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The Effect of Live Yeast and Yeast Extracts on Antimicrobial Susceptibilities of Fecal *Escherichia coli* of Nursery Pigs Weaned from Sows Fed Diets with or without Yeast Additives

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Summary

A total of 340 weaned pigs (Line 241 × 600, DNA; initially 11.2 lb BW) were used in a 45-d study to evaluate previous sow treatment (control vs. yeast additives) and nursery diets with or without added yeast-based pre- and probiotics (Phileo by Lesaffre, Milwaukee, WI) on antimicrobial resistance (AMR) patterns of fecal *Escherichia coli*. At placement in the nursery, pigs were housed by pen based on sow treatment and randomly assigned to 1 of 2 dietary treatments with 5 pigs per pen and 17 pens per treatment. Treatments were arranged in a 2 × 2 factorial with main effects of sow treatment (control vs. yeast-based pre- and probiotic diet; 0.10% ActiSaf Sc 47 HR+ and 0.025% SafMannan) and nursery treatment (control vs. yeast-based pre- and probiotic diet; 0.10% ActiSaf Sc 47 HR+, 0.05% SafMannan, and 0.05% NucleoSaf from d 0 to 7, then concentrations were lowered by 50% from d 7 to 24). All pigs were fed a common diet from d 24 to 45 post-weaning. The *E. coli* was isolated from fecal samples and species confirmation was accomplished by PCR detection of *uidA* and *clpB* genes. Microbroth dilution method (Sensititre CMV3AGNF panel plates) was used to determine the minimum 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 guidelines. A three-way interaction of sow treatment × nursery treatment × sampling day was observed ($P < 0.05$) for ciprofloxacin, gentamicin, sulfisoxazole, and trimethoprim/sulfamethoxazole. Fecal *E. coli* isolated from pigs of the yeast-supplemented sow group had increased ($P = 0.034$) MIC to nalidixic acid and a tendency for increased MIC to ciprofloxacin ($P = 0.065$) and gentamicin ($P = 0.054$). Yet, when yeast additives were fed in the nursery there was reduced ($P < 0.05$) fecal *E. coli* AMR to azithromycin and

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chloramphenicol. All fecal *E. coli* isolates were considered susceptible to all antimicrobials, except tetracycline on d 5. In conclusion, feeding sows live yeast and yeast extracts could potentially impact fecal *E. coli* AMR in their progeny. Furthermore, feeding live yeast and yeast additives in the nursery may alleviate the AMR of azithromycin and chloramphenicol of *E. coli* isolated from nursery pig fecal material.

Introduction

Yeast-based pre- and probiotics have been considered a potential alternative to in-feed antibiotics and pharmacological levels of zinc in the nursery because of their ability to positively modulate gut microflora, which may lead to improved immunity, nutrient digestion and absorption, and growth performance.⁵ This report is a companion to our previous study where we evaluated the effects of the live yeast *Saccharomyces cerevisiae* strain NCYC Sc 47 and yeast-based prebiotics derived from *Saccharomyces cerevisiae* on nursery pigs weaned from sows fed a diet with or without yeast additives on weanling pig growth performance.⁶ The objective of this study was to evaluate effects of the live yeast *Saccharomyces cerevisiae* strain NCYC Sc 47 and yeast-based prebiotics derived from *Saccharomyces cerevisiae* on nursery pigs weaned from sows fed a diet with or without yeast additives extracts on the AMR patterns for *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 Swine Teaching and Research Center in Manhattan, KS. The facility is completely enclosed, environmentally controlled, and mechanically ventilated. Each pen contained a 4-hole, dry self-feeder and a nipple waterer to provide *ad libitum* access to feed and water. Pens (4 × 4 ft) had metal tri-bar floors and allowed approximately 2.7 ft²/pig.

