The effect of feeding live yeast and yeast extracts on growth performance and antimicrobial susceptibility of fecal *Escherichia coli* in sows and nursery pigs

by

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Abstract

This thesis is comprised of 3 chapters involving studies evaluating live yeast and yeast extracts with or without the addition of pharmacological levels of Zn, feeding sows live yeast and a yeast extract and following their offspring into the nursery where progeny were fed varying inclusions of yeast additives and direct fed microbials (DFM). Chapter 1 involved 360 weanling barrows to determine the effects of feeding live yeast and yeast extracts with or without pharmacological Zn on nursery pig growth performance, fecal dry matter (DM), and antimicrobial susceptibilities of fecal *E.coli*. The results suggest that feeding pharmacological Zn for 21-d post-weaning is an effective strategy to optimize growth performance, economic criteria, and increase fecal DM for the first few days following weaning. Although all isolates were classified as susceptible to ciprofloxacin, the minimal inhibitory concentration (MIC) of fecal *E.coli* tended to be increased when pigs were fed pharmacological levels of Zn but no difference was observed to the remaining thirteen antimicrobials that were evaluated. Thus, the short-term use of pharmacological levels of Zn did not increase antimicrobial resistance (AMR). There was no benefit for any of the growth, economic, fecal DM, or AMR response criteria when live yeast and yeast extracts were included in the diets. Chapter 2 involved three batch-farrowing groups where 80 sows were used to determine the effects of feeding live yeast and a yeast extract's impact on sow and litter performance. One of the three sow groups (27 sows) were used to determine the yeast additives impact on the antimicrobial susceptibility of sow fecal *E.coli*. The results suggest that feeding live yeast and a yeast extract, from d 110 of gestation through lactation, may increase sow feed intake but had no impact on any other sow or litter performance criteria. A diet by sampling day interaction revealed that fecal *E.coli* isolates gained resistance to the antimicrobial cefoxitin over time when the yeast additives were included in the diet, but the

main effect of diet had no impact on any of the fourteen antibiotics tested. Chapter 3 consisted of two experiments which used 670 weaned pigs to evaluate previous sow treatment (control vs yeast additives) and nursery diets with varying combinations of yeast additives and DFM. In Exp. 1, subsequent offspring from one of the sow groups in chapter 2 were fed either a control diet or a diet that contained live yeast and yeast extracts to evaluate growth performance and the AMR patterns of fecal *E.coli*. In Exp 2., subsequent offspring from one of the sow groups in chapter 2 were fed either a control diet, a diet containing yeast extracts (DFM 1), or a diet with *Bacillus* spp. and yeast extracts (DFM 2). Results from both studies suggest that feeding sows yeast additives from d 110 of gestation through lactation can improve growth performance of their offspring in the nursery. In Exp. 1, feeding live yeast and yeast extracts in the nursery appeared to hinder growth performance. However, in Exp. 2, pigs that were fed DFM 2 reported optimized growth in the late nursery period. In Exp 1., results suggest that progeny from sows that were fed yeast might increase the potential of fecal *E.coli* MIC to nalidixic acid, ciprofloxacin, and gentamicin. Yet, feeding live yeast and yeast extracts in the nursery may lower the MIC of azithromycin and chloramphenicol of fecal *E.coli.*

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"Be patient but persistent." – Dr. Ronald E. Chance

CHAPTER 1- Live yeast and yeast extracts with and without pharmacological levels of zinc on nursery pig growth performance and antimicrobial susceptibilities of fecal *Escherichia coli*

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 1 of 4 dietary treatments with 5 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 d 0 to 7, then concentrations were lowered by 50% from d 7 to 21) and pharmacological levels of Zn (110 vs. 3,000 mg/kg from d 0 to 7, and 2,000 mg/kg from d 7 to 21 with added Zn provided by ZnO). All pigs were fed a common diet from d 21 to 42 post-weaning. There were no yeast × Zn interactions or effects from yeast additives observed on any response criteria. From d 0 to 21, and 0 to 42, pigs fed pharmacological levels of Zn had increased ($P < 0.001$) ADG and ADFI. The dietary addition of Zn improved ($P < 0.05$) most economic criteria. Fecal samples were collected on d 4, 21, and 42 from the same three pigs per pen for fecal dry matter (DM) and AMR patterns of *E.coli*. On d 4, pigs fed pharmacological levels of Zn had greater fecal DM ($P = 0.043$); however, no differences were observed on d 21 or 42. *E.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 economic criteria 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

ATCC: American Type Culture Collection

BW: Body weight

CFU: Colony-forming unit

CLSI: Clinical and Laboratory Standards Institute

CP: Crude protein

DFM: Direct-fed microbial

DM: Dry matter

G:F: Gain-to-feed ratio

IOFC: Income over feed cost

ME: Metabolizable energy

MIC: Minimal inhibitory concentration

NARMS: National Antimicrobial Resistance Monitoring System

NE: Net energy

NRC: National Research Council

PCR: Polymerase chain reaction

PWD: Post-weaning diarrhea

SCFA: Short-chain fatty acid

SEM: Standard error of the mean

SID: Standardized ileal digestible

STTD: Standardized total tract digestible

WHO: World Health Organization

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., 2010b). 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; Bonetti et al., 2021).

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 (Jacela et al., 2010a). Feeding probiotics can alter the gut's microflora by introducing live cultures of beneficial microorganisms into the digestive tract and can aid in competitively exclude or suppress pathogens (Liao and Nyachoti, 2017). The improved microbial profile in the gut may allow for more protection against enteric diseases while subsequently improving growth performance (Doyle, 2001; Jacela et al., 2010a). 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 5 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 1 of

4 dietary treatments in a randomized complete block design with pens blocked by BW. 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 d 0 to 7, then concentrations were lowered by 50% from d 7 to 21) and pharmacological levels of Zn (110 mg/kg vs. 3,000 mg/kg from d 0 to 7 and 2,000 mg/kg from d 7 to 21; added Zn provided by ZnO; Table 1-1). 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 d 7 and then offered phase 2 diets from d 7 to 21 (Table 1-1). A common phase 3 diet, without yeast additives or pharmacological levels of ZnO, was fed to all pigs from d 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 1-2).

Economics

Total feed cost per pig, cost per kg of gain, revenue, and income over feed cost (IOFC) were calculated to evaluate the economics behind including yeast additives and ZnO. Feed cost per pig placed was determined by multiplying total feed intake by diet cost then divided by number of pigs placed. Feed cost per kg of gain was calculated by dividing the total feed cost per pig by the total weight gained per pig. Revenue per pig placed was determined by total gain times $1.47/kg carcass price then multiplied by the assumed dressing percentage (75%) in order to convert to a live price. Income over feed cost was calculated using revenue per pig placed minus feed cost per pig placed. For all economic evaluations, the following ingredients prices

were used: corn = $7.06/bushel ($278/metric ton); soybean meal = $404/metric ton; L-Lys HCl = $1.76/kg; DL-methionine = $4.85/kg; MHA =$4.96/kg; L-threonine = $2.23/kg; L-tryptophan = $8.80/kg; L-valine = $5.10/kg; ZnO = $2.41/kg; ActiSaf Sc 47 HR+ = $6.17/kg; SafMannan = $3.09/kg; and NucleoSaf = $18.08/kg.

Fecal collection

Fecal samples were collected on d 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 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 hours. Fecal DM was calculated as:

$$\text{Fecal DM \%} = \frac{\text{Dry sample weight at 48 h} - \text{ pan weight}}{\text{Initial wet sample weight} - \text{ pan weight}} \times 100$$

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. 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 1-3) 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, fecal dry matter, and economics 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. 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 (Table 1-4 & Table 1-5). In phases 1 (d 0 to 7) and 2 (d 7 to 21), pigs fed pharmacological levels of Zn had increased ($P < 0.05$) ADG, ADFI, and heavier d 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 (d 0 to 21), pigs fed pharmacological levels of Zn had increased ($P < 0.001$) ADG and ADFI leading to increased ($P < 0.001$) d 21 BW. However, there was no evidence for difference ($P > 0.10$) in G:F between those fed diets with or without pharmacological levels of Zn.

During the common period (d 21 to 42), pigs previously fed pharmacological levels of Zn had increased ($P = 0.002$) BW on d 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 (d 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.

Economics

No interactions between the addition of live yeast and pharmacological Zn were observed for any economic criteria (Table 1-4). There was a tendency ($P = 0.062$) for increased feed cost per pig when yeast additives were included in the diet compared to a diet without yeast; however, there was no evidence for difference ($P = 0.923$) for feed cost per kg of gain. There was no statistical difference ($P > 0.10$) between diets with or without yeast-based pre- and probiotics, pigs fed yeast had a numerical increase in revenue (+$0.53; Table 5) and IOFC (+$0.19; Table 1-5). Pigs fed pharmacological levels of Zn, provided by ZnO, had a tendency ($P = 0.076$) for increased feed cost per pig compared to those fed diets without added ZnO; yet, pigs fed pharmacological levels of Zn had lower ($P = 0.014$) feed cost per kg of gain. Furthermore, pigs fed added ZnO had increased ($P < 0.005$) revenue (+$0.99) and IOFC (+$0.67) compared to pigs diets without pharmacological Zn (Table 1-5).

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. On d 4, pigs fed 3,000 mg/kg of Zn had greater ($P = 0.043$) fecal DM than those without added Zn (Table 1-5). However, no differences were observed on d 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 1-6). Thus, MIC values of fecal *E.coli* isolates in response to the inclusion of yeast-based pre- and probiotics, pharmacological levels of Zn, and sampling day were further explored (Table 1-7).

All fecal *E.coli* isolates were susceptible to azithromycin, ciprofloxacin, nalidixic acid, sulfisoxazole, and trimethoprim/sulfamethoxazole at all three sampling time points (d 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 d 4 and 42 but intermediate on d 21. On d 4 and 21, fecal *E.coli* isolates were considered intermediate to ceftriaxone but were resistant on d 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 d 4 to 21 but then increased ($P < 0.05$) from d 21 to 42. Chloramphenicol MIC values increased ($P < 0.05$) from d 21 to 42 with d 4 values being intermediate while MIC values for gentamicin increased ($P < 0.05$) from d 4 to 21 with d 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 d 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; Menegat et al., 2019b). 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 (Cameron and McAllister, 2019). 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 (Liao and Nyachoti, 2017; Cameron and McAllister, 2019). 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. 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 (Kogan and Kocher, 2007), 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 intestinal integrity of the epithelial lining, and reduce periods of diarrhea by promoting beneficial microbiota colonization in the gastrointestinal tract (Stein and Kil, 2006; Liu et al., 2018). 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; Bonetti et al., 2021). The NRC (2012) requirements for Zn is 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 days immediately following weaning, has 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 d 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; 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 (Liu et al., 2018; Bonetti et al., 2021). 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 d 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; Liu et al., 2018). For example, in two

experiments conducted by van Heughten 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 is 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 our knowledge, there is 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 while optimizing feed cost per kg of gain, revenue, and IOFC. 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*.

Table 1-1. 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

continued

Table 1-1. (cont.)

	Phase 1	Phase 2	Phase 3
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 d 0 to 7 (approximately 5.6 to 6.1 kg) and phase 2 diets were fed from d 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, without ZnO or yeast probiotics, was fed during phase 3 from d 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- & 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).

Table 1-2. 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- & 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).

Table 1-3. 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.

