19 ratio	Highly important	≤ 2/38	N/A	≥ 4/76

[1]Breakpoints established by Clinical and Laboratory Standards Institute (CLSI, 2018) which are categorized as susceptible (treatable), intermediate (possibly treatable with higher doses), and resistant (not treatable). MIC values greater than the susceptible breakpoint but lower than the resistant breakpoint were considered intermediate.
[2]World Health Organization (WHO) categorization of antimicrobials according to importance for human medicine (WHO, 2018).
[3]N/A, not applicable. The National Antimicrobial Resistance Monitoring System has not established breakpoints; therefore, there is no Clinical and Laboratory Standards Institute resistant breakpoint.

ADFI leading to increased ($P < 0.001$) day 21 BW. However, there was no evidence for difference ($P > 0.10$) in G:F between pens of pigs fed diets with or without pharmacological levels of Zn.

During the common period (days 21 to 42), pigs previously fed pharmacological levels of Zn had increased ($P = 0.002$) BW on day 42 compared those fed diets without added Zn. There was no evidence for statistical difference ($P > 0.10$) between any of the previous treatment combinations on any of the remaining growth criteria.

For the overall study (days 0 to 42), pigs fed pharmacological levels of Zn had increased ($P < 0.05$) ADG, ADFI, G:F, and heavier BW. There were no differences observed for pigs fed yeast-based pre- and probiotics.

Fecal dry matter

There were no interactions observed between the dietary addition of pharmacological levels of Zn and yeast-based pre- and probiotics or for the main effect of yeast additives for fecal dry matter (Table 4). On day 4, pigs fed 3,000 mg/kg of Zn had greater ($P = 0.043$) fecal DM than those without added Zn. However, no differences were observed on day 21 or 42 between any of the dietary treatments for fecal DM.

Antimicrobial susceptibility

There were no two-way or three-way interactions observed for any of the antimicrobials among the *E. coli* isolates tested (Table 5). All fecal *E. coli* isolates were susceptible to azithromycin, ciprofloxacin, nalidixic acid, sulfisoxazole, and trimethoprim/sulfamethoxazole at all three sampling time points (days 4, 21, and 42) regardless of the inclusion of live yeast and yeast extracts or pharmacological levels of Zn. Regardless of diet or sampling day, fecal *E. coli* isolates were intermediate to amoxicillin:clavulanic acid, ampicillin, cefoxitin, ceftiofur, and chloramphenicol. *E. coli* isolates from all dietary treatments were resistant to streptomycin at all three sampling time points. Interestingly, fecal *E. coli* was susceptible to gentamicin on days 4 and 42 but intermediate on day 21. On days 4 and 21, fecal *E. coli* isolates were considered intermediate to ceftriaxone but were resistant on day 42.

There was evidence for increased ($P < 0.05$) MIC values over time for ampicillin, cefoxitin, ceftriaxone, ciprofloxacin, nalidixic acid, and sulfisoxazole. Values for azithromycin and trimethoprim/sulfamethoxazole decreased ($P < 0.05$) from days 4 to 21 but then increased ($P < 0.05$) from days 21 to 42. Chloramphenicol MIC values increased ($P < 0.05$) from days 21 to 42 with day 4 values being intermediate while MIC values for gentamicin increased ($P < 0.05$) from days 4 to 21 with day 42 values being intermediate.

The MICs of antimicrobials were not affected by the dietary addition of yeast-based pre- and probiotics. Only fecal *E. coli* isolated from pigs fed pharmacological levels of Zn from days

Table 4. Main effects of yeast pre- and probiotics and pharmacological levels of Zn on nursery pig performance[1]

	Yeast additives				Zinc			
Item	No yeast	Yeast	SEM	P-value	Low Zn	High Zn	SEM	P-value
BW, kg								
d 0	5.64	5.64	0.024	0.779	5.64	5.64	0.024	0.901
d 7	5.95	5.99	0.060	0.508	5.86	6.07	0.060	0.001
d 21	11.21	11.31	0.122	0.533	10.96	11.56	0.122	< 0.001
d 42	23.58	23.66	0.202	0.744	23.22	24.01	0.202	0.002
Phase 1 (days 0 to 7)								
ADG, g	44	50	7.3	0.489	32	62	7.3	0.001
ADFI, g	80	81	5.0	0.847	74	87	5.0	0.042
G:F, g/kg	397	547	73.1	0.077	297	646	73.1	< 0.001
Phase 2 (days 7 to 21)								
ADG, g	373	380	5.9	0.401	361	392	5.9	< 0.001
ADFI, g	464	472	9.0	0.507	445	492	9.0	< 0.001
G:F, g/kg	805	808	8.4	0.810	814	799	8.4	0.169
Experimental period (days 0 to 21)								
ADG, g	262	270	5.6	0.288	251	281	5.6	< 0.001
ADFI, g	335	342	7.3	0.461	321	356	7.3	< 0.001
G:F, g/kg	782	792	7.2	0.247	782	791	7.2	0.282
Phase 3 common period (days 21 to 42)								
ADG, g	589	588	5.8	0.947	584	593	5.8	0.264
ADFI, g	875	868	8.1	0.573	870	873	8.1	0.811
G:F, g/kg	674	677	3.9	0.502	672	680	3.9	0.158
Overall (days 0 to 42)								
ADG, g	424	429	4.4	0.433	417	437	4.4	0.001
ADFI, g	603	605	7.0	0.830	594	614	7.0	0.031
G:F, g/kg	704	709	3.5	0.284	702	712	3.5	0.043
Fecal dry matter, %[2]								
d 4	18.9	18.8	0.82	0.955	17.7	20.0	0.82	0.043
d 21	22.0	22.9	0.81	0.397	22.6	22.3	0.81	0.786
d 42	24.5	23.6	0.85	0.437	24.0	24.2	0.85	0.891