Animals and treatment structure

A total of 340 weaned pigs (DNA 241 × 600, DNA; initially 11.2 ± 0.07 lb BW), offspring of sows fed either a control diet or a diet containing yeast-based pre- and probiotics from d 110 of gestation through weaning, were used in a 45-d nursery study with 5 pigs per pen and 17 pens (replications) per treatment. Details regarding pig allotment, experimental design, and diet preparation can be found in Chance et al.⁶

Briefly, dietary treatments were arranged in a 2 × 2 factorial with sow treatment (control vs. yeast additives; 0.10% ActiSaf Sc 47 HR+ and 0.025% SafMannan; Phileo by Lesaffre, Milwaukee, WI) and nursery treatment (control vs. yeast additives; 0.10% ActiSaf Sc 47 HR+, 0.05% SafMannan, and 0.05% NucleoSaf from d 0 to 7 then concentrations were lowered by 50% from d 7 to 24; Phileo by Lesaffre, Milwaukee,

⁵ Menegat, M. B., R. D. Goodband, J. M. DeRouchey, M. D. Tokach, J. C. Woodworth, and S. S. Dritz. 2019. Kansas State University Swine Nutrition Guide: Feed Additives in Swine Diets.

⁶ Chance, J. A., J. T. Gebhardt, J. M. DeRouchey, M. D. Tokach, J. C. Woodworth, R. D. Goodband, and J. A. Loughmiller. 2021. The Effect of Live Yeast and Yeast Extracts on Growth Performance of Nursery Pigs Weaned from Sows Fed Diets with or without Yeast Additives. *Kansas Experimental Station Research Reports*: Vol. 7, Issue 11.

WI). Thus, half of the pigs from each sow group was fed either a control diet or a diet with yeast additives. The live yeast *Saccharomyces cerevisiae* strain NCYC Sc 47 (ActiSaf Sc 47 HR+) served as the yeast-based probiotic. The yeast-based prebiotics included a yeast cell wall fraction with concentrated mannan-oligosaccharides and β -glucans from *Saccharomyces cerevisiae* (SafMannan) and a yeast extract containing $\geq 6\%$ unbound nucleotides from *Saccharomyces cerevisiae* (NucleoSaf).

Fecal collection

Fecal samples were collected on d 5, 24, and 45 of the experiment for antimicrobial resistance profiles of fecal *E. coli*. 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 and kept on ice until delivered to the laboratory on the same day of collection. Fecal samples were transported to the laboratory of Dr. Raghavendra Amachawadi at the Kansas State University College of Veterinary Medicine for bacterial isolation and antimicrobial susceptibility testing.

E. coli isolation

Approximately 1 g of fecal sample was suspended in 9 mL of phosphate-buffered saline. Fifty microliters of the fecal suspension were then spread-plated onto a MacConkey agar (Becton Dickinson, Sparks, MD) for the isolation of *E. coli*. Two lactose-fermenting colonies were picked from each MacConkey agar; each colony was individually streaked onto a blood agar plate (Remel, Lenexa, KS) and incubated at 98.6°F for 24 h. An indole test was conducted and indole-positive isolates were stored in cryo-protect beads (Cryocare, Key Scientific Products, Round Rock, TX) at -112°F. Species confirmation of *E. coli* was by polymerase chain reaction (PCR) assay for *uidA* and *clpB* genes.

Antimicrobial susceptibility testing of E. coli isolates

Antimicrobial susceptibility testing was done on *E. coli* isolates recovered on days 5, 24, and 45. The microbroth dilution method as outlined by the Clinical and Laboratory Standards Institute (CLSI, 2018)⁷ was used to determine the minimal inhibitory concentrations (MIC) of antibiotics. The antimicrobials evaluated included: amoxicillin/clavulanic acid 2:1 ratio, 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 98.6°F for 24 h. Individual colonies were suspended in demineralized water (Trek Diagnostic Systems, Cleveland, OH) and turbidity was adjusted to 0.5 McFarland turbidity standards. Then, 10 μ L of the bacterial inoculum was added to Mueller–Hinton broth and vortexed to mix. A Sensititre automated inoculation delivery system (Trek Diagnostics Systems) was used to dispense 100 μ L 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) strains were included as quality controls for *E. coli* susceptibility testing. Plates were incubated at 98.6°F for 18 h and bacterial growth was assessed

⁷ 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. CLSI, Wayne, PA.

using Sensititre ARIS and Vizion systems (Trek Diagnostic Systems). Clinical and Laboratory Standards Institute (CLSI, 2018; Table 1) guidelines were used to classify each isolate as susceptible, intermediate, or resistant according to the breakpoints established for each antimicrobial. The MIC values greater than the susceptible breakpoint but lower than the resistant breakpoint were considered intermediate.