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

	No yeast additives		Yeast additives			*P* =		
Item	Low Zn	High Zn	Low Zn	High Zn	SEM	Yeast × Zn	Yeast	Zn
BW, kg								
d 0	5.64	5.64	5.64	5.64	0.025	0.963	0.779	0.901
d 7	5.81	6.08	5.91	6.07	0.075	0.359	0.508	0.001
d 21	10.87	11.56	11.05	11.56	0.159	0.531	0.533	< 0.001
d 42	23.17	23.98	23.28	24.03	0.266	0.912	0.744	0.002
Phase 1 (d 0 to 7)								
ADG, g	25	63	39	61	9.8	0.360	0.489	0.001
ADFI, g	70	90	78	85	6.7	0.324	0.847	0.042
G:F, g/kg	190	603	404	689	94.0	0.446	0.077	< 0.001
Phase 2 (d 7 to 21)								
ADG, g	355	391	367	393	8.3	0.546	0.401	< 0.001
ADFI, g	437	492	452	492	12.3	0.538	0.507	< 0.001
G:F, g/kg	814	796	813	802	11.1	0.777	0.810	0.169
Experimental period (d 0 to 21)								
ADG, g	244	281	258	282	7.6	0.400	0.288	< 0.001
ADFI, g	314	356	328	356	9.8	0.456	0.461	< 0.001
G:F, g/kg	776	787	788	795	9.4	0.806	0.247	0.282
Phase 3 common period (d 21 to 42)								
ADG, g	586	591	582	594	7.8	0.685	0.947	0.264
ADFI, g	877	872	863	874	11.1	0.451	0.573	0.811
G:F, g/kg	668	679	675	680	5.5	0.619	0.502	0.158
Overall (d 0 to 42)								
ADG, g	413	436	420	438	6.2	0.721	0.433	0.001
ADFI, g	593	613	595	615	9.5	0.982	0.830	0.031
G:F, g/kg	697	710	706	713	5.0	0.518	0.284	0.043
Economics, $								
Feed cost/pig[2]	11.41	11.74	11.76	12.08	0.181	0.997	0.062	0.076
Feed cost/kg gain[3]	0.675	0.651	0.668	0.657	0.0079	0.345	0.923	0.014
Revenue[4]	18.76	19.91	19.45	20.29	0.355	0.631	0.100	0.003
IOFC[5]	7.35	8.17	7.70	8.21	0.236	0.451	0.344	0.002

Fecal dry matter,%[6]								
d 4	18.0	19.8	17.5	20.1	1.13	0.708	0.955	0.043
d 21	22.8	21.3	22.5	23.4	1.10	0.281	0.397	0.786
d 42	24.5	24.6	23.5	23.8	1.20	0.909	0.437	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 18 pens per treatment. Yeast pre- & 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.

[2] Feed cost per pig = total feed cost ÷ pigs placed in the pen.

[3] Feed cost per kg gain = feed cost per pig ÷ body weight gain per pig.

[4] Revenue = (gain per pig x $1.47/kg) x assumed 75% yield.

[5] Income over feed cost = revenue – feed cost per pig.

[6] Fecal samples from the same 3 pigs/pen were collected on d 4, 21, and 42. Zinc × yeast × day, $P = 0.790$; Zinc × day, $P = 0.220$; Yeast × day, $P = 0.515$.

Table 1-5. 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* =	Low Zn	High Zn	SEM	*P* =
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 (d 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 (d 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 (d 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 (d 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 (d 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
Economics, $								
Feed cost/pig[2]	11.58	11.92	0.129	0.062	11.58	11.91	0.129	0.076
Feed cost/kg gain[3]	0.663	0.662	0.0062	0.923	0.671	0.654	0.0062	0.014
Revenue[4]	19.34	19.87	0.274	0.100	19.11	20.10	0.274	0.003
IOFC[5]	7.76	7.95	0.188	0.344	7.52	8.19	0.188	0.002

Fecal dry matter, %[6]								
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- & 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.

[2] Feed cost per pig = total feed cost ÷ pigs placed in the pen.

[3] Feed cost per kg gain = feed cost per pig ÷ body weight gain per pig.

[4] Revenue = (gain per pig x $1.47/kg) x assumed 75% yield.

[5] Income over feed cost = revenue – feed cost per pig.

[6] Fecal samples from the same 3 pigs/pen were collected on d 4, 21, and 42. Zinc × yeast × day, $P = 0.790$; Zinc × day, $P = 0.220$; Yeast × day, $P = 0.515$.

Table 1-6. Interactive effects of yeast pre- and probiotics and pharmacological levels of Zn over time on antimicrobial susceptibilities of fecal *Escherichia coli* according to National Antimicrobial Resistance Monitoring System (CLSI, 2018) established breakpoints[1]

	No yeast additives		Yeast additives[3]		*P* =			
Item	Low Zn	High Zn[4]	Low Zn	High Zn	Yeast × Zn	Yeast × Day	Zn × Day	Yeast × Zn × Day
Amoxicillin:clavulanic acid 2:1 ratio[5]					0.342	0.445	0.644	0.461
d 4	10.5 ± 2.1	14.8 ± 2.9	10.1 ± 2.0	10.5 ± 2.1				
d 21	11.8 ± 2.3	14.8 ± 2.9	18.0 ± 3.5	13.7 ± 2.7				
d 42	16.0 ± 3.2	13.7 ± 2.7	14.3 ± 2.8	14.8 ± 2.9				
Ampicillin					0.625	0.720	0.480	0.757
d 4	18.0 ± 4.1	19.4 ± 4.4	14.8 ± 3.4	21.8 ± 5.0				
d 21	21.0 ± 3.4	21.8 ± 3.6	25.4 ± 4.2	24.4 ± 4.0				
d 42	32.0 ± 1.2	29.6 ± 1.1	32.0 ± 1.2	32.0 ± 1.2				
Azithromycin					0.874	0.559	0.558	0.929
d 4	11.3 ± 1.7	13.2 ± 2.0	10.5 ± 1.6	11.8 ± 1.8				
d 21	6.6 ± 1.0	7.4 ± 1.1	5.9 ± 0.9	7.4 ± 1.1				
d 42	9.3 ± 1.4	9.0 ± 1.3	10.5 ± 1.6	10.1 ± 1.5				
Cefoxitin					0.294	0.704	0.672	0.865
d 4	10.1 ± 1.8	13.7 ± 2.4	11.8 ± 2.1	12.2 ± 2.2				
d 21	11.8 ± 2.1	13.7 ± 2.4	16.6 ± 2.9	14.8 ± 2.6				
d 42	14.3 ± 2.5	18.7 ± 3.3	14.8 ± 2.6	18.7 ± 3.3				
Ceftiofur					0.807	0.670	0.658	0.970
d 4	2.33 ± 0.69	2.24 ± 0.66	2.00 ± 0.59	2.02 ± 0.60				
d 21	2.52 ± 0.75	1.92 ± 0.57	3.43 ± 1.02	2.33 ± 0.69				
d 42	2.83 ± 0.84	3.17 ± 0.94	3.43 ± 1.02	3.30 ± 0.98				
Ceftriaxone					0.307	0.763	0.572	0.794
d 4	1.53 ± 0.68	2.42 ± 1.08	2.33 ± 1.04	1.46 ± 0.65				
d 21	1.85 ± 0.82	2.08 ± 0.92	3.17 ± 1.41	2.83 ± 1.26				
d 42	6.11 ± 2.71	3.56 ± 1.58	7.13 ± 3.17	3.70 ± 1.65				
Chloramphenicol					0.999	0.189	0.952	0.828
d 4	21.0 ± 3.5	22.6 ± 3.7	18.0 ± 3.0	18.0 ± 3.0				
d 21	16.0 ± 3.0	16.0 ± 3.0	21.8 ± 4.1	20.2 ± 3.8				
d 42	24.4 ± 3.1	22.6 ± 2.9	22.6 ± 2.9	24.4 ± 3.1				

Continued

Table 1-6. (cont.)

	No yeast additives		Yeast additives[2]		*P* =			
Item	Low Zn	High Zn[3]	Low Zn	High Zn	Yeast × Zn	Yeast × Day	Zn × Day	Yeast × Zn × Day
Ciprofloxacin					0.566	0.714	0.542	0.877
d 4	0.0221 ± 0.0057	0.0313 ± 0.0081	0.0182 ± 0.0047	0.0269 ± 0.0070				
d 21	0.0213 ± 0.0055	0.0314 ± 0.0081	0.0197 ± 0.0051	0.0447 ± 0.0116				
d 42	0.0428 ± 0.0156	0.0462 ± 0.0169	0.0413 ± 0.0151	0.0541 ± 0.0197				
Gentamicin					0.066	0.559	0.722	0.631
d 4	3.30 ± 1.01	1.71 ± 0.53	2.33 ± 0.72	3.17 ± 0.98				
d 21	4.00 ± 1.23	2.52 ± 0.77	5.66 ± 1.74	4.67 ± 1.43				
d 42	4.16 ± 1.28	2.62 ± 0.80	2.83 ± 0.87	4.49 ± 1.38				
Nalidixic acid					0.524	0.957	0.701	0.638
d 4	2.72 ± 0.47	3.05 ± 0.53	2.94 ± 0.51	3.05 ± 0.53				
d 21	2.62 ± 0.38	3.17 ± 0.46	2.52 ± 0.37	3.30 ± 0.48				
d 42	3.56 ± 0.72	4.49 ± 0.91	4.16 ± 0.84	3.56 ± 0.72				
Streptomycin					0.932	0.771	0.415	0.582
d 4	47.0 ± 6.5	54.9 ± 7.6	48.9 ± 6.8	56.7 ± 7.9				
d 21	47.0 ± 6.5	54.9 ± 7.6	52.8 ± 7.3	50.8 ± 7.0				
d 42	54.9 ± 7.6	43.5 ± 6.0	57.0 ± 7.9	57.0 ± 7.9				
Sulfisoxazole					0.879	0.183	0.215	0.410
d 4	174 ± 34	246 ± 48	138 ± 27	161 ± 31				
d 21	228 ± 31	188 ± 26	256 ± 35	211 ± 29				
d 42	256 ± 21	219 ± 18	228 ± 19	256 ± 21				
Trimethoprim/sulfamethoxazole 1:19 ratio[4]					0.366	0.904	0.902	0.259
d 4	0.179 ± 0.037	0.236 ± 0.049	0.193 ± 0.040	0.178 ± 0.037				
d 21	0.146 ± 0.025	0.152 ± 0.026	0.125 ± 0.021	0.185 ± 0.032				
d 42	0.377 ± 0.131	0.696 ± 0.242	0.555 ± 0.193	0.474 ± 0.165				

[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 18 pens per treatment. Fecal samples from the same 3 pigs/pen were collected on d 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 0.10%, 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).

[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.

Table 1-7. 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* =	Low Zn	High Zn	*P* =	4	21	42	*P* =
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 d 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,b,c] Superscripts signify a statistical difference of $P < 0.05$.