[1]A total of 360 barrows (initially 5.6 ± 0.03 kg) were used in a 42-d growth study with 5 pigs per pen and 36 pens per treatment. Yeast pre- and probiotics included ActiSaf Sc 47 HR+ at 0.10.%, SafMannan at 0.05% and NucleoSaf at 0.05% in phase 1 diets and then concentrations were lowered by 50% in phase 2 diets (Phileo by Lesaffre, Milwaukee, WI). ZnO was fed to supply 3,000 mg/kg of Zn for the duration of phase 1 and 2,000 mg/kg of Zn for the duration of phase 2. All yeast additive × zinc interaction, $P > 0.05$.
[2]Fecal samples from the same 3 pigs/pen were collected on days 4, 21, and 42. Zinc × yeast × day, $P = 0.790$; Zinc × day, $P = 0.220$; Yeast × day, $P = 0.515$.

0 to 21 had a marginally significant effect ($P = 0.051$) where the AMR to ciprofloxacin was higher compared to those that were not fed added Zn. However, all median MICs were still well under the CLSI (2018) resistant breakpoint for ciprofloxacin.

Discussion

The dietary addition of prebiotics provides a substrate that is indigestible by the host but is fermented by gut bacteria, thereby selectively stimulating the growth of a beneficial microbial population in the gastrointestinal tract (Gibson et al., 2004). Inulin, lactulose, fructo-olgosaccharides, and transgalacto-oligosaccharides are some of the most common prebiotics used in swine diets because favorable gut bacteria can ferment them readily and produce short-chain fatty acids (SCFA; Gibson et al., 2004). In this study, we evaluated prebiotic benefits of a yeast cell wall fraction with concentrated mannan-oligosaccharides and β-glucans derived from *S. cerevisiae* (SafMannan; Phileo by Lesaffre) and a yeast extract containing ≥ 6% unbound nucleotides derived from *S. cerevisiae* (NucleoSaf; Phileo by Lesaffre). Feeding a probiotic, a live microorganism, can alter the gut's microflora by introducing live cultures of favorable microbes into the digestive tract that can aid in competitive exclusion or suppression of pathogens (Bajagai,et al., 2016). The production of lactic acid and SCFA can lower intestinal pH, thus promoting intestinal villi growth and epithelial integrity, which may improve nutrient digestibility and nutrient absorption and suppress enteric pathogens to mitigate subclinical infections (Pollmann et al., 1990; Bajagai et al., 2016). Most probiotics can be categorized into one of three groups: *Bacillus*, lactic acid producing bacteria, or yeasts (Stein and Kil, 2006). The live yeast *S. cerevisiae* strain NCYC Sc 47 (ActiSaf Sc 47 HR+; Phileo by Lesaffre) was evaluated in the present study as the probiotic source. Live yeast (probiotics) and yeast extracts (prebiotics) are of particular interest due to the β-glucans and α-mannans found in yeast cell walls along with unbound nucleotides. Yeast cell walls may improve the colonization of good bacteria in the gut by preventing the binding of enteric pathogens to the intestinal mucosa (Kogan and Kocher, 2007). Additionally, live yeast and yeast cell walls have the potential to improve immunity (Perez-Sotelo et al., 2011; Zanello et al., 2011; Badia et al., 2012), bind toxins (Yiannikouris et al., 2004; Šrobárová et al., 2005), and reduce the instances of enteric infections (Kiarie et al., 2011; Che et al., 2017; Trevisi et al., 2017), thus contributing to improved growth performance in the nursery (Shen et al., 2009; Kiros et al., 2018). Furthermore, live yeast and yeast extracts contain free nucleotides. Feeding unbound nucleotides in the early nursery has demonstrated to increase feed intake, improve