Statistical analysis

The MIC data of each antimicrobial were analyzed using a linear mixed model. Fixed effects of the model included sow diet, nursery pig diet, sampling day, and their second- and third-order interactions. Pen was included in the model as a random effect. The variance-covariance structure of pen was taken as either compound symmetry, first-order autoregressive or unstructured according to the model fitting criteria. To better satisfy model assumptions, data underwent natural log transformation before statistical modeling. Treatment effect was assessed via back-transformed least squares means, the geometric means of the MIC values. Statistical analysis was performed using SAS (v. 9.4, SAS Inst.; Cary, NC) PROC MIXED with option DDFM=KR in the MODEL statement. Differences between treatments were considered significant at $P \leq 0.05$ and marginally significant at $0.05 < P \leq 0.10$.

Results and Discussion

A three-way interaction of sow treatment $\times$ nursery treatment $\times$ sampling day was observed ($P < 0.05$) for ciprofloxacin, gentamicin, sulfisoxazole, and trimethoprim/sulfamethoxazole (Table 2). *E. coli* isolated from feces of pigs from sows fed yeast additives and fed yeast-based pre- and probiotics through the nursery had reduced ($P = 0.044$) MIC values to ciprofloxacin on d 45 with a tendency ($P = 0.081$) for reduced AMR on d 24 compared to pigs from the same sow treatment group but fed a control nursery diet. However, there was evidence for a marginal increase ($P = 0.061$) in MIC values of *E. coli* to ciprofloxacin on d 5 from progeny of sows fed yeast which were also fed live yeast and yeast extracts in the nursery. For gentamicin, MIC values of fecal *E. coli* isolated from pigs of the yeast-fed sow and yeast nursery treatment were higher ($P = 0.021$) on d 5 but lower ($P = 0.018$) on d 24 compared to the yeast sow and control nursery treatment. On d 45, *E. coli* isolated from feces collected from progeny of the control sows that were then fed yeast-based pre- and probiotics in the nursery had lower ($P = 0.005$) MIC values to sulfisoxazole compared to pigs that were also from the control sow group but fed a control diet in the nursery. Fecal *E. coli* had lower ($P = 0.004$) MIC values on d 5 to trimethoprim/sulfamethoxazole from the control sow and yeast nursery treatment compared to the control sow and control nursery treatment. It is important to note that all fecal *E. coli* isolates had a low MIC values for ciprofloxacin, gentamicin, sulfisoxazole, and trimethoprim/sulfamethoxazole and thus, all values would be classified as susceptible for each respective antimicrobial. There were no further three- or two-way interactions observed; thus, the main effects of sow treatment, nursery treatment, and sampling day were explored (Table 3).

For the sow portion of this study, regardless of dietary treatment, sow fecal *E. coli* had increased ($P < 0.001$) AMR to tetracycline at weaning compared to AMR at the entry into the farrowing house.⁸ Interestingly, this effect carried over into the nursery. All fecal *E. coli* isolates had significantly ($P < 0.001$) higher MIC values to tetracycline on d 5 post-weaning, which then decreased on d 24 and then slightly increased on d 45. No matter the dietary treatment combination, all *E. coli* isolated were resistant to tetracycline on d 5 but were intermediate on d 24 and 45. Fecal *E. coli* isolates were considered susceptible or intermediate for the remaining 13 antimicrobials at all three sampling timepoints (d 5, 24, and 45) regardless of the sow or nursery treatment's inclusion of live yeast and yeast extracts.