CHAPTER 2- The effect of live yeast and yeast extracts included in lactation diets on sow and litter performance and antimicrobial susceptibility of fecal *Escherichia coli*: I Sow performance

Abstract

A total of 80 sows (Line 241; DNA Genetics) across three farrowing groups were used in a study to evaluate the effect of feeding live yeast and yeast extracts to lactating sows on sow and litter performance and antimicrobial resistance (AMR) patterns of sow fecal *E.coli.* Sows were blocked by farrowing group, BW, and parity on d 110 of gestation and allotted to 1 of 2 dietary treatments. Dietary treatments consisted of a standard lactation diet or a diet that contained yeast-based pre- and probiotics (0.10% Actisaf Sc 47 HR+ and 0.025% SafMannan; Phileo by Lesaffre, Milwaukee, WI). Diets were fed from d 110 of gestation until weaning (approximately d 19 post-farrow). A tendency ($P = 0.073$) was observed for increased feed intake through lactation when sows were fed a diet with yeast additives compared to the control diet. There was no evidence ($P > 0.10$) that treatment influenced any other sow or litter performance criteria. Fecal samples were collected from the first farrowing group (27 sows) to determine the AMR patterns of *E.coli* upon entry into the farrowing house and at weaning. *E.coli* was isolated from fecal samples and species confirmation was by PCR detection of *uidA* and *clpB* genes. 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 guidelines. An interaction ($P = 0.026$) of diet × sampling day was observed for cefoxitin where fecal *E.coli*

showed no evidence of treatment differences ($P = 0.237$) in MIC values at entry, but sows fed the control diet had lower ($P = 0.035$) MIC values at weaning compared to sows fed yeast additives. There were no diet main effects ($P > 0.10$) on the AMR of fecal *E.coli*. There was an increased ($P < 0.02$) trend towards resistance for 11 of the 14 antimicrobials over time. Fecal *E.coli* were resistant to tetracycline and ceftriaxone at weaning. Fecal *E.coli* were considered susceptible or intermediate across sampling day to the remaining antimicrobials. In conclusion, feeding live yeast and yeast extracts tended to increase feed intake during lactation but did not influence either sow or litter performance measurements or the AMR of fecal *E.coli* during lactation except for cefoxitin which had a higher MIC at the end of lactation when yeast additives were present in the diet.

Key Words: Antimicrobial resistance, sows, litter performance, live yeast, yeast extract

Abbreviations

ADFI: Average daily feed intake

ADG: Average daily gain

AMR: Antimicrobial resistance

ATCC: American Type Culture Collection

BF: Back fat

BW: Body weight

CFU: Colony-forming unit

CLSI: Clinical and Laboratory Standards Institute

CP: Crude protein

MIC: Minimal inhibitory concentration

NARMS: National Antimicrobial Resistance Monitoring System

NE: Net energy

NRC: National Research Council

PCR: Polymerase chain reaction

PWM: Preweaning mortality

SEM: Standard error of the mean

SID: Standardized ileal digestible

STTD: Standardized total tract digestible

WEI: Wean-to-estrus interval

WHO: World Health Organization

Introduction

Supplementing live yeast and yeast extracts in sow diets has been researched due to potential for a healthier/heavier piglet, which may be better equipped to handle weaning stress leading to improved nursery performance. The inclusion of live yeast has positively influenced IgG in sow plasma and colostrum allowing increased maternal transfer of immunity to their offspring (Zanello et al., 2012; Peng et al., 2020). Furthermore, feeding live yeast and yeast extracts may positively modulate sow gut microflora, which may provide piglets with exposure to more beneficial and less pathogenic bacteria through the sow's feces (Hasan et al., 2018). Additionally, feeding *Saccharomyces cerevisiae* through gestation and lactation has shown to improve average daily gain (ADG), increase body weight (BW), and improve gross energy digestibility of offspring in the nursery (Lu et al., 2019).

While there are many studies exploring the effects of feeding live yeast to sows and its influence on litter performance in the farrowing house, there is little-to-no data related to the impacts of feeding live yeast and yeast extracts on the antimicrobial resistance (AMR) of gut bacteria in sows. Thus, the objective of this study was to evaluate the effects of feeding the live yeast *Saccharomyces cerevisiae* strain NCYC Sc 47 and a yeast cell wall fraction with concentrated mannan-oligosaccharides and β-glucans from *S. cerevisiae* on sow and litter performance and antimicrobial susceptibility of *E.coli* isolated from the feces of sows. Our hypothesis was that supplementing live yeast and yeast extracts to sows would lessen the AMR of fecal *E.coli* to antimicrobials that are important to human and animal medicine and may have a positive impact on sow and litter performance.

Materials and Methods

Animals and treatment structure

The Kansas State University Institutional Care and Use Committee approved the protocol used in this experiment. A total of 80 mixed-parity sows (DNA 241, DNA Genetics) were used across three batch farrowing groups with 40 sows per treatment at the Kansas State University Swine Teaching and Research Center in Manhattan, KS. On d 110 of gestation, sows were weighed and moved into the farrowing house. Sows were blocked by farrowing group, parity, and BW and allotted to one of two dietary treatments. Dietary treatments consisted of a standard corn-soybean meal-based lactation diet or a diet that contained yeast-based pre- and probiotics (0.10% Actisaf Sc 47 HR+ and 0.025% SafMannan; Phileo by Lesaffre, Milwaukee, WI). The live yeast *S. cerevisiae* strain NCYC Sc 47 (ActiSaf Sc 47 HR+) served as the yeast-based probiotic. The yeast-based prebiotic included a yeast cell wall fraction with concentrated mannan-oligosaccharides and β-glucans from *S. cerevisiae* (SafMannan). Both diets were formulated to meet or exceed National Research Council (NRC, 2012) requirement estimates (Table 2-1).

From d 110 until farrowing (approximately d 115), sows were fed approximately 2.7 kg of their respective treatment diets. Post farrowing, sows were allowed *ad libitum* access to feed during lactation which was recorded by weighing the amount of feed placed in the feeder and the amount remaining at weaning. The diets for the first farrowing group were manufactured at the Kansas State University O.H. Kruse Feed Technology Innovation Center (Manhattan, KS) and the diets for the following two farrowing groups were manufactured at a commercial feed mill (Hubbard Feeds; Beloit, KS).

Sow BW was measured at entry into the farrowing house, 24 h after farrowing, and at weaning. Sow back fat (BF) depth (measured 7 cm from the midline at the last rib) was measured at entry to the farrowing house and at weaning. Cross-fostering of piglets was performed to equalize litter size within sow treatment group within 48 h after birth. Litters were weighed on d 2, 10, and at weaning. Pre-weaning mortality was calculated as the total mortality (d 0 to wean) per sow divided by the total born alive per sow with cross-fostered pigs accounted for in the calculations.

Chemical analysis

Complete diet samples were taken from every fifth 23 kg bag using a feed probe. Complete diet samples were stored at -20°C until they were homogenized, subsampled, and submitted for quantification (Analabs; Fulton, IL; method 997.02; AOAC International, 1998) of active live yeast (Table 2-1).

Fecal collection

Fecal samples were collected from the first farrowing group (27 sows) to determine the antimicrobial resistance patterns of *E.coli* upon entry into the farrowing house and at weaning. Fecal samples were collected directly from the rectum of each sow using a sterile, single-use cotton tipped applicator (Fisher Healthcare, Pittsburgh, PA) from 13 or 14 sows per treatment. Samples were stored in a clean, single-use zipper storage bag and kept on ice until delivered to the Kansas State University College of Veterinary Medicine for bacterial isolation and further characterization.

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 and then individually streaked onto a blood agar plate (Remel, Lenexa, KS) and incubated at 37°C for 24 h. 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 accomplished 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 when sows entered the farrowing house (approximately d 110 of gestation) and at weaning (approximately 19 d post-farrowing). 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 several 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 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 standards. Then, 10 µL 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 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) strains were included as quality controls for *E.coli* susceptibility testing. 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 1-3) guidelines were used to classify each isolate as susceptible, intermediate, or resistant according to the breakpoints established for each antimicrobial. MIC values greater than the susceptible breakpoint but lower than the resistant breakpoint were considered intermediate.

Statistical analysis

Sow and litter performance. Performance data were analyzed using the *lme4* package of R (Version 4.0.0, R Foundation for Statistical Computing, Vienna, Austria) as a randomized complete block design. Blocking structure accounted for group, parity, and BW. For all analyses, sow was considered the experimental unit. Treatment was included as a fixed effect with block included as a random effect. Performance related to bodyweight, lactation length, and body fat was modeled by normal distribution with identity link. Count of total born, litter size, and parity were modeled by both Poisson and negative binomial distributions with log link and model fit was superior using the negative binomial response distribution through evaluation of the Bayesian Information Criterion. Proportion of piglets within each litter born alive, stillborn, or mummified, and pre-weaning mortality was modeled by a binomial distribution with logit link.

Differences between treatments were considered significant at $P \leq 0.05$ and marginally significant at $0.05 < P \leq 0.10$.

Antimicrobial susceptibility. The MIC data of each antimicrobial were analyzed using a linear mixed model. Fixed effects of the model included diet, sampling day, and their interaction. Random effects included block and sow (i.e., the error term vector corresponding to repeated measurement over sampling day). The variance-covariance structure of sow was taken as either compound symmetry 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, i.e., geometric means. Comparisons were carried out using the 2-sided test. Statistical analysis was performed using Statistical Analysis Software (SAS version 9.4; Cary, NC) PROC MIXED with option DDFM=KR in the MODEL statement. Differences between treatments were considered significant at $P \leq 0.05$.

Results

Sow & litter performance

Inclusion of yeast additives from d 110 of gestation through weaning resulted in no statistical difference ($P > 0.10$) for sow BW or BW change throughout lactation (Table 2-2). Furthermore, there was no evidence of treatment differences ($P > 0.10$) for sow BF at entry or weaning, or the loss in BF from entry to weaning. There was a tendency ($P = 0.073$) for increased feed intake from farrowing to weaning when sows were fed the diet with yeast additives compared to the control diet. There was no evidence of treatment difference ($P > 0.10$) in wean-to-estrus interval (WEI).

There was no evidence ($P > 0.10$; Table 2-3) that the addition of a live yeast and a yeast extract in sow diets influenced litter characteristics including litter size, litter weight, or mean piglet BW on d 2 post-farrowing, d 10 post-farrowing, or at weaning. Furthermore, the addition of yeast additives showed no evidence of a difference ($P > 0.10$) on litter or piglet ADG, or preweaning mortality (PWM).

Antimicrobial resistance

An interaction ($P = 0.026$) of diet × sampling day was observed for AMR for the antimicrobial cefoxitin (Table 2-4). It was observed that fecal *E.coli* isolates from sows fed the control diet had lower ($P = 0.035$) MIC values at weaning compared to sows fed the diet with added yeast-based pre- and probiotics. However, there was no significant ($P > 0.10$) difference in MIC values for cefoxitin between the two dietary treatments at entry into the farrowing house. There were no further interactions observed ($P > 0.10$). There was no evidence ($P > 0.10$) that the dietary inclusion of yeast additives influenced the AMR of fecal *E.coli* isolates compared to the control diet for any of the 14 antimicrobials evaluated (Table 2-5).

Fecal *E.coli* isolates from sows fed either dietary treatment were mostly resistant to tetracycline. Based on CLSI (2018) guidelines, the MIC of *E.coli* isolates were considered intermediate to tetracycline from fecal samples collected at entry into the farrowing house; however, MIC values increased ($P < 0.001$) by weaning with isolates being classified as resistant (Resistant isolates: 14/14 for the control diet; 11/13 for the yeast diet). Interestingly, this effect carried over into the nursery (Chance et al., 2021c). All nursery pig fecal *E.coli* isolates had higher ($P < 0.001$) MIC values to tetracycline on d 5 post-weaning which then decreased on d 24 and then slightly increased on d 45 in the nursery. Fecal *E.coli* was susceptible to ceftriaxone at

entry into the farrowing house but resistant at weaning. The remaining 12 antimicrobials were considered susceptible or intermediate for both treatments across both sampling days.