Table 5. Main effects of yeast pre- and probiotics, pharmacological levels of Zn, and day of sampling on antimicrobial susceptibilities of fecal Escherichia coli according to National Antimicrobial Resistance Monitoring System (CLSI, 2018) established breakpoints[1]

	Yeast additives[2]			Zn[3]			Day			
Item	No yeast	Yeast	P-value	Low Zn	High Zn	P-value	4	21	42	P-value
Amoxicillin:clavulanic acid 2:1 ratio[5]	13.5 ± 1.0	13.3 ± 1.0	0.905	13.1 ± 1.0	13.6 ± 1.0	0.721	11.3 ± 1.1	14.4 ± 1.4	14.7 ± 1.4	0.134
Ampicillin	23.1 ± 1.7	24.3 ± 1.8	0.625	22.9 ± 1.7	24.4 ± 1.8	0.541	18.3 ± 2.1^{a}	23.1 ± 1.9^{a}	31.4 ± 0.6^{b}	< 0.001
Azithromycin	9.21 ± 0.64	9.10 ± 0.63	0.876	8.75 ± 0.60	9.58 ± 0.66	0.272	11.65 ± 0.96^{b}	6.79 ± 0.56^{a}	9.70 ± 0.80^{b}	< 0.001
Cefoxitin	13.5 ± 1.0	14.6 ± 1.1	0.367	13.0 ± 1.0	15.1 ± 1.1	0.112	11.9 ± 1.1^{a}	14.1 ± 1.3^{ab}	16.5 ± 1.6^{b}	0.030
Ceftiofur	2.47 ± 0.30	2.67 ± 0.32	0.606	2.70 ± 0.32	2.44 ± 0.29	0.505	2.14 ± 0.33	2.50 ± 0.39	3.17 ± 0.49	0.189
Ceftriaxone	2.60 ± 0.49	3.05 ± 0.58	0.443	3.11 ± 0.59	2.55 ± 0.48	0.336	1.88 ± 0.46^{a}	2.42 ± 0.60^{a}	4.90 ± 1.20^{b}	0.011
Chloramphenicol	20.2 ± 1.3	20.7 ± 1.3	0.774	20.4 ± 1.3	20.4 ± 1.3	0.999	19.8 ± 1.6^{ab}	18.3 ± 1.7^{a}	23.5 ± 1.5^{b}	0.087
Ciprofloxacin	0.031 ± 0.004	0.031 ± 0.004	0.996	0.026 ± 0.004	0.038 ± 0.005	0.051	0.0242 ± 0.0031^{a}	0.0277 ± 0.0036^{a}	0.0458 ± 0.0084^{b}	0.007
Gentamicin	2.92 ± 0.43	3.68 ± 0.54	0.232	3.56 ± 0.52	3.02 ± 0.44	0.386	2.54 ± 0.41^{a}	4.04 ± 0.65^{b}	3.43 ± 0.55^{ab}	0.072
Nalidixic acid	3.22 ± 0.23	3.22 ± 0.23	1.000	3.04 ± 0.21	3.41 ± 0.24	0.253	2.94 ± 0.25^{a}	2.88 ± 0.21^{a}	3.92 ± 0.40^{b}	0.029
Streptomycin	50.1 ± 2.8	53.8 ± 3.0	0.288	51.1 ± 2.9	52.7 ± 3.0	0.633	51.7 ± 3.9	51.3 ± 3.8	52.8 ± 4.0	0.958
Sulfisoxazole	217 ± 13	203 ± 12	0.447	208 ± 12	211 ± 13	0.879	176 ± 17^{a}	219 ± 15^{ab}	239 ± 10^{b}	0.010
Trimethoprim/ Sulfamethoxazole 1:19 ratio[4]	0.250 ± 0.025	0.244 ± 0.025	0.848	0.226 ± 0.023	0.270 ± 0.027	0.210	0.195 ± 0.020^{b}	0.151 ± 0.013^{a}	0.512 ± 0.089^{c}	< 0.001

[1]A total of 360 barrows (initial BW of 5.6 ± 0.03 kg) were used in a 42-d study with 5 pigs per pen and 36 pens per treatment. Fecal samples from the same 3 pigs/pen were collected on days 4, 21, and 42 for *E. coli* isolation and further characterization. Data reported as geometric mean of MIC ± SEM.
[2]Yeast pre- & probiotics included ActiSaf Sc 47 HR+ at 0.1%, SafMannan at 0.05% and NucleoSaf at 0.05% in phase 1 diets and then concentrations were lowered by 50% in phase 2 diets (Phileo by Lesaffre, Milwaukee, WI).
[3]ZnO was fed to supply 3,000 mg/kg of Zn for the duration of phase 1 and 2,000 mg/kg of Zn for the duration of phase 2.
[4]The MIC numerator of the ratio was reporter for the antimicrobial's amoxicillin:clavulanic acid and trimethoprim/sulfamethoxazole.
[a–c]Superscripts signify a statistical difference of $P < 0.05$.

intestinal integrity of the epithelial lining, and reduce periods of diarrhea by promoting beneficial microbiota colonization in the gastrointestinal tract (Yu et al., 2002; Mateo, 2005). While many studies have shown positive impacts from the dietary addition of pre- and probiotics due to the modulation of the gut's microbial population and host immunity, there are still inconsistent results on the impact of growth criteria (Zimmerman et al., 2016).