E. coli isolated from feces of the progeny of sows fed yeast-based pre- and probiotics had increased ($P = 0.034$) MIC values to nalidixic acid and a tendency for increased AMR to ciprofloxacin ($P = 0.065$) and gentamicin ($P = 0.054$). Fecal *E. coli* isolates had reduced AMR to azithromycin ($P = 0.037$) and chloramphenicol ($P = 0.031$) when live yeast and yeast extracts were supplemented in the nursery. Again, all fecal *E. coli* isolates would be classified as susceptible or intermediate for each respective antimicrobial as tetracycline was the only antibiotic that displayed resistance in this study.

There was evidence for decreased ($P < 0.05$) AMR over time in fecal *E. coli* for azithromycin, cefoxitin, and streptomycin regardless of yeast-based pre- and probiotic supplementation in the sow or nursery treatment. Axomicillin:clavulanic acid 2:1 ratio, chloramphenicol, and trimethoprim/sulfamethoxazole had increased ($P < 0.10$) MIC values from d 5 to 24 and then reduced MIC values from d 24 to 45. This differs from gentamicin, nalidixic acid, and tetracycline which had reduced ($P < 0.10$) AMR from d 5 to 24 and then an increase in MIC values from d 24 to 45.

In conclusion, progeny from sows that were fed yeast-based pre- and probiotics had increased potential of fecal *E. coli* AMR to nalidixic acid, ciprofloxacin, and gentamicin. Yet, feeding live yeast and yeast extracts in the nursery reduced the AMR of azithromycin and chloramphenicol of fecal *E. coli*. Interestingly, fecal *E. coli* samples isolated from all the weaned pigs were resistant to tetracycline with MIC values decreasing over time. In the sow portion of this study, regardless of dietary treatment, fecal *E. coli* isolated from sows had a significant increase in AMR to tetracycline at weaning compared to entry into the farrowing house. It is important to reiterate that all fecal *E. coli* isolates were considered susceptible or intermediate to the remaining 13 antimicrobials based on CLSI (2018) guidelines.

Brand names appearing in this publication are for product identification purposes only. No endorsement is intended, nor is criticism implied of similar products not mentioned. Persons using such products assume responsibility for their use in accordance with current label directions of the manufacturer.

⁸ Chance, J. A., J. T. Gebhardt, J. M. DeRouche, R. G. Amachawadi, V. Ishengoma, T. G. Nagaraja, M. D. Tokach, J. C. Woodworth, R. D. Goodband, Q. Kang, and J. A. Loughmiller. 2021. The Effect of Live Yeast and Yeast Extracts on Sow and Litter Performance and Sow Antimicrobial Susceptibility of Fecal *Escherichia coli*. *Kansas Experimental Station Research Reports*: Vol. 7, Issue 11.

Table 1. 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 (Clinical and Laboratory Standards Institute (CLSI). 2018. Performance standards for antimicrobial disk and dilution susceptibility tests for bacteria isolated from animals. Approved standards (5th ed. CLSI supplement VET08. CLSI, Wayne, PA), which are categorized as susceptible (treatable), intermediate (possibly treatable with higher doses), and resistant (not treatable). Minimal inhibitory concentration (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).

³N/A = not applicable. The National Antimicrobial Resistance Monitoring System has not established breakpoints; therefore, there is no Clinical and Laboratory Standards Institute resistant breakpoint.