E.coli isolated from sow feces had increased ($P < 0.02$) MIC values for amoxicillin/clavulanic acid, ampicillin, azithromycin, cefoxitin, ceftiofur, ceftriaxone, ciprofloxacin, nalidixic acid, streptomycin, tetracycline, and trimethoprim/sulfamethoxazole at weaning compared to when sows entered the farrowing house. In fact, fecal *E.coli* isolates were susceptible to amoxicillin/clavulanic acid, ampicillin, cefoxitin, ceftiofur, ceftriaxone, and streptomycin upon entry into the farrowing house but showed trends towards resistance over time at weaning. Whereas fecal *E.coli* isolates were susceptible at both time points for azithromycin, ciprofloxacin, nalidixic acid, streptomycin, and trimethoprim/sulfamethoxazole. Thus, the only antimicrobials that fecal *E.coli*'s MIC values did not significantly ($P > 0.10$) change over time and were considered susceptible at both time points were for chloramphenicol, gentamicin, and sulfisoxazole.

Discussion

Genetic selection of highly prolific sows and shortened lactation periods has led to pigs entering the nursery at a lighter BW. Lower entry BW has often been associated with underdeveloped gastrointestinal tracts and immune systems leading to a lag in performance and chances of enteric infections (Moeser et al., 2017). Thus, feeding strategies to increase litter weight have been sought out in order to wean a heavier pig who is more physiologically equipped to handle the stress of weaning. Feeding sows live yeast (probiotic) and yeast extracts (prebiotic) has been shown to increase immunity and growth of progeny (Shen et al., 2011; Gao et al., 2021). We evaluated the live *S. cerevisiae* strain NCYC Sc 47 (ActiSaf HR+; Phileo by

Lesaffre, Milwaukee, WI) as the yeast-based probiotic and a yeast cell wall fraction with concentrated mannan-oligosaccharides and β-glucans derived from *S. cerevisiae* (SafMannan; Phileo by Lesaffre, Milwaukee, WI) as a yeast-based prebiotic in the present study. Probiotics are live microorganisms which are designed to withstand the harsh environment of the stomach and can flourish in the gastrointestinal tract while outcompeting enteric pathogens (Cameron and McAllister, 2019). While prebiotics have similar modes of action as probiotics, they differ in the sense that prebiotics are not live microbes. Instead, prebiotics are a food source that can selectively stimulate beneficial gut microorganisms (Menegat et al., 2019b).

In this study we observed that feeding live yeast and a yeast extract tended to increase feed intake during the lactation period. This response is similar to a recent study by Tan et al. (2021) which reported an increase in feed intake in the first week of lactation as the inclusion of a yeast extract increased from 0 to 10 g/kg in the diet from d 90 of gestation through lactation. However, many studies that evaluate the inclusion of yeast additives in sow diets report no statistical impact on sow feed intake during lactation (Kim et al., 2010; Chen et al., 2020; Gao et al., 2021). Unlike Tan et al. (2021), there was no evidence of treatment difference in sow BW or BW change in the present study. There were no evidence of treatment differences in sow BF thickness or change in BF thickness in studies by Shen et al. (2011), Zanello et al. (2012), and Peng et al. (2020) which is consistent with our observations. Interestingly, Jang et al. (2012) reported reduced WEI and increased percentage of estrus detection by d 7 post-weaning as the inclusion of live yeast and length of feeding live yeast increased. Similarly, Kim et al. (2008) found it required 2 d less for successful breeding post-weaning when sows were supplemented with the live yeast *S. cerevisiae* from d 35 of gestation through lactation compared to sows fed a

control diet. This differs from the present study as no impact on subsequent reproductive criteria was observed.

Feeding yeast and yeast extracts to sows has previously been reported to affect the sow's offspring. Unlike many studies, the inclusion of live yeast and a yeast extract did not impact any litter performance parameters in the present study. A number of studies have reported improved litter weight gain and heavier weaning weights when yeast additives were fed to their dam (Kim et al., 2008; Shen et al., 2011; Hasan et al., 2018). In many studies, sows did not have increased feed intake; thus, the improvement in litter performance may be attributed to yeast's impact on colostrum quality and yield (Peng et al., 2020), maternal transfer of immunity (Zanello et al., 2012; Gao et al., 2021), increased exposure to a more diverse fecal microflora (Trckova et al., 2014; Hasan et al., 2018), and/or improved nutrient digestibility (Lu et al., 2019). Some studies have reported increased total born alive (Mariella et al., 2009; Chen et al., 2020) and reduced stillborns (Peng et al., 2020), mummies (Zanello et al., 2012), and PWM (Mariella et al., 2009) when sows were supplemented with yeast additives, but this was not observed in our study. However, these studies report feeding yeast for a longer duration during gestation than the present study which could be a potential reason we did not observe a response for the respective litter characteristics.

There is limited data regarding the AMR of gut bacteria in swine when fed antibiotic alternatives. To our knowledge, this is one of the few studies reporting the AMR of fecal *E.coli* in sows when fed a diet containing yeast-based pre- and probiotics. Ouwehand et al. (2016) speculated that positively modulating gut bacteria through probiotic supplementation may reduce the need for antibiotics; thus, reducing the chances of further contribution to AMR. Yet, in chapter 1 we observed no statistical impact on the AMR of fecal *E.coli* when nursery pig diets

were supplemented with live yeast and yeast extracts (Chance et al., 2021a). In the present study, an interaction revealed increased AMR to cefoxitin over time when sows were fed yeast additives. However, when MIC values were averaged across the two sampling timepoints, the inclusion of yeast did not impact the AMR of sow fecal *E.coli* for any of the antibiotics tested. Although no antibiotics were administered during lactation, MIC values to 11 of the 14 antimicrobials tested increased over time regardless of dietary treatment. Literature reports evidence that the resistance of *E.coli* and other gut microbes in sows can be passed down to progeny (Mathew et al., 2005; Stannarius et al., 2009; Callens et al., 2014). We observed that sows developed resistance to tetracycline during lactation, passing off the resistance to their offspring in the nursery which then decreased over time (Chance et al., 2021c). Our findings agree with other cross-sectional studies on AMR where high AMR gene levels reported among young pigs were attributed to sow population. This is possibly due to either vertical or horizontal transmission of resistance of bacteria at, or shortly after, birth and similarities in microbiome abundance in diversity (Sekirov et al., 2010; Marchant and Moreno, 2013; Lanza et al., 2015). However, more research is warranted to fully comprehend the impacts of live yeast and yeast extract's impact on sow fecal AMR and its subsequent impact on the AMR of gut bacteria in progeny.

In conclusion, feeding live yeast and yeast extracts from d 110 of gestation through lactation tended to increase lactation feed intake but did not affect any other sow or litter performance criteria. Furthermore, yeast additives had minimal effect on the antimicrobial resistance of fecal *E.coli* except for cefoxitin which had higher MIC values at the end of lactation when the live yeast and yeast extracts were present in the diet. Regardless of the diet, 11 of the 14 antimicrobials had increased MIC values at weaning compared to entry into the farrowing

house with most classified as susceptible upon entry but classified as intermediate or resistant at weaning even though none of these antibiotics were used during the lactation period.

Table 2-1. Composition of lactation diets (as-fed basis)[1]

Ingredients, %	
Corn	64.4
Soybean meal, 46.5% CP	30.0
Oil	2.00
Monocalcium P, 21% P	1.15
Calcium carbonate	0.90
Salt	0.50
L-Lys-HCl	0.20
DL-Met	0.05
L-Thr	0.07
L-Trp	0.01
Vitamin premix without phytase[2]	0.25
Sow vitamin pack[3]	0.25
Trace mineral premix[4]	0.15
Phytase[5]	0.08
Yeast additives[6]	±
Total	100
Calculated analysis	
SID amino acids, %	
Lys	1.07
Ile:Lys	67
Leu:Lys	140
Met:Lys	30
Met and Cys:Lys	56
Thr:Lys	63
Trp:Lys	20.7
Val:Lys	73
His:Lys	44
Total Lys, %	1.21
NE, kcal/kg	2,508
SID Lys:NE, g/Mcal	4.25
CP, %	19.9
Ca, %	0.77
P, %	0.63
STTD P, %	0.50
Live yeast, CFU/g[7]	76,133 or 14,866,666

[1] Feed was manufactured at the O.H. Kruse Feed Technology Innovation Center (Manhattan, KS) for the first farrowing group and then feed was manufactured by a commercial feed mill (Hubbard Feeds; Beloit, KS).

[2] 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.

[3] Provided per kg of premix: 1,653,465 IU vitamin A; 4,409 IU vitamin E; 88 mg biotin; 882 mg folic acid; 397 mg pyridoxine; 220,462 mg choline; 19,842 mg carnitine; 79 mg chromium.

[4] 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.

[5] Ronozyme HiPhos 2700 (DSM Nutritional Products, Parsippany, NJ) provided 2,027 FTU/kg and a STTD P release of 0.12%.

[6] Live yeast was provided by 0.10% Actisaf Sc 47 HR+ and yeast extracts were provided by 0.025% SafMannan (Phileo by Lesaffre, Milwaukee, WI) at the expense of corn.

[7] Average quantification between feed samples taken from the three farrowing groups. The control diet had 76,133 CFU/g of live yeast and the diets with added yeast had 14,866,666 CFU/g of live yeast detected.

Table 2-2. Effects of including live yeast and a yeast extract in lactation diets on sow performance[1]

Item	Control	Yeast[2]	SEM	*P* =
Count, *n*	40	40	---	---
Parity	2.2	2.2	0.24	0.999
Lactation length, d	18.7	18.7	0.15	0.603
Sow BW, kg				
Entry	245.0	245.0	5.00	0.978
Farrow	223.7	224.0	4.95	0.920
Wean	217.5	218.9	5.11	0.694
Sow BW change, kg				
Entry to farrow	-21.2	-21.1	1.46	0.974
Farrow to wean	-6.1	-5.3	1.42	0.663
Entry to wean	-27.3	-26.4	2.07	0.750
Sow back fat, mm				
Entry	12.7	12.5	0.35	0.684
Wean	10.1	10.3	0.35	0.705
Change (entry to wean)	-2.6	-2.2	0.24	0.197
Sow ADFI, kg				
Farrow to wean	5.65	5.90	0.121	0.073
Wean-estrus interval, d	4.4	4.3	0.14	0.748

[1] A total of 80 mixed-parity sows (DNA 241, DNA Genetics) and litters were used in a lactation study from d 110 of gestation until weaning with 40 sows and litters per treatment. Litters were cross-fostered to equalize litter size up to 48-h post-farrowing within treatment group.

[2] Live yeast was provided by 0.10% Actisaf Sc 47 HR+ and yeast extracts were provided by 0.025% SafMannan (Phileo by Lesaffre, Milwaukee, WI).

Table 2-3. Effects of including live yeast and a yeast extract in lactation diets on litter performance[1]

Item	Control	Yeast[2]	SEM	*P* =
Litter characteristics				
Total born, *n*	16.2	16.6	0.65	0.639
Born alive, %	91.4	91.1	4.50	0.960
Stillborn, %	7.0	5.4	4.04	0.764
Mummy, %	1.5	3.5	2.90	0.575
Litter size, *n*				
d 2	14.2	14.3	0.60	0.836
d 10	13.3	13.9	0.59	0.448
Wean	12.9	13.5	0.58	0.498
Litter weight, kg				
d 2	23.23	23.42	0.530	0.797
d 10	46.33	46.19	1.739	0.946
Wean	71.35	72.67	1.961	0.635
Mean piglet BW, kg				
d 2	1.65	1.64	0.033	0.849
d 10	3.48	3.34	0.118	0.312
Wean	5.51	5.41	0.119	0.579
Litter ADG d 2 to wean, kg/day	2.59	2.64	0.973	0.741
Piglet ADG d 2 to wean, g/day	198	196	6.2	0.786
Preweaning mortality, %	10.7	9.6	4.88	0.873
Wean age	18.7	18.7	0.15	0.603

[1] A total of 80 mixed-parity sows (DNA 241, DNA Genetics) and litters were used in a lactation study from d 110 of gestation until weaning with 40 sows and litters per treatment. Litters were cross-fostered to equalize litter size up to 48-h post-farrowing within treatment group.