Zinc is an imperative micronutrient for many physiological body functions. Some functions include enzyme performance for metabolism, digestion, and cellular signaling, normal skin accretion, along with proper body maintenance and reproductive development (Reese and Hill, 2010). The NRC (2012) requirements for Zn are 26.6 to 46.8 mg/kg of Zn for a 4- to 12-kg pig. Flohr et al. (2016) found that the average for Zn inclusion in the United States swine industry was 3,032 mg/kg Zn and 2,081 mg/kg Zn in phase 1 (weaning to 7 kg BW) and phase 2 (7 to 11 kg BW) diets, respectively. These pharmacological levels of Zn are well above the pig's physiological requirement; however, elevated levels of Zn in the diet, for 10 to 21 d immediately following weaning, have been proven to have positive implications on growth performance and controlling PWD in a young pig. We observed increased ADG, ADFI, G:F, BW, and fecal DM on day 4 post-weaning when weaned pigs were fed 3,000 mg/kg Zn in phase 1 and 2,000 mg/kg Zn in phase 2 with the added Zn provided by ZnO. Many studies support the positive attributes that pharmacological Zn, in the form of ZnO, has on improved growth, increased intake, and reduced occurrence of PWD (Hill et al., 2000; Reese and Hill, 2010; Sales, 2013). Other forms of Zn ($ZnSO_4$ and Zn-Lys) have shown inconsistent results when fed at pharmacological levels (Hahn and Baker, 1993). As reviewed by Liu et al. (2018) and Bonetti et al. (2021), zinc oxide has unique modes of action which include antimicrobial tendencies, antioxidant properties, improved digestion, and nutrient absorption because of increased secretion of ghrelin in the stomach and digestive enzymes in the pancreas, as well as improved intestinal epithelial integrity, hence improved gut barrier function and enhanced immune responses. Even though ZnO has proved to be a beneficial additive in the early nursery for growth and controlling PWD, alternative feeding strategies are being explored.

One such strategy is the use of yeast-based pre- and probiotics. In the present study, we observed a tendency for improved G:F immediately following weaning from days 0 to 7 but no further statistical impact from the dietary addition of live yeast and yeast extracts for any of the remaining growth criteria during the experimental, post-treatment, or overall study period. The lack of statistical growth response from added yeast-based pre- and/or probiotics is consistent with results found by Perez-Sotelo et al. (2011), Trevisi et al. (2015), and Williams et al. (2016). However, others have found that supplementing the live yeast *S. cerevisiae* has increased growth parameters such as ADG, ADFI, and BW (Shen et al., 2009; Kiarie et al., 2011; Kiros et al., 2018). As previously discussed, the results from the dietary addition of yeast additives are inconsistent and variable (Zimmerman et al., 2016;). For example, in two experiments conducted by van Heugten et al. (2003), there was no added benefit for any growth criteria when pigs were fed *S. cerevisiae* in the first experiment; however, in the second experiment, they observed heavier BW and increased ADG when the live yeast was supplemented with antibiotics and pharmacological Zn and Cu compared to when yeast was not included. The variability in literature regarding growth performance and inclusion of yeast additives can be attributed to multiple factors. Some of these factors may include yeast strain, inclusion rate, and/or the duration of feeding the yeast additive(s), product inconsistency, as well as external factors such as genetics, herd health status, and general stockmanship (Liao and Nyachoti, 2017).