Table 2. Effects of sow and nursery pig dietary treatment over time on antimicrobial susceptibilities of fecal *Escherichia coli* according to National Antimicrobial Resistance Monitoring System (CLSI, 2018) established breakpoints^{1,2}

Sow treatment: ³	Control		Yeast		<i>P</i> =							
Nursery treatment: ⁴	Control	Yeast	Control	Yeast	Sow	Nursery	Day	Sow × nursery	Sow × day	Nursery × day	Sow × nursery × day	
Amoxicillin:clavulanic acid 2:1 ratio ⁵					0.455	0.389	0.024	0.389	0.438	0.656	0.849	
d 5	4.9 ± 1.1	5.1 ± 1.1	6.3 ± 1.3	6.0 ± 1.3								
d 24	6.8 ± 1.5	8.0 ± 1.7	10.2 ± 2.2	8.0 ± 1.7								
d 45	6.8 ± 1.5	5.5 ± 1.2	6.3 ± 1.3	4.5 ± 1.0								
Ampicillin					0.925	0.85	0.191	0.220	0.697	0.226	0.856	
d 5	7.7 ± 2.2	9.0 ± 2.5	7.7 ± 2.2	7.4 ± 2.1								
d 24	7.4 ± 2.1	11.1 ± 3.1	10.2 ± 2.9	12.0 ± 3.4								
d 45	7.7 ± 2.2	6.8 ± 1.9	9.0 ± 2.5	4.3 ± 1.2								
Azithromycin					0.291	0.037	0.034	0.480	0.484	0.909	0.328	
d 5	5.1 ± 0.46	5.1 ± 0.46	5.3 ± 0.48	4.5 ± 0.41								
d 24	4.5 ± 0.32	4.0 ± 0.28	4.5 ± 0.32	4.5 ± 0.32								
d 45	4.2 ± 0.24	4.0 ± 0.23	4.9 ± 0.28	4.2 ± 0.24								
Cefoxitin					0.434	0.372	0.006	0.823	0.352	0.543	0.781	
d 5	10.2 ± 2.0	8.3 ± 1.6	9.4 ± 1.8	9.8 ± 1.9								
d 24	8.0 ± 1.5	8.0 ± 1.5	10.6 ± 2.1	11.1 ± 2.1								
d 45	7.4 ± 1.4	6.0 ± 1.2	7.4 ± 1.4	5.3 ± 1.0								
Ceftiofur					0.438	0.877	0.962	0.485	0.708	0.374	0.073	
d 5	0.96 ± 0.30	0.64 ± 0.20	0.69 ± 0.22	1.70 ± 0.53								
d 24	0.92 ± 0.29	0.88 ± 0.28	0.96 ± 0.30	1.08 ± 0.34								
d 45	0.92 ± 0.29	0.92 ± 0.29	1.28 ± 0.40	0.61 ± 0.19								
Ceftriaxone					0.687	0.762	0.279	0.481	0.194	0.519	0.509	
d 5	0.42 ± 0.19	0.48 ± 0.21	0.82 ± 0.36	1.13 ± 0.50								
d 24	1.04 ± 0.46	1.13 ± 0.50	0.96 ± 0.43	0.88 ± 0.39								
d 45	0.69 ± 0.31	0.78 ± 0.35	0.96 ± 0.43	0.33 ± 0.15								

continued

Table 2. Effects of sow and nursery pig dietary treatment over time on antimicrobial susceptibilities of fecal *Escherichia coli* according to National Antimicrobial Resistance Monitoring System (CLSI, 2018) established breakpoints^{1,2}