[2] Live yeast was provided by 0.10% Actisaf Sc 47 HR+ and yeast extracts were provided by 0.025% SafMannan (Phileo by Lesaffre, Milwaukee, WI).

Table 2-4. Interactive effects of including live yeast and a yeast extract in lactation diets over time on antimicrobial susceptibilities of fecal *Escherichia coli* in sows according to National Antimicrobial Resistance Monitoring System (CLSI, 2018) established breakpoints[1]

			P =		
Item	Control	Yeast[2]	Diet	Day	Diet × Day
Amoxicillin:clavulanic acid 2:1 ratio[3]			0.854	< 0.001	0.876
Entry	4.0 ± 0.55	4.0 ± 0.55			
Wean	19.5 ± 4.32	20.8 ± 4.79			
Ampicillin			0.276	< 0.001	0.946
Entry	3.8 ± 0.75	3.0 ± 0.59			
Wean	27.6 ± 5.45	22.1 ± 4.54			
Azithromycin			0.318	0.016	0.966
Entry	4.6 ± 0.66	5.1 ± 0.73			
Wean	6.6 ± 0.93	7.3 ± 1.08			
Cefoxitin[4]			0.186	< 0.001	0.026
Entry	7.6 ± 0.88	6.3 ± 0.72			
Wean	16.0 ± 2.88	28.6 ± 5.36			
Ceftiofur			0.822	< 0.001	0.225
Entry	0.50 ± 0.090	0.41 ± 0.074			
Wean	4.64 ± 0.836	6.12 ± 1.147			
Ceftriaxone			0.919	< 0.001	0.275
Entry	0.35 ± 0.087	0.25 ± 0.061			
Wean	7.61 ± 3.315	11.62 ± 5.269			
Chloramphenicol			0.338	0.742	0.468
Entry	8.8 ± 0.95	8.8 ± 0.95			
Wean	8.4 ± 0.90	10.1 ± 1.12			
Ciprofloxacin			0.491	0.002	0.974
Entry	0.017 ± 0.0015	0.020 ± 0.0018			
Wean	0.043 ± 0.0143	0.051 ± 0.0175			
Gentamicin			0.774	0.268	0.276
Entry	1.05 ± 0.106	0.95 ± 0.096			
Wean	0.91 ± 0.072	0.95 ± 0.078			
Nalidixic acid			0.369	0.009	0.859
Entry	2.1 ± 0.27	2.8 ± 0.36			
Wean	4.4 ± 1.51	5.4 ± 1.93			
Streptomycin			0.657	0.017	0.345
Entry	10.8 ± 2.3	14.5 ± 3.1			
Wean	23.8 ± 5.1	20.7 ± 4.6			
Sulfisoxazole			0.912	0.345	0.910
Entry	172 ± 44	164 ± 42			
Wean	210 ± 36	211 ± 38			
Tetracycline			0.618	< 0.001	0.055
Entry	8.4 ± 2.3	14.5 ± 4.0			
Wean	32.0 ± 4.6	23.3 ± 3.5			
Trimethoprim/sulfamethoxazole 1:19 ratio[3]			0.366	0.010	0.949
Entry	0.12 ± 0.021	0.15 ± 0.027			
Wean	0.30 ± 0.119	0.40 ± 0.165			

[1] A total of 27 mixed-parity sows (DNA 241, DNA Genetics) and litters were used in a lactation study from d 110 of gestation until weaning with 13 or 14 sows per treatment. Fecal samples were collected upon entry into the farrowing

house (approximately d 110 of gestion) and prior to weaning (approximately d 18 post-farrowing). Data reported as geometric mean of MIC ± SEM.

[2] Yeast-based pre- and probiotics included Actisaf Sc 47 HR+ at 0.10% and SafMannan at 0.025% (Phileo by Lesaffre, Milwaukee, WI) from d 110 of gestation until weaning.

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

[4] Interaction of diet × day where sows fed a control diet had lower (P = 0.035) MIC to cefoxitin at weaning compared to sows fed yeast additives. There were no evidence for treatment differences (P = 0.237) observed at the entry into the farrowing house.

Table 2-5. Main effects of including live yeast and a yeast extract in lactation diets and time of sample collection on antimicrobial susceptibilities of fecal *Escherichia coli* in sows according to National Antimicrobial Resistance Monitoring System (CLSI, 2018) established breakpoints[1]

Antimicrobial	Control	Yeast[2]	*P* =	Entry	Wean	*P* =
Amoxicillin:clavulanic acid 2:1 ratio[3]	8.8 ± 1.1	9.1 ± 1.1	0.854	4.0 ± 0.40	20.1 ± 3.27	< 0.001
Ampicillin	10.2 ± 1.5	8.1 ± 1.2	0.276	3.4 ± 0.47	24.7 ± 3.52	< 0.001
Azithromycin	5.5 ± 0.58	6.1 ± 0.66	0.318	4.9 ± 0.56	6.9 ± 0.81	0.016
Cefoxitin	11.0 ± 1.1	13.4 ± 1.4	0.186	6.9 ± 0.6	21.4 ± 2.8	< 0.001
Ceftiofur	1.5 ± 0.18	1.6 ± 0.20	0.822	0.45 ± 0.058	5.33 ± 0.693	< 0.001
Ceftriaxone	1.6 ± 0.43	1.7 ± 0.46	0.919	0.30 ± 0.052	9.41 ± 2.962	< 0.001
Chloramphenicol	8.6 ± 0.56	9.4 ± 0.62	0.338	8.8 ± 0.67	9.2 ± 0.71	0.742
Ciprofloxacin	0.027 ± 0.0043	0.032 ± 0.0052	0.491	0.019 ± 0.0012	0.047 ± 0.0112	0.002
Gentamicin	0.98 ± 0.076	0.95 ± 0.075	0.774	1.00 ± 0.079	0.93 ± 0.062	0.268
Nalidixic acid	3.1 ± 0.59	3.9 ± 0.78	0.369	2.4 ± 0.22	4.9 ± 1.21	0.009
Streptomycin	16.0 ± 2.3	17.3 ± 2.5	0.657	12.5 ± 2.0	22.2 ± 3.6	0.017
Sulfisoxazole	190 ± 27	186 ± 27	0.912	168 ± 30	210 ± 26	0.345
Tetracycline	16.4 ± 2.6	18.4 ± 2.9	0.618	11.0 ± 2.1	27.3 ± 2.8	< 0.001
Trimethoprim/sulfamethoxazole 1:19 ratio[3]	0.19 ± 0.039	0.25 ± 0.053	0.366	0.14 ± 0.017	0.34 ± 0.099	0.010

[1] A total of 27 mixed-parity sows (DNA 241, DNA Genetics) and litters were used in a lactation study from d 110 of gestation until weaning with 13 or 14 sows per treatment. Fecal samples were collected upon entry into the farrowing house (approximately d 110 of gestation) and prior to weaning (approximately d 18 post-farrowing). Data reported as geometric mean of MIC ± SEM.

[2] Yeast-based pre- and probiotics included Actisaf Sc 47 HR+ at 0.10% and SafMannan at 0.025% (Phileo by Lesaffre, Milwaukee, WI) from d 110 of gestation until weaning.

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

CHAPTER 3- The effect of live yeast and yeast extracts included in lactation diets on sow and litter performance and antimicrobial susceptibility of fecal *Escherichia coli*: II Nursery performance

Abstract

Two experiments were conducted to determine the impact of various combinations of yeast-based direct fed microbials (DFM) in diets fed to nursery pigs weaned from sows fed lactation diets with or without yeast additives. In Exp. 1, 340 weaned pigs, initially 5.1 kg ± 0.02, were used to evaluate previous sow treatment (control vs yeast additives) and nursery diets with or without added yeast-based DFM on growth performance and antimicrobial resistance (AMR) patterns of fecal *Escherichia coli*. 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, Phileo by Lesaffre, Milwaukee, WI) 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 decreased by 50% from d 7 to 24) with 5 pigs per pen and 17 replications per treatment. Progeny from sows fed yeast additives had increased ($P < 0.05$) average daily gain (ADG) from d 0 to 24 and d 0 to 45. However, pigs that were fed yeast additives for the first 24 d in the nursery tended to have decreased d 0 to 45 ADG ($P = 0.079$). Fecal *E.coli* isolated from pigs from the sows fed yeast group had increased ($P = 0.034$) AMR to nalidixic acid and a tendency for increased AMR to ciprofloxacin ($P = 0.065$) and gentamicin ($P = 0.054$). Yet, when yeast additives were added in the nursery there was reduced ($P < 0.05$) fecal *E.coli* AMR to azithromycin and chloramphenicol. In Exp. 2, 330 weaned pigs,

initially 5.8 kg ± 0.03, were used to evaluate diets with two different combinations of DFM on growth performance. Treatments were arranged in a 2 × 3 factorial with main effects of sow treatment (same as described in Exp. 1) and nursery treatment (control; DFM 1, 0.05% of SafMannan from d 0 to 38 and NucleoSaf at 0.05% from d 0 to 10 and 0.025% from d 10 to 24; or DFM 2, 0.10% MicroSaf from d 0 to 38 and NucleoSaf at 0.05% from d 0 to 10 and 0.025% from d 10 to 24) with 6 pigs per pen and 8 to 10 replications per treatment. From d 0 to 10 post-weaning, progeny of sows fed yeast additives had increased ($P < 0.05$) ADG and feed efficiency. In conclusion, feeding sows yeast through lactation improved offspring growth performance in the nursery. While feeding live yeast and yeast extracts reduced nursery pig performance in Exp. 1, feeding DFM 2 improved growth later in the nursery period in Exp. 2.

Key Words: Antimicrobial resistance, *Bacillus*, growth, live yeast, nursery pigs, yeast extract

Abbreviations

ADFI: Average daily feed intake

ADG: Average daily gain

AMR: Antimicrobial resistance

ATCC: American Type Culture Collection

BW: Body weight

CFU: Colony-forming unit

CLSI: Clinical and Laboratory Standards Institute

CP: Crude protein

DFM: Direct-fed microbial

ETEC: Enterotoxigenic *E.coli*

G:F: Gain-to-feed ratio/feed efficiency

ME: Metabolizable energy

MIC: Minimal inhibitory concentration

NARMS: National Antimicrobial Resistance Monitoring System

NRC: National Research Council

NE: Net energy

PCR: Polymerase chain reaction

PWD: Post-weaning diarrhea

SCFA: Short-chain fatty acid

SEM: Standard error of the mean

SID: Standardized ileal digestible

STTD: Standardized total tract digestible

WHO: World Health Organization

Introduction

The post-weaning period is one of the most stressful periods in a pig's life. Separation from the sow, transitioning from a liquid to solid diet, and a new environment with new pen-mates are contributing factors that lead to the post-weaning growth lag and diarrhea (PWD; Pluske, 2013). During this time, it is common for the colonization of enterotoxigenic *E.coli* (ETEC) in the gut which is one of the main causes for PWD (Fairbrother et al., 2005). Antibiotics were used for many years to help control the occurrences of PWD caused by ETEC; however, the ban of antibiotics for growth promotion purposes in the EU in 2006 (Regulation (EC) No. 1831/2003) and the implementation of the veterinary feed directive in the US in 2017 (FD&C Act (21 U. S. C. 354 (a) (1))) have led to research in alternative strategies to help mitigate the negative effects that follow weaning.