The five main concerns to the dietary addition of pharmacological levels of ZnO are environmental pollution, co-selection of resistance to antibiotics that are important to human and animal medicine, heavy metal tolerance of gut bacteria, microflora modification in the gut, and Zn toxicity to the pig (Bonetti et al., 2021). Because of these concerns, the European Union had put a ban on feeding pharmacological levels of Zn beginning in June of 2022 with the legal limit being 150 mg/kg of Zn in a complete feed (EMA, 2017). In a study on the effect of heavy metals in liquid swine manure on AMR, Hölzel et al. (2012) observed that Zn was linked to the resistance of doxycycline, tetracycline, piperacillin, ampicillin, and multi- drug resistant *E. coli*. Multi-drug resistance is characterized when the bacteria are resistant to three or more antimicrobial classes (Schwarz et al., 2010). Furthermore, Bednorz et al. (2013) observed that 18.6% of *E. coli* isolates, cultured from digesta originating from the ileum of nursery pigs, were multi-drug resistant when pigs were fed 2,500 mg/kg of Zn while no multi-drug resistant isolates were identified for pigs fed a diet containing 50 mg/kg of Zn. There are several other studies that report increased prevalence of AMR genes when pharmacological Zn is included in the diet (Slifierz et al., 2015; Ciesinksi et al., 2018; Muurinen et al., 2021). Conversely, we observed that the inclusion of pharmacological Zn had little impact on the AMR to 13 of the 14 antibiotics tested. When MIC values were averaged across the three sampling time points, fecal *E. coli* tended to be more resistant to ciprofloxacin when pigs were fed pharmacological levels of Zn for the first 21 d post-weaning; however, all isolates were still considered susceptible based off its CLSI (2018) breakpoint. Ciprofloxacin is a fluoroquinolone class of antimicrobial that is not approved for use in food animals. However, ciprofloxacin is a broad-spectrum antibiotic in human medicine used to treat both Gram-negative and Gram-positive bacterial infections (Davis et al., 1996).

To the best of our knowledge, there are limited data evaluating yeast-based pre- and probiotics' impact on the AMR of gut bacteria. However, Ouwehand et al. (2016) wrote a comprehensive review on bacterial probiotics potential to prevent AMR which concluded that it is still unknown if probiotics could prevent the development and spread of AMR organisms. Nonetheless, it was hypothesized that there could be reduced persistence and evolution of AMR because probiotics can positively modulate gut bacteria and reduce enteric pathogens, thus reducing the need for antibiotic interventions to control diarrhea. Williams et al. (2018) observed that the direct fed microbial (DFM) blend of *Bacillus licheniformis* and *Bacillus subtilus* or DFM blend of *Enterococcus faecium*, *Lactobacillus reuteri*, *Lactobacillus salivarius*, and *Pediococcus acidilactici* had no impact on the AMR of nursery pig fecal *E. coli* isolates to the same 14 antimicrobials that were evaluated in this study. Similarly, we observed that dietary addition of live yeast and yeast extracts had no impact on the AMR of 14 antibiotics that are important to human and animal health.

In conclusion, adding pharmacological levels of Zn proved to be a useful additive to stimulate intake, increase growth, and improve fecal consistency in the early nursery period. Although feeding high levels of Zn did tend to increase the MIC of fecal *E. coli* to ciprofloxacin, all fecal *E. coli* isolates were well under the CLSI (2018) resistance breakpoint. Thus, the short-term use of pharmacological levels of Zn did not increase antimicrobial

resistance. There was no statistical response observed from the dietary addition of live yeast and yeast extracts for any of the growth, economic, fecal DM, or AMR profiles of fecal *E. coli*.

Acknowledgments

Contribution no. 22-035-J of the Kansas Agricultural Experiment Station, Manhattan, 66506-0201. We would like to thank Phileo by Lesaffre, Milwaukee, WI, for providing the yeast additives and partial financial support of this study.

Conflict of interest statement

The authors declare no conflict of interest; however, J.A.L. and B.H. are employees of Phileo by Lesaffre, the company who provided partial financial support for this project.

Literature Cited

Amachawadi, R. G., F. Giok, X. Shi, J. Soto, S. K. Narayanan, M. D. Tokach, M. D. Apley, and T. G. Nagaraja. 2018. Antimicrobial resistance of *Enterococcus faecium* strains isolated from commercial probiotic products used in cattle and swine. *J. Anim. Sci.* **96**:912–920. doi:10.1093/jas/sky056

AOAC International. 1998. *Official methods of analysis of AOAC International*. 8th ed. Arlington (VA): Assoc. Off. Anal. Chem.

AOAC International. 2019. *Official methods of analysis of AOAC International*. 21st ed. Arlington (VA): Assoc. Off. Anal. Chem.

Badia, R., R. Lizardo, P. Martinez, I. Badiola, and J. Brufau. 2012. The influence of dietary locust bean gum and live yeast on some digestive immunological parameters of piglets experimentally challenged with *Escherichia coli*. *J. Anim. Sci.* **90**(Suppl 4):260–262. doi:10.2527/jas.53894

Bajagai, Y., A. Klieve, P. Dart, and W. Bryden. 2016. *Probiotics in animal nutrition: production, impact and regulation*. Rome (Italy): FAO. http://www.fao.org/3/i5933e/i5933e.pdf

Bednorz, C., K. Oelgeschläger, B. Kinnemann, S. Hartmann, K. Neumann, R. Pieper, A. Bethe, T. Semmler, K. Tedin, P. Schierack, *et al.* 2013. The broader context of antibiotic resistance: zinc feed supplementation of piglets increases the proportion of multi-resistant *Escherichia coli* in vivo. *Int. J. Med. Microbiol.* **303**:396–403. doi:10.1016/j.ijmm.2013.06.004

Bonetti, A., B. Tugnoli, A. Piva, and E. Grilli. 2021. Towards zero zinc oxide: feeding strategies to manage post-weaning diarrhea in piglets. *Ani.* **11**:642. doi:10.3390/ani11030642.