Sow treatment: ³	Control		Yeast		<i>P</i> =							
Nursery treatment: ⁴	Control	Yeast	Control	Yeast	Sow	Nursery	Day	Sow × nursery	Sow × day	Nursery × day	Sow × nursery × day	
Chloramphenicol					0.299	0.031	<0.001	0.136	0.966	0.180	0.701	
d 5	9.0 ± 0.97	7.1 ± 0.76	9.0 ± 0.97	6.5 ± 0.70								
d 24	9.4 ± 1.01	11.1 ± 1.19	10.2 ± 1.09	8.7 ± 0.93								
d 45	7.4 ± 0.79	7.1 ± 0.76	7.4 ± 0.79	6.3 ± 0.67								
Ciprofloxacin ⁶					0.065	0.557	0.790	0.291	0.419	0.495	0.010	
d 5	0.020 ± 0.0043	0.015 ± 0.0032	0.018 ± 0.0040	0.033 ± 0.0071								
d 24	0.015 ± 0.0032	0.017 ± 0.0037	0.029 ± 0.0062	0.017 ± 0.0037								
d 45	0.018 ± 0.0038	0.025 ± 0.0053	0.028 ± 0.0060	0.015 ± 0.0032								
Gentamicin ⁷					0.054	0.638	< 0.001	0.736	0.379	0.065	0.045	
d 5	0.96 ± 0.210	0.89 ± 0.194	0.96 ± 0.210	2.00 ± 0.437								
d 24	0.48 ± 0.086	0.48 ± 0.086	0.72 ± 0.129	0.39 ± 0.070								
d 45	0.72 ± 0.071	0.61 ± 0.060	0.78 ± 0.077	0.67 ± 0.065								
Nalidixic acid					0.034	0.648	0.075	0.648	0.061	0.551	0.201	
d 5	2.0 ± 0.45	2.0 ± 0.45	3.1 ± 0.71	4.2 ± 0.94								
d 24	2.2 ± 0.13	2.1 ± 0.13	2.4 ± 0.15	2.1 ± 0.13								
d 45	2.2 ± 0.35	3.0 ± 0.49	2.9 ± 0.47	2.5 ± 0.40								
Streptomycin					0.493	0.600	< 0.001	0.444	0.147	0.391	0.393	
d 5	14.2 ± 3.23	21.3 ± 4.86	13.1 ± 2.98	16.0 ± 3.65								
d 24	7.1 ± 2.56	12.5 ± 4.53	11.6 ± 4.17	8.3 ± 3.01								
d 45	6.5 ± 1.68	4.7 ± 1.21	9.0 ± 2.32	9.0 ± 2.32								

continued

Table 2. Effects of sow and nursery pig dietary treatment over time on antimicrobial susceptibilities of fecal *Escherichia coli* according to National Antimicrobial Resistance Monitoring System (CLSI, 2018) established breakpoints^{1,2}

Sow treatment: ³	Control		Yeast		<i>P</i> =						
Nursery treatment: ⁴	Control	Yeast	Control	Yeast	Sow	Nursery	Day	Sow × nursery	Sow × day	Nursery × day	Sow × nursery × day
Sulfisoxazole ⁸					0.881	1.000	0.363	0.159	0.989	0.416	0.035
d 5	67 ± 20	78 ± 24	69 ± 21	85 ± 26							
d 24	48 ± 15	64 ± 20	57 ± 17	57 ± 17							
d 45	109 ± 33	32 ± 10	44 ± 14	78 ± 24							
Tetracycline					0.540	0.624	< 0.001	0.223	0.580	0.985	0.645
d 5	25.1 ± 3.7	30.7 ± 4.5	26.1 ± 3.9	18.8 ± 2.8							
d 24	6.8 ± 1.5	7.4 ± 1.7	8.3 ± 1.9	6.5 ± 1.5							
d 45	8.7 ± 2.1	8.3 ± 2.0	8.3 ± 2.0	8.3 ± 2.0							
Trimethoprim/sulfamethoxazole ^{5,9}					0.781	0.304	0.069	0.973	0.415	0.208	0.042
d 5	0.42 ± 0.126	0.12 ± 0.036	0.24 ± 0.074	0.24 ± 0.074							
d 24	0.28 ± 0.083	0.37 ± 0.111	0.30 ± 0.091	0.21 ± 0.063							
d 45	0.12 ± 0.036	0.18 ± 0.055	0.22 ± 0.068	0.18 ± 0.055							

¹A total of 340 pigs (initially 11.0 or 11.5 ± 0.07 lb) were used in a 45-d nursery trial with 5 pigs per pen and 17 pens per treatment. Pigs were weaned at approximately 19 d of age and allotted to treatment in completely randomized design. Dietary treatments were arranged in a 2 × 2 factorial with sow treatment (control or yeast-based probiotics) and nursery pig treatment (control or yeast-based probiotics). Data were reported as geometric mean of minimal inhibitory concentration (MIC) ± standard error of the mean.