Yeast-based pre- and probiotics, also known as direct fed microbials (DFM), have been considered an alternative of interest because of their potential to positively modulate gut microflora which may lead to improved immunity, nutrient digestion and absorption, and growth performance (Liao and Nyachoti, 2017; Menegat et al., 2019b). These beneficial attributes may be heightened during a stressful stage of life, such as weaning. Supplementing live yeast (*Saccharomyces cerevisiae*) and/or yeast extracts derived from *S. cerevisiae* following weaning has alleviated the shedding of ETEC, shortened diarrhea occurrences, and improved nursery body weight (BW; Stuyven et al., 2009; Trckova et al., 2014). Lu et al. (2019) recently reported that feeding *S. cerevisiae* through gestation and lactation improved ADG, increased BW, and improved gross energy digestibility of offspring in the nursery.

There is little data exploring the impacts of feeding live yeast and yeast extracts in late gestation through lactation and its impact on subsequent offspring growth performance and

antimicrobial susceptibilities of fecal *E.coli* in the nursery. Our hypothesis was that the addition of yeast-based DFM would provide additive growth, from both the sow and nursery supplementation, and may lessen the instances of antimicrobial resistance (AMR) of antibiotics that are meaningful to human and animal medicine in nursery pigs.

Materials and Methods

General

The Kansas State University Institutional Animal Care and Use Committee approved the protocols used in two experiments to evaluate various yeast-based DFM supplementation when pigs were weaned from sows fed a diet with or without yeast additives. Both studies were conducted at the Kansas State University Swine Teaching and Research Center in Manhattan, KS. A single nursery room was used in Exp. 1 and Exp. 2 was conducted between two identical nursery rooms. All nursery rooms utilized are 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 (1.3 × 1.3 m) had metal tri-bar floors and allowed approximately 0.34 m^2/pig in Exp. 1 and 0.28 m^2/pig in Exp. 2.

Experiment 1

Animals and treatment structure. The objective of Exp. 1 was to evaluate the live yeast *S. cerevisiae* strain NCYC Sc 47 and yeast-based prebiotics derived from *S. cerevisiae* on nursery pigs weaned from sows fed a diet with or without yeast additives on growth performance and antimicrobial susceptibilities of *E.coli* isolated from nursery pig fecal matter. A total of 340 weaned pigs (DNA 241 × 600, DNA; initially 5.1 ± 0.03 kg 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 (Chance et al., 2021b). Only ten weaned pigs (7 from control litters and 3 from yeast additive litters) were not included in the nursery study to maintain an even number of replications per treatment and/or because of poor health. Pigs within the same sow treatment were kept together and allotted to pens, pens were then allotted to treatment with 5 pigs per pen and 17 replications per treatment in a completely randomized design.

Dietary treatments were arranged in a 2 × 2 factorial with main effects of 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). 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 *S. cerevisiae* (SafMannan) and a yeast extract containing ≥ 6% unbound nucleotides from *S. cerevisiae* (NucleoSaf).

Diet preparation. Pigs were fed experimental phase 1 diets from placement until d 7 and then offered experimental phase 2 diets from d 7 to 24 (Table 3-1). A common phase 3 diet without live yeast or yeast extracts was fed to all pigs from d 24 to 45 (Table 3-1). Phase 1 diets were formulated to 1.40% standardized ileal digestible (SID) Lys and phase 2 and 3 diets were formulated to 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 O.H. Kruse Feed Technology Innovation Center (Manhattan, KS)

and the common phase 3 diet was manufactured by a commercial feed mill (Hubbard Feeds; Beloit, KS). 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 obtain 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. Samples per dietary treatment were analyzed (Analabs; Fulton, IL; method 997.02; AOAC International, 1998) for active live yeast in phase 1 (Control: 2,000 CFU/g vs. Yeast: 19,000,000 CFU/g) and phase 2 (Control: 1,000 CFU/g vs. Yeast: 8,000,000 CFU/g) diets.

Fecal collection. Fecal samples were collected on d 5, 24, and 45 of the experiment for fecal antimicrobial resistance profiles of *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 at the Kansas State University College of Veterinary Medicine for bacterial isolation and further characterization.

***E.coli* isolation and antimicrobial susceptibility testing.** Fecal samples were subjected to the culture method, as described in chapter 2 (Chance et al., 2021b), to isolate and identify *E coli.* Antimicrobial susceptibility testing was conducted on one *E.coli* isolate per fecal sample recovered on days 5, 24, and 45. Briefly, the microbroth dilution method as outlined by the

Clinical and Laboratory Standards Institute (CLSI, 2018: Table 1-3) was used to determine the minimal inhibitory concentrations (MIC) of antibiotics. Clinical and Laboratory Standards Institute guidelines were used to classify each isolate as susceptible, intermediate, or resistant according to the breakpoints established for each antimicrobial. Minimal inhibitory concentration values greater than the susceptible breakpoint but lower than the resistant breakpoint were considered intermediate. 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.

Experiment 2

Animals and treatment structure. The objective of Exp. 2 was to evaluate feeding diets with two different combinations of *Bacillus* spp. and yeast extracts derived from *S. cerevisiae* on nursery pigs weaned from sows fed a diet with or without yeast additives on nursery pig growth performance. A total of 330 weaned pigs (DNA 241 × 600, DNA; initially 5.8 ± 0.03 kg BW), progeny of sows fed either a control diet or a diet containing yeast additives from d 110 of gestation through weaning, were used in a 38-d nursery study (Chance et al., 2021b). Only twelve weaned pigs (6 pigs from each sow treatment) were not included in the nursery study due to being either a fall behind needing extra care or pigs that were well above the average weight at weaning. Pigs within the same sow treatment were randomly allotted to pens, pens were then allotted to treatment with 6 pigs per pen and 8 to 10 replications per treatment.

Dietary treatments were fed in 3 phases and arranged in a 2 × 3 factorial with main effects of 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; DFM 1, 0.05% of SafMannan from d 0 to 38 and NucleoSaf at 0.05% from d 0 to 10 and 0.025% from d 10 to 24; or DFM 2, 0.10% MicroSaf from d 0 to 38 and NucleoSaf at 0.05% from d 0 to 10 and 0.025% from d 10 to 24; SafMannan, NucleoSaf, and MicroSaf; Phileo by Lesaffre, Milwaukee, WI). Thus, one third of the pigs from each sow group were fed either a control diet, a diet with the DFM 1 additives, or a diet with the DFM 2 additives. Direct fed microbial 1 included a yeast cell wall fraction with concentrated mannan-oligosaccharides and β-glucans from *S. cerevisiae* (SafMannan) and DFM 2 included a blend of *Bacillus* spp. and a yeast cell wall fraction (MicroSaf). Both DFM 1 and DFM 2 included a yeast extract containing ≥ 6% unbound nucleotides from *S. cerevisiae* (NucleoSaf). A respiratory disease challenge occurred from approximately d 8 to 20 of the study; thus, removals were recorded and analyzed (Table 3-6 & 3-7).

Diet preparation. Pigs were fed phase 1 diets from placement until d 10, phase 2 diets were fed from d 10 to 24, and phase 3 diets fed from d 24 to 38 (Table 3-1). Phase 1 diets were formulated to 1.40% SID Lys and phase 2 and 3 diets were formulated to 1.35% SID Lys. All other nutrients were formulated to meet or exceed NRC (2012) requirement estimates. The phase 1 control diet was manufactured by a commercial feed mill (Hubbard Feeds; Beloit, KS) then DFM 1 and DFM 2 were added at their respective amounts for phase 1 and mixed at the O.H. Kruse Feed Technology Innovation Center (Manhattan, KS). All phase 2 and 3 diets were manufactured by the same commercial feed mill with the DFM added at the expense of corn. Feed samples were collected from every fourth, 23 kg bag using a feed probe to obtain a representative sample for each respective diet and phase. All three phases were fed in meal form.

Pens of pigs were weighed, and feed disappearance recorded weekly to determine ADG, ADFI and G:F.

Statistical analysis

In both experiments, growth performance data were analyzed using the *nlme* package of R (Version 4.0.0, R Foundation for Statistical Computing, Vienna, Austria) as a completely randomized design with pen as the experimental unit. Fixed effects included sow treatment, nursery treatment, and their interaction. Nursery room served as the random effect in Exp. 2. The main effects of sow treatment and nursery treatment, as well as their interactions, were tested. In Exp. 2, the proportion of pigs removed from test pens was analyzed using a binomial distribution using a logit link function. Differences between treatments were considered significant at $P \leq 0.05$ and marginally significant at $0.05 < P \leq 0.10$.

In Exp. 1, the MIC data of each antimicrobial was 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, i.e. geometric means of the MIC values. Statistical analysis was performed using Statistical Analysis Software (SAS version 9.4; Cary, NC) PROC MIXED with option DDFM=KR in the MODEL statement. Comparisons were carried out using the two-sided test. No multiplicity adjustment was applied.

Results

Experiment 1

Growth Performance. There were no interactions observed between sow treatment and nursery treatment for any growth performance criteria (Table 3-2). In phase 1 (d 0 to 7), there were no main effects ($P > 0.30$) observed for ADG, ADFI, or G:F for sow or nursery treatments (Table 3-2 & 3-3). Pigs weaned from sows fed the yeast-based pre- and probiotics entered the nursery at a heavier BW ($P < 0.001$; 5.0 vs 5.2 kg) compared to offspring from the control sows. There was statistical difference ($P < 0.001$) in d 7 BW with offspring from sows fed the yeast-based pre- and probiotics having a heavier BW at the end of phase 1.

In phase 2 (d 7 to 24) and for the overall experimental period (d 0 to 24), progeny from sows fed the yeast-based pre- and probiotics had increased ($P < 0.05$) ADG, ADFI, and d 24 BW; however, there was no evidence for difference ($P = 0.162$) in G:F. There was no statistical difference ($P > 0.10$) observed for nursery dietary treatment on any growth criteria.

During the common period (d 24 to 45), there were main effects ($P < 0.05$) of both sow and nursery treatments on ADG. Offspring from sows fed the yeast-based pre- and probiotics had increased ($P = 0.003$) ADG, heavier ($P < 0.001$) d 45 BW, and a tendency ($P = 0.057$) for increased ADFI compared to progeny from sows fed the control diet. Pigs fed the control diet in the nursery had increased ($P = 0.011$) ADG and a tendency ($P = 0.060$) for increased ADFI compared to those fed the diet containing live yeast and yeast extracts. There was no evidence for statistical difference ($P > 0.10$) in G:F for sow or nursery treatment.

For the overall period (d 0 to 45), progeny from sows fed the yeast-based products had increased ($P < 0.05$) BW, ADG, ADFI, and improved G:F compared to pigs from sows fed the control diet. There was a tendency for increased ($P = 0.079$) ADG and increased ($P = 0.086$) BW

for pigs fed the control diet in the nursery compared to those fed the yeast-based pre- and probiotics. There was no statistical difference ($P > 0.10$) in ADFI or G:F for nursery treatment.

Antimicrobial Susceptibilities. A three-way interaction of sow treatment × nursery treatment × sampling day was observed ($P < 0.05$) for ciprofloxacin, gentamicin, sulfisoxazole, and trimethoprim/sulfamethoxazole (Table 3-4). *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 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 lower 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 3-5).