Campbell, C. R., and C. O. Plank. 1991. Sample preparation. In: C. Owen, editor, *Plant analysis reference procedures for the southern region of the United States*. Southern Cooperative Series Bulletin 368. Athens, GA: University of Georgia; p. 1–11. http://www.cropsoil.uga.edu/~oplank/sera368.pdf

Che, L., Q. Xu, C. Wu, Y. Luo, X. Huang, B. Zhang, E. Auclair, T. Kiros, Z. Fang, Y. Lin, *et al.* 2017. Effects of dietary live yeast supplementation on growth performance, diarrhoea severity, intestinal permeability and immunological parameters of weaned piglets challenged with enterotoxigenic *Escherichia coli* K88. *Br. J. Nutr.* **118**:949–958. doi:10.1017/S0007114517003051

Ciesinski, L., S. Guenther, R. Pieper, M. Kalisch, C. Bednorz, and L. H. Wieler. 2018. High dietary zinc feeding promotes persistence of multi-resistant *E. coli* in the swine gut. *PLoS One* **13**:e0191660. doi:10.1371/journal.pone.0191660

Clinical and Laboratory Standards Institute (CLSI). 2018. *Performance standards for antimicrobial disk and dilution susceptibility tests for bacteria isolated from animals*. Approved standard, 5th ed. CLSI supplement VET08. Wayne (PA): CLSI

Davis, R., A. Markham, and J. A. Balfour. 1996. Ciprofloxacin. *Drugs*. **51**:1019–1074. doi:10.2165/00003495-199651060-00010

Doyle, M. E. 2001. *Alternatives to antibiotic use for growth promotion in animal husbandry*. Food Research Institute. Available from https://www.iatp.org/sites/default/files/Alternatives_to_Antibiotic_Use_for_Growth_Prom.pdf.

EMA. 2017. *CVMP. EMA—Zinc Oxide—Annex II—Scientific Conclusions and Grounds for the Refusal of the Marketing Authorization and for Withdrawal of the Existing Marketing Authorizations*. Amsterdam (The Netherlands): EMEA.

Flohr, J. R., J. M. DeRouchey, J. C. Woodworth, M. D. Tokach, R. D. Goodband, and S. S. Dritz. 2016. A survey of current feeding regimens for vitamins and trace minerals in the US swine industry. *J. Swine. Health. Prod.* **24**:290–303.

Gibson, G. R., H. M. Probert, J. V. Loo, R. A. Rastall, and M. B. Roberfroid. 2004. Dietary modulation of the human colonic microbiota: updating the concept of prebiotics. *Nutr. Res. Rev.* **17**:259–275. doi:10.1079/NRR200479

Hahn, J. D., nd D. H. Baker. 1993. Growth and plasma zinc responses of young pigs fed pharmacological levels of zinc. *J. Anim. Sci.* **71**:3020–3024. doi:10.2527/1993.71113020x

Hill, G. M., G. L. Cromwell, T. D. Crenshaw, C. R. Dove, R. C. Ewan, D. A. Knabe, A. J. Lewis, G. W. Libal, D. C. Mahan, G. C. Shurson, *et al.* 2000. Growth promotion effects and plasma changes from feeding high dietary concentrations of zinc and copper to weanling pigs (regional study). *J. Anim. Sci.* **78**:1010–1016. doi:10.2527/2000.7841010x

Hölzel, C. S., C. Müller, K. S. Harms, S. Mikolajewski, S. Schäfer, K. Schwaiger, and J. Bauer. 2012. Heavy metals in liquid pig manure in light of bacterial antimicrobial resistance. *Environ. Res.* **113**:21–27. doi:10.1016/j.envres.2012.01.002

Jacela, J. Y., J. M. DeRouchey, M. D. Tokach, R. D. Goodband, J. L. Nelssen, D. G. Renter, and S. S. Dritz. 2010. Feed additives for swine: fact sheets –high dietary levels of copper and zinc for young pigs, and phytase. *J. Swine Health Prod.* **18**:87–91.