²Fecal samples from the same 3 pigs/pen were collected on d 5, 24, and 45.

³Sow treatment consisted of providing a control diet or a yeast-based pre- and probiotic diet supplemented with ActiSaf Sc 47 HR+ at 0.10% and SafMannan at 0.03% (Phileo by Lesaffre, Milwaukee, WI) from d 110 of gestation until weaning. Sow fecal samples were collected on ~ d 110 of gestation and d 18 post-farrowing.

⁴Nursery treatment consisted of providing a control diet or a yeast-based pre- and probiotic diet supplemented with 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).

⁵The MIC numerator of the ratio was reported for the antimicrobial's amoxicillin:clavulanic acid 2:1 ratio and trimethoprim/sulfamethoxazole 1:19 ratio.

⁶A three-way interaction of sow treatment × nursery treatment × day was observed (*P* = 0.010). On d 24 (*P* = 0.081) and on d 45 (*P* = 0.045), pigs that were fed yeast in the nursery and came from the yeast sow group had reduced MIC values compared to nursery pigs fed a control diet who were also reared from sows fed yeast. There was marginal evidence on d 5 (*P* = 0.061) for the yeast sow group offspring fed yeast additives having increased MIC values compared to pigs fed a control diet who were also offspring of sows fed yeast.

⁷A three-way interaction of sow treatment × nursery treatment × day was observed (*P* = 0.045). The MIC values of fecal *E. coli* isolated from pigs of the yeast sow and yeast nursery treatment were higher (*P* = 0.021) on d 5 but lower (*P* = 0.018) on d 24 compared to the yeast sow and control nursery treatment. There was no evidence for difference (*P* > 0.10) between dietary treatments on d 45.

⁸Three-way interaction of sow treatment × nursery treatment × day was observed (*P* = 0.035). On d 45, pigs that came from the control sow treatment and yeast nursery treatment had lower (*P* = 0.005) MIC values compared to pigs that were also from the control sow group but fed a control diet in the nursery. There was no evidence for difference (*P* > 0.10) between dietary treatments on d 5 or d 24.

⁹Three-way interaction of sow treatment × nursery treatment × day was observed (*P* = 0.042). On d 5, pigs that came from the control sow treatment and yeast nursery treatment had lower (*P* = 0.004) MIC values compared to the control sow and control nursery treatment. There was no evidence for difference (*P* > 0.10) between dietary treatments on d 24 or d 45.

Table 3. Main effects of sow and nursery pig dietary treatment over time on antimicrobial susceptibilities of fecal *Escherichia coli* according to National Antimicrobial Main Resistance Monitoring System (CLSI, 2018) established breakpoints^{1,2}