The dams of the pigs used in this study had increased ($P < 0.001$) fecal *E.coli* AMR to tetracycline at weaning compared to at the entry into the farrowing house, regardless of dietary treatment (Chance et al., 2021b). 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 dietary 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, 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.

Experiment 2

There were no interactions observed between previous sow treatment and nursery treatment (Table 3-6). Thus, the main effects of sow and nursery treatment are reported (Table 3-7).

In phase 1 (d 0 to 10), pigs weaned from sows fed yeast additives had increased ($P < 0.03$) ADG, ADFI, and G:F. Offspring from the sows fed yeast additives had lighter BW at weaning ($P < 0.001$) compared to the control sow's progeny; however, by d 10 there was no difference ($P = 0.753$) in nursery pig BW between the two sow treatments. There was no evidence for difference ($P > 0.10$) for nursery dietary treatment on any growth criteria from d 0 to 10. In phase 2 (d 10 to 24), there was no evidence ($P > 0.10$) for difference for either sow or nursery treatments on any of the response criteria.

In phase 3 (d 24 to 38), there was a tendency ($P = 0.090$) for increased ADFI for progeny of sows that were fed the control diet. There was no difference ($P > 0.10$) for previous sow treatment on ADG, G:F, or d 38 BW. Interestingly, pigs fed the DFM 2 treatment in the nursery had increased ($P < 0.05$) ADG, G:F, and d 38 BW compared to the control treatment with pigs fed DFM 1 intermediate.

For the overall period (d 0 to 38), a tendency ($P = 0.080$) was observed for improved G:F of offspring from sows fed yeast additives from d 110 of gestation through weaning. As mentioned previously, pigs fed the DFM 2 treatment in the nursery had greater ($P < 0.05$) ending BW compared to the control treatment with pigs fed DFM 1 intermediate. Regardless of dietary treatment, there was no difference ($P > 0.05$) in ADG or ADFI for the overall period. There was no evidence for statistical difference ($P > 0.10$) for the percentage of removals between treatments in this study.

Discussion

Probiotics are beneficial, live microorganisms that are designed to withstand the acidic pH of the stomach and once in the gastrointestinal tract can manipulate its microbial population. Probiotics increase the desirable microbes in the gut while subsequently out-competing enteric pathogens, which can lead to increased short-chain fatty acid (SCFA) production, improved intestinal lining integrity, increased nutrient absorption, and ultimately improved growth (Liao and Nyachoti, 2017; Liu et al., 2017; Cameron and McAllister, 2019). Most probiotics are often classified as: *Bacillus*, lactic acid producing bacteria (*Lactobacillus*, *Bifidobacterium*, and *Pediococcus*) or yeast (*S. cerevisiae*; Stein and Kil, 2006; Cameron and McAllister, 2019). Although similar to probiotics, prebiotics are not live microorganisms. Instead, prebiotics function as a food source, through fermentation and further SCFA production, to selectively stimulate the favorable gut microorganisms (Gibson et al., 2004). Inulin, lactulose, fructo-olgosaccharides, and transgalacto-oligosaccharides can be easily fermented; thus, they are some of the most commonly used prebiotics in nursery pig diets (Gibson et al., 2004; Liu et al; 2017).

In Exp. 1, live *S. cerevisiae* strain NCYC Sc 47 was evaluated as the yeast-based probiotic (ActiSaf Sc 47 HR+; Phileo by Lesaffre, Milwaukee, WI). We evaluated two yeast-based prebiotics in both Exp. 1 and Exp. 2, which included a yeast cell wall fraction with concentrated mannan-oligosaccharides and β-glucans (SafMannan; Phileo by Lesaffre, Milwaukee, WI) and a yeast extract containing ≥ 6% unbound nucleotides (NucleoSaf; Phileo by Lesaffre, Milwaukee, WI). The unique qualities of live yeast and yeast extracts is partially due to the β-glucans and α-mannans in the yeast cell wall and the fact that they encompass free nucleotides. Some of the benefits from feeding nursery pigs live yeast (probiotic) and yeast extracts (prebiotic) include: enhanced immunity (Perez-Sotelo et al., 2011; Zanello et al., 2011;

Badia et al., 2012), minimized ETEC challenges (Kiarie et al., 2011; Che et al., 2017; Trevisi et al., 2017), adsorption of mycotoxins in the feed (Kogan and Kocher, 2007), and increased growth (Shen et al., 2009; Kiros et al., 2018).

In Exp. 2, DFM 1 contained the same yeast extracts as in Exp. 1 (SafMannan and NucleoSaf) but did not contain a probiotic source. Direct fed microbial 2 contained a blend of *Bacillus* spp. and yeast extracts (MicroSaf and NucleoSaf; Phileo by Lesaffre, Milwaukee, WI). *Bacillus*-based probiotics are Gram-positive, spore-forming organisms that can withstand the acidic pH of the stomach and high temperatures of pelleting making them one of the most utilized probiotics in swine diets (Stein and Kil, 2006). *Bacillus* spores are active at the more neutral pH of the small intestine allowing for a higher likelihood for colonization and production of enzymes leading to an increase in SCFAs (Liu et al., 2017). Increasing SCFA production in a young pig can lower the digesta pH which helps control enteric pathogens and can lead to reduced occurrences of PWD (Liao and Nyachoti, 2017; Liu et al., 2017).

Although feeding pre- and probiotics has promising results on growth performance in the nursery, there is still inconsistency in literature (Zimmerman et al., 2016). Many studies have observed increased ADG, ADFI, and BW when live *S. cerevisiae* and yeast extracts were fed in the nursery (Shen et al., 2009; Kiarie et al., 2011; Kiros et al., 2018). In contrast, we observed reduced ADG during the common (d 24 to 45) and overall (d 0 to 45) periods with little statistical impact on any of the remaining growth criteria when pigs were fed the live yeast *S. cerevisiae* and yeast extracts in Exp.1. Similarly, there was no difference in growth performance criteria when pigs were fed only yeast extracts (DFM 1) compared to pigs fed a control diet in Exp. 2. Like our findings, feeding live yeast and/or yeast extracts did not affect nursery pig growth performance in some studies (Perez-Sotelo et al., 2011; Trevisi et al., 2015; and Williams

et al., 2016). When pigs were fed a *Bacillus* spp. and yeast extract blend (DFM 2) they had improved ADG and G:F in phase 3 (d 24 to 38) and heavier end of nursery BW. When Lee et al. (2011) fed a yeast-*Bacillus* blend for 35 d post-weaning, they saw no added growth benefit from the inclusion of the probiotic blend.

Some literature does not report improvement in ADG, ADFI, or BW when *Bacillus* was included in nursery diets (Williams et al., 2018; Menegat et al., 2019a; Wang et al., 2021); however, other studies report an improvement in G:F in the early nursery period (Cai et al., 2015; Wang et al, 2021). A possible explanation for the improvement in some growth performance criteria in Exp. 2 for DFM 2 could be because there was a synergistic effect of the *Bacillus* spp. and the yeast cell wall fraction (MicroSaf), without the inclusion of the unbound nucleotides (NucleoSaf), which resulted in an improvement in the later nursery period, regardless of sow treatment. The results from both experiments further exemplifies the variability in results when feeding pre- and probiotics in the nursery.

Some studies have observed an increase in sow ADFI during lactation when yeast products were included in the diet (Chance et al., 2021b, Tan et al., 2021). It is generally observed that when sows have increased intake during lactation, they tend to wean heavier pigs (Eissen et al., 2003; Krahn et al., 2021). Even though Chance et al. (2021b) and Tan et al. (2021) observed increased sow ADFI in lactation, they did not observe an improvement in litter or individual pig weaning weight. However, feeding sows yeast additives has shown to improve offspring immunity (Zanello et al., 2012; Gao et al., 2021), increase exposure to beneficial microorganisms through the sow feces (Hasan et al., 2018), and increase growth pre-weaning (Kim et al., 2008; Shen et al., 2011). These benefits may allow for the offspring to be more physiologically prepared for the stressful weaning period. In the present study, pigs were weaned

from sows that were fed the live yeast *S. cerevisiae* strain NCYC Sc 47 (ActiSaf HR+; Phileo by Lesaffre, Milwaukee, WI) which served as the yeast-based probiotic from entry into the farrowing house (approximately d 110 of gestation) through lactation. A yeast cell wall fraction with concentrated mannan-oligosaccharides and β-glucans derived from *Saccharomyces cerevisiae* (SafMannan; Phileo by Lesaffre, Milwaukee, WI) was also fed and considered a yeast-based prebiotic.

The immunological and microfloral benefits observed pre-weaning may be the main contributing factors to the improvement in growth post-weaning as a few studies have observed improved growth in the nursery when pigs were weaned from sows fed yeast. Both Lu et al. (2019) and Loughmiller et al. (2021) reported increased ADG and ADFI in the nursery when pigs were weaned from sows that were fed live yeast through gestation and lactation, which is consistent with the results from d 0 to 24 and d 0 to 45 in Exp. 1 and d 0 to 10 in Exp. 2. Both of the present experiments showed the potential for improved G:F when pigs were weaned from sows supplemented with yeast which was consistent with Lu et al. (2019) but not observed by Loughmiller et al. (2021).

To our knowledge, there is no data following the offspring of sows fed a lactation diet with or with yeast on the antimicrobial resistance of gut bacteria. A sow treatment × nursery treatment × sampling day interaction was observed for gentamicin in the current study. This interaction revealed fecal *E.coli* from progeny of yeast-fed sows that were also fed yeast in the nursery had higher MIC on d 5 post-weaning but lower MIC on d 24 compared to pigs from the yeast-fed sows but fed a control diet in the nursery. Furthermore, offspring of sows fed yeast tended to have increased AMR to gentamicin than offspring of sows fed the control diet in the farrowing house. It is important to note that, while there were statistical differences, all fecal

E.coli were considered susceptible to gentamicin. Gentamicin is an aminoglycoside class antimicrobial, and its key function is to target the 30s ribosomal subunit to prevent protein synthesis (Yoshizawa et al., 1998). It is commonly used to treat Gram-negative infections but can also be used to treat a select few Gram-positive bacteria in both humans and animals.

Ciprofloxacin and nalidixic acid are in the fluoroquinolone antibiotic family. Ciprofloxacin is used as broad-spectrum antimicrobial and nalidixic acid is used to treat primarily Gram-negative infections (Crumplin and Smith, 1975; Davis et al., 1996). Both antibiotics are used in human medicine; however, neither are utilized in animal agriculture. Fluoroquinolone class antibiotics prevent bacteria DNA synthesis by inhibiting the DNA gyrase enzyme ultimately resulting in cell death (Paton and Reeves, 1988). We observed a sow treatment × nursery treatment × sampling day interaction for ciprofloxacin. Fecal *E.coli* of offspring of yeast-fed sows that were fed yeast in the nursery appeared to have higher MIC on d 24 and 45 but lower MIC on d 5 compared to pigs also weaned from sows fed yeast but were fed the control diet in the nursery. Furthermore, progeny of sows fed yeast in the farrowing house tended to have increased AMR to ciprofloxacin and nalidixic acid. However, regardless of sow treatment, all fecal *E.coli* were considered susceptible to ciprofloxacin and nalidixic acid at all sampling time points.