Kiarie, E., S. Bhandari, M. Scott, D. O. Krause, and C. M. Nyachoti. 2011. Growth performance and gastrointestinal microbial ecology responses of piglets receiving *Saccharomyces cerevisiae* fermentation products after an oral challenge with *Escherichia coli* (K88). *J. Anim. Sci.* **89**:1062–1078. doi:10.2527/jas.2010-3424

Kiros, T. G., H. Derakhshani, E. Pinloche, R. D'Inca, J. Marshall, E. Auclair, E. Khafipour, and A. Van Kessel. 2018. Effect of live yeast *Saccharomyces cerevisiae* (Actisaf Sc 47) supplementation on the performance and hindgut microbiota composition of weanling pigs. *Sci. Rep.* **8**:5315. doi:10.1038/s41598-018-23373-8

Kogan, G., and A. Kocher. 2007. Role of yeast cell wall polysaccharides in pig nutrition and health protection. *Livest. Sci.* **109**:161–165. doi:10.1016/j.livsci.2007.01.134

Liao, S. F., and M. Nyachoti. 2017. Using probiotics to improve swine gut health and nutrient utilization. *Anim. Nutr.* **3**:331–343. doi:10.1016/j.aninu.2017.06.007

Liu, Y., C. D. Espinosa, J. J. Abelilla, G. A. Casas, L. V. Lagos, S. A. Lee, W. B. Kwon, J. K. Mathai, D. M. D. L. Navarro, N. W. Jaworski, *et al.* 2018. Non-antibiotic feed additives in diets for pigs: a review. *Anim. Nutr.* **4**:113–125. doi:10.1016/j.aninu.2018.01.007

Mateo, C. D. 2005. *Aspects of nucleotide nutrition in pigs*. PhD. Dissertation. Brookings (SD): South Dakota State University.

Muurinen, J., J. Richert, C. L. Wickware, B. Richert, and T. A. Johnson. 2021. Swine growth promotion with antibiotics or alternatives can increase antibiotic resistance gene mobility potential. *Sci. Rep.* **11**:5485. doi:10.1038/s41598-021-84759-9

National Research Council. 2012. *Nutrient requirements of swine*. 11th revised ed. Washington, DC: The National Academies Press. doi:10.17226/13298

Nguyen, C. C., C. N. Hugie, M. L. Kile, and T. Navab-Daneshmand. 2019. Association between heavy metals and antibiotic-resistant human pathogens in environmental reservoirs: a review. *Front. Environ. Sci. Eng.* **13**:46. doi:10.1007/s11783-019-1129-0

Ouwehand, A. C., S. Forssten, A. A. Hibberd, A. Lyra, and B. Stahl. 2016. Probiotic approach to prevent antibiotic resistance. *Ann. Med.* **48**:246–255. doi:10.3109/07853890.2016.1161232

Perez-Sotelo, L., G. Vaughan, R. Fajardo, Y. Gonzalez, H. Monroy, and J. C. Vazquez. 2011. Modulator effects of dietary supplementation with *Saccharomyces Cerevisiae* on Coliform counts, adaptive general immunologic response and growth-performance in pigs. *Indian. J. Anim. Nutr.* **28**:191–197.

Pollmann, D. S., D. M. Danielson, and E. R. Peo. 1990. Effects of microbial feed additives on performance of starter and growing-finishing pigs. *J. Anim. Sci.* **65**:577–581. doi:10.2527/jas1980.513577x

Reese, D. E., and G. M. Hill. 2010. *National Swine nutrition guide: trace minerals and vitamins for Swine diets*. Ames, IA: U.S. Pork Center of Excellence.

Sales, J. 2013. Effects of pharmacological concentrations of dietary zinc oxide on growth of post-weaning pigs: a meta-analysis. *Biol. Trace Elem. Res.* **152**:343–349. doi:10.1007/s12011-013-9638-3

Schwarz, S., P. Silley, S. Simjee, N. Woodford, E. van Duijkeren, A. P. Johnson, and W. Gaastra. 2010. Editorial: assessing the antimicrobial susceptibility of bacteria obtained from animals. *J. Antimicrob. Chemother.* **65**:601–604. doi:10.1093/jac/dkq037

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. doi:10.2527/jas.2008-1512

Slifierz, M. J., R. Friendship, and J. S. Weese. 2015. Zinc oxide therapy increases prevalence and persistence of methicillin-resistant *Staphylococcus aureus* in pigs: a randomized controlled trial. *Zoonoses Public Health* **62**:301–308. doi:10.1111/zph.12150

Srobárová, A., G. Kogan, and S. Eged. 2005. Yeast polysaccharide affects fusaric acid content in maize root rot. *Chem. Biodivers.* **2**:1685–1690. doi:10.1002/cbdv.200590138

Stein, H. H., and D. Y. Kil. 2006. Reduced use of antibiotic growth promoters in diets fed to weanling pigs: dietary tools, part 2. *Anim. Biotechnol.* **17**:217–231. doi:10.1080/10495390600957191

Stuyven, E., E. Cox, S. Vancaeneghem, S. Arnouts, P. Deprez, and B. M. Goddeeris. 2009. Effect of beta-glucans on an ETEC infection in piglets. *Vet. Immunol. Immunopathol.* **128**:60–66. doi:10.1016/j.vetimm.2008.10.311