Item	Sow treatment ³			Nursery treatment ⁴			Day			
	Control	Yeast	P =	Control	Yeast	P =	5	24	45	P =
Amoxicillin:clavulanic acid 2:1 ratio ⁵	6.1 ± 0.51	6.7 ± 0.55	0.455	6.7 ± 0.56	6.1 ± 0.50	0.389	5.5 ± 0.59 ^a	8.2 ± 0.87 ^b	5.7 ± 0.61 ^a	0.024
Ampicillin	8.2 ± 0.83	8.1 ± 0.82	0.925	8.2 ± 0.83	8.0 ± 0.81	0.850	7.9 ± 1.1	10.0 ± 1.4	6.7 ± 0.9	0.191
Azithromycin	4.5 ± 0.12	4.7 ± 0.13	0.291	4.7 ± 0.13	4.4 ± 0.12	0.037	5.0 ± 0.23 ^b	4.4 ± 0.16 ^a	4.3 ± 0.12 ^a	0.034
Cefoxitin	7.9 ± 0.67	8.7 ± 0.74	0.434	8.7 ± 0.75	7.8 ± 0.67	0.372	9.4 ± 0.91 ^b	9.3 ± 0.90 ^b	6.5 ± 0.62 ^a	0.006
Ceftiofur	0.87 ± 0.11	0.99 ± 0.12	0.438	0.94 ± 0.12	0.92 ± 0.11	0.877	0.92 ± 0.14	0.96 ± 0.15	0.90 ± 0.14	0.962
Ceftriaxone	0.71 ± 0.14	0.79 ± 0.15	0.687	0.78 ± 0.15	0.72 ± 0.14	0.762	0.66 ± 0.15	1.00 ± 0.22	0.65 ± 0.14	0.279
Chloramphenicol	8.4 ± 0.35	7.9 ± 0.33	0.299	8.7 ± 0.36	7.6 ± 0.32	0.031	7.8 ± 0.42 ^a	9.8 ± 0.52 ^b	7.0 ± 0.37 ^a	< 0.001
Ciprofloxacin	0.018 ± 0.0015	0.022 ± 0.0018	0.065	0.021 ± 0.0017	0.019 ± 0.0016	0.557	0.021 ± 0.0022	0.019 ± 0.0020	0.021 ± 0.0022	0.790
Gentamicin	0.67 ± 0.047	0.81 ± 0.058	0.054	0.75 ± 0.053	0.72 ± 0.051	0.638	1.13 ± 0.124 ^c	0.51 ± 0.045 ^a	0.69 ± 0.034 ^b	< 0.001
Nalidixic acid	2.2 ± 0.16	2.8 ± 0.20	0.034	2.4 ± 0.18	2.5 ± 0.19	0.648	2.7 ± 0.30 ^b	2.2 ± 0.07 ^a	2.6 ± 0.21 ^b	0.075
Streptomycin	9.7 ± 1.1	10.9 ± 1.3	0.493	9.8 ± 1.2	10.7 ± 1.3	0.600	15.8 ± 1.8 ^b	9.6 ± 1.7 ^a	7.1 ± 0.9 ^a	< 0.001
Sulfisoxazole	61.9 ± 7.9	63.6 ± 8.1	0.881	62.7 ± 8.0	62.7 ± 8.0	1.000	74.6 ± 11.4	56.1 ± 8.6	59.0 ± 9.0	0.363
Tetracycline	11.9 ± 0.93	11.1 ± 0.87	0.540	11.8 ± 0.92	11.2 ± 0.87	0.624	24.8 ± 1.83 ^b	7.2 ± 0.82 ^a	8.4 ± 1.03 ^a	< 0.001
Trimethoprim/ Sulfamethoxazole ⁵	0.22 ± 0.028	0.23 ± 0.029	0.781	0.25 ± 0.031	0.20 ± 0.026	0.304	0.23 ± 0.035 ^b	0.28 ± 0.043 ^b	0.17 ± 0.026 ^a	0.069

¹A total of 340 pigs (initially 11.0 or 11.5 ± 0.07 lb) were used in a 45-d nursery trial with 5 pigs per pen and 17 pens per treatment. Pigs were weaned at approximately 19 d of age and allotted to treatment in completely randomized design. Dietary treatments were arranged in a 2 × 2 factorial with main effects of sow treatment (control or yeast-based probiotics) and nursery pig treatment (control or yeast-based probiotics). Data reported as geometric mean of minimal inhibitory concentration (MIC) ± standard error of the mean.

²Fecal samples from the same 3 pigs/pen were collected on d 5, 24, and 45. Sow fecal samples were collected on ~ d 110 of gestation and d 18 post-farrowing.

³Sow treatment consisted of providing a control diet or a yeast-based pre- and probiotic diet supplemented with ActiSaf Sc 47 HR+ at 0.10% and SafMannan at 0.03% (Phileo by Lesaffre, Milwaukee, WI) from d 110 of gestation until weaning.

⁴Nursery treatment consisted of providing a control diet or a yeast-based pre- and probiotic diet supplemented with 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).

⁵The MIC numerator of the ratio was reported for the antimicrobial's amoxicillin:clavulanic acid 2:1 ratio and trimethoprim/sulfamethoxazole.