Trckova, M., M. Faldyna, P. Alexa, Z. Sramkova Zajacova, E. Gopfert, D. Kumprechtova, E. Auclair, and R. D'Inca. 2014. The effects of live yeast *Saccharomyces cerevisiae* on postweaning diarrhea, immune response, and growth performance in weaned piglets. *J. Anim. Sci.* **92**:767–774. doi:10.2527/jas.2013-6793

Trevisi, P., M. Colombo, D. Priori, L. Fontanesi, G. Galimberti, G. Calò, V. Motta, R. Latorre, F. Fanelli, M. Mezzullo, *et al.* 2015. Comparison of three patterns of feed supplementation with live *Saccharomyces cerevisiae* yeast on postweaning diarrhea, health status, and blood metabolic profile of susceptible weaning pigs orally challenged with *Escherichia coli* F4ac. *J. Anim. Sci.* **93**:2225–2233. doi:10.2527/jas.2014-8539

Trevisi, P., R. Latorre, D. Priori, D. Luise, I. Archetti, M. Mazzoni, R. D'Inca, and P. Bosi. 2017. Effect of feed supplementation with live yeast on the intestinal transcriptome profile of weaning pigs orally challenged with *Escherichia coli* F4. *Animal* **11**:33–44. doi:10.1017/S1751731116001178

van Heugten, E., D. W. Funderburke, and K. L. Dorton. 2003. Growth performance, nutrient digestibility, and fecal microflora in weanling pigs fed live yeast. *J. Anim. Sci.* **81**:1004–1012. doi:10.2527/2003.8141004x

Williams, H. E., J. C. Woodworth, J. M. DeRouchey, S. S. Dritz, M. D. Tokach, and R. D. Goodband. 2016. Effects of evosure on nursery pig performance. Kansas agricultural experiment station research reports. **2**:17. doi:10.4148/2378-5977.1294

Williams, H. E., M. D. Tokach, S. S. Dritz, J. C. Woodworth, J. M. DeRouchey, T. G. Nagaraja, R. D. Goodband, J. R. Pluske, K. Chitakasempornkul, N. M. Bello, *et al.* 2018. Effects of chlortetracycline alone or in combination with direct fed microbials on nursery pig growth performance and antimicrobial resistance of fecal *Escherichia coli*. *J. Anim. Sci.* **96**:5166–5178. doi:10.1093/jas/sky370

World Health Organization. 2018. *Critically important antimicrobials for human medicine*. 6th rev ed. Geneva (Switzerland): WHO Document Production Services.

Yazdankhah, S., K. Rudi, and A. Bernhoft. 2014. Zinc and copper in animal feed – development of resistance and co-resistance to antimicrobial agents in bacteria of animal origin. *Microb. Ecol. Health Dis.* **25**:25862. doi:10.3402/mehd.v25.25862

Yiannikouris, A., J. François, L. Poughon, C. G. Dussap, G. Bertin, G. Jeminet, and J. P. Jouany. 2004. Adsorption of zearalenone by β-D-glucans in the *Saccharomyces cerevisiae* cell wall. *J. Food Prot.* **67**:1195–1200. doi:10.4315/0362-028X-67.6.1195

Yu, I. T., J. F. Wu, P. C. Wang, C. Y. Liu, D. N. Lee, and H. T. Yen. 2002. Roles of glutamine and nucleotides in combination in growth, immune response, and FMD antibody titres of weaned pigs. *Anim. Sci.* **75**:379–385. doi:10.1017/S1357729800053157

Zanello, G., F. Meurens, M. Berri, C. Chevaleyre, S. Melo, E. Auclair, and H. Salmon. 2011. *Saccharomyces cerevisiae* decreases inflammatory responses induced by F4+ enterotoxigenic *Escherichia coli* in porcine intestinal epithelial cells. *Vet. Immunol. Immunopathol.* **141**:133–138. doi:10.1016/j.vetimm.2011.01.018

Zhang, Y., J. Zhou, Z. Dong, G. Li, J. Wang, Y. Li, D. Wan, H. Yang, and Y. Yin. 2019. Effect of dietary copper on intestinal microbiota and antimicrobial resistance profiles of *Escherichia coli* in weaned piglets. *Front. Microbiol.* **10**:2808. doi:10.3389/fmicb.2019.02808

Zimmermann, J. A., M. L. Fusari, E. Rossler, J. E. Blajman, A. Romero-Scharpen, D. M. Astesana, C. R. Olivero, A. P. Berisvil, M. L. Signorini, M. V. Zbrun, L. S. Frizzo, and L. P. Soto. 2016. Effects of probiotics in swine growth performance: a meta-analysis of randomized controlled trials. *Anim. Feed Sci. Tech.* **219**:280–293. doi:10.1016/j.anifeedsci.2016.06.021