Sulfisoxazole and trimpethorim/sulfamethoxazole are in the sulfonamide antimicrobial class. They are broad-spectrum antibiotics and both inhibit the dihydropteroate enzyme during folic acid metabolism which normally aids in nucleic acids production for DNA (Kapoor et al., 2017). Sulfisoxazole and trimpethorim/sulfamethoxazole are commonly used antibiotics in both human and livestock medicine. A sow treatment × nursery treatment × sampling day interaction was observed for both sulfisoxazole and trimpethorim/sulfamethoxazole in our study. Progeny of

the control sows that were fed live yeast and yeast extracts in the nursery had lower MIC values to sulfisoxazole on d 45 and to trimpethorim/sulfamethoxazole on d 5 compared to offspring that were also from the control sows but were fed a control diet in the nursery. Once again, all fecal *E.coli* was susceptible to both sulfisoxazole and trimpethorim/sulfamethoxazole regardless of treatment or sampling day.

Azithromycin is in the azalide family, a more specific class of macrolide antimicrobials (Bakheit et al., 2014). Chloramphenicol is a partially synthesized antibiotic from *Streptomyces venequelae* in the phenicol class (National Center for Biotechnology Information, 2021). Both azithromycin and chloramphenicol are broad-spectrum antibiotics that interfere with protein synthesis by binding to the 50s ribosomal subunit resulting in bacterial cell death (Bakheit et al., 2014; National Center for Biotechnology Information, 2021). However, azithromycin is commonly utilized in both humans and animals while chloramphenicol is rarely used in human medicine and prohibited in animal agriculture. We observed a decrease in MIC values for the antimicrobial's azithromycin and chloramphenicol in nursery pig fecal *E.coli* when live yeast and yeast extracts were included in the diet. All MIC values were well under the CLSI (2018) breakpoint for azithromycin and chloramphenicol and were considered either susceptible or intermediate. Adversely, the addition of the same combination of live yeast and yeast extracts used in Exp. 1 did not impact the AMR of fecal *E.coli* in nursery pigs in chapter 1 (Chance et al., 2021a). Using the same 14 antimicrobials evaluated in our study, Williams et al. (2018) also observed no difference in the AMR of fecal *E.coli* from nursery pigs that were fed a *bacillus*-based DFM or a blend of lactic acid producing DFM compared to pigs fed a control diet.

In conclusion, for Exp. 1, when sows were fed a live yeast and yeast extract from d 110 of gestation through weaning, their progeny were heavier at weaning and had increased ADG,

ADFI, and heavier BW throughout the nursery period. However, feeding yeast additives in the nursery tended to reduce ADG and lower nursery ending BW. Offspring from sows that were fed yeast might increase the potential of fecal *E.coli* AMR to nalidixic acid, ciprofloxacin, and gentamicin. Yet, feeding live yeast and yeast extracts in the nursery may lessen the AMR of azithromycin and chloramphenicol of fecal *E.coli*. In Exp. 2, feeding yeast additives from d 110 of gestation through lactation improved progeny nursery growth performance from d 0 to 10 post-weaning and tended to improve overall G:F. Additionally, feeding DFM 2 in nursery diets improved final BW and late nursery ADG and G:F compared to pigs not fed a DFM. Thus, in Exp. 2, the addition of yeast additives in sow diets had more impact on offspring's growth performance in the early nursery while the inclusion of DFMs in the nursery had more influence on growth later in the nursery.

Table 3-1. Diet composition (as-fed basis)[1,2]

	Exp. 1			Exp. 2		
Item	Phase 1	Phase 2	Phase 3	Phase 1	Phase 2	Phase 3
Ingredients, %						
Corn	44.36	57.40	64.73	44.15	56.75	64.75
Soybean meal, 46.5% CP	18.12	26.35	31.30	18.20	25.85	31.30
Whey powder	25.00	10.00	---	25.00	10.00	---
Fish meal	4.50	---	---	4.50	2.00	---
Enzymatically-treated soybean meal[3]	3.75	2.00	---	3.75	---	---
Oil	1.50	---	---	1.50	1.50	---
Calcium carbonate	0.30	0.90	0.85	0.30	0.63	0.85
Monocalcium phosphate, 21% P	0.48	1.10	1.00	0.48	0.85	1.00
Salt	0.30	0.55	0.60	0.30	0.55	0.60
L-Lys-HCl	0.43	0.51	0.52	0.43	0.51	0.52
DL-Met	0.22	0.22	0.21	0.22	0.22	0.21
L-Thr	0.18	0.21	0.22	0.18	0.22	0.22
L-Trp	0.07	0.06	0.06	0.07	0.06	0.06
L-Val	0.13	0.14	0.13	0.13	0.15	0.13
L-Ile	---	---	---	---	0.02	---
Vitamin premix[4]	0.25	0.25	---	---	---	---
Vitamin premix with phytase[5]	---	---	0.25	0.25	0.25	0.25
Trace mineral premix[6]	0.15	0.15	0.15	0.15	0.15	0.15
Zinc oxide	---	---	---	0.40	0.27	---
Phytase[7]	0.08	0.08	---	---	---	---
DFM [8,9]	±	±	---	±	±	±
Total	100	100	100	100	100	100

Table 3-1. (cont.)

	Exp. 1			Exp. 2		
	Phase 1	Phase 2	Phase 3	Phase 1	Phase 2	Phase 3
Calculated analysis						
SID amino acids, %						
Lys	1.40	1.35	1.35	1.40	1.35	1.35
Ile:Lys	56	55	55	56	55	55
Leu:Lys	109	112	114	109	110	114
Met:Lys	38	36	36	38	37	36
Met and Cys:Lys	57	57	57	57	57	57
Thr:Lys	63	63	63	63	63	63
Trp:Lys	20.6	20.2	20.3	20.6	20.0	20.3
Val:Lys	69	69	69	69	69	69
His:Lys	32	34	36	32	34	36
Total Lys, %	1.54	1.48	1.49	1.54	1.49	1.49
ME, kcal/kg	3,425	3,282	3,278	3,419	3,373	3,280
NE, kcal/kg	2,582	2,440	2,421	2,577	2,529	2,423
SID Lys:NE, g/Mcal	5.42	5.53	5.57	5.43	5.34	5.57
CP, %	20.9	20.5	21.2	20.9	20.3	21.2
Ca, %	0.69	0.77	0.69	0.69	0.70	0.69
P, %	0.68	0.66	0.61	0.68	0.64	0.61
STTD P, %	0.63	0.58	0.50	0.63	0.57	0.50

[1] In Exp. 1, phase 1 diets were fed from d 0 to 7 (approximately 5.1 to 5.5 kg BW) and phase 2 diets were fed from d 7 to 24 (approximately 5.5 to 11.9 kg BW). A common diet, without yeast additives, was fed during phase 3 from d 24 to 45 (approximately 11.9 to 27.1 kg BW).

[2] In Exp. 2, phase 1 diets were fed from d 0 to 10 (approximately 5.8 to 6.7 kg BW), phase 2 diets were fed from d 10 to 24 (approximately 6.7 to 13.3 kg BW), and phase 3 diets were fed from d 24 to 38 (approximately 13.3 to 21.5 kg BW).

[3] HP 300, Hamlet Protein, Findlay, OH.

[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 2,027 FTU/kg in phases 1 and 2 and 1,250 FTU/kg in phase 3 with an expected STTD P release of 0.16% in phases 1 and 2 and 0.14% in phase 3. 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 918 FTU/lb and an estimated release of 0.16% STTD P.

[8] In Exp. 1, DFM 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).

[9] In Exp 2, DFM 1 was a yeast-extract blend with SafMannan (0.05% in phases 1, 2, and 3) and NucleoSaf (0.05% in phase 1, 0.025% in phase 2 and 0% in phase 3) or DFM 2 was a *Bacillus* spp. and yeast-extract blend with MicroSaf (0.10% in phases 1, 2, and 3) and NucleoSaf (0.05% in phase 1, 0.025% in phase 2 and 0% in phase 3). SafMannan, NucleoSaf, and MicroSaf; Phileo by Lesaffre, Milwaukee, WI.

Table 3-2. Exp.1– Interactive effects of yeast-fed sows and yeast-fed nursery pigs on growth performance of nursery pigs[1]

	Sow treatment[2]/Nursery treatment[3]							
	Control		Yeast			*P* =		
Item	Control	Yeast	Control	Yeast	SEM	Sow	Nursery	Sow × Nursery
BW, kg								
d 0	5.02	4.97	5.21	5.21	0.034	< 0.001	0.507	0.507
d 7	5.40	5.34	5.62	5.59	0.070	0.001	0.516	0.825
d 24	11.51	11.38	12.33	12.22	0.211	< 0.001	0.569	0.968
d 45	26.54	26.18	28.23	27.35	0.356	< 0.001	0.086	0.476
Phase 1 (d 0 to 7)								
ADG, g	54	46	53	53	7.9	0.719	0.604	0.654
ADFI, g	113	113	122	116	6.4	0.351	0.585	0.637
G:F, g/kg	464	368	397	415	56.9	0.858	0.497	0.315
Phase 2 (d 7 to 24)								
ADG, g	359	354	390	387	9.7	0.002	0.653	0.920
ADFI, g	499	493	535	523	14.6	0.026	0.530	0.864
G:F, g/kg	722	719	732	742	11.4	0.162	0.781	0.580
Experimental period (d 0 to 24)								
ADG, g	269	263	291	288	8.1	0.006	0.560	0.839
ADFI, g	386	380	413	403	11.4	0.031	0.479	0.822
G:F, g/kg	701	690	705	717	10.8	0.153	0.974	0.308
Phase 3 common diet (d 24 to 45)								
ADG, g	716	700	754	721	9.6	0.003	0.011	0.369
ADFI, g	1,085	1,059	1,122	1,086	16.3	0.057	0.060	0.747
G:F, g/kg	660	661	673	664	5.2	0.123	0.446	0.337
Overall (d 0 to 45)								
ADG, g	477	465	504	489	7.4	0.001	0.079	0.868
ADFI, g	711	695	739	720	12.3	0.037	0.163	0.940
G:F, g/kg	671	670	683	680	5.1	0.040	0.599	0.862

[1] A total of 340 pigs (initial BW of 5.1 ± 0.03 kg) 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 additives) and nursery pig treatment (control or yeast additives).

[2] Sow treatment consisted of providing a control diet or a yeast-based pre- & probiotic diet supplemented with ActiSaf Sc 47 HR+ at 0.10% and SafMannan at 0.025% (Phileo by Lesaffre, Milwaukee, WI) from d 110 of gestation until weaning.

[3] Nursery treatment consisted of providing a control diet or a yeast-based pre- & 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).

Table 3-3. Exp.1– Main effects of yeast-fed sows and yeast-fed nursery pigs on growth performance of nursery pigs[1]

	Sow treatment[2]				Nursery treatment[3]			
Item	Control	Yeast	SEM	*P* =	Control	Yeast	SEM	*P* =
BW, kg								
d 0	5.00	5.21	0.024	< 0.001	5.11	5.09	0.024	0.507
d 7	5.37	5.61	0.049	0.001	5.51	5.46	0.049	0.516
d 24	11.44	12.27	0.149	< 0.001	11.92	11.80	0.149	0.569
d 45	26.36	27.79	0.251	< 0.001	27.38	26.76	0.251	0.086
Phase 1 (d 0 to 7)								
ADG, g	50	53	5.6	0.719	54	50	5.6	0.604
ADFI, g	113	119	4.5	0.351	118	114	4.5	0.585
G:F, g/kg	416	406	40.2	0.858	430	391	40.2	0.497
Phase 2 (d 7 to 24)								
ADG, g	357	388	6.8	0.002	375	370	6.8	0.653
ADFI, g	496	529	10.3	0.026	517	508	10.3	0.530
G:F, g/kg	721	737	8.1	0.162	727	730	8.1	0.781
Experimental period (d 0 to 24)								
ADG, g	266	289	5.7	0.006	280	275	5.7	0.560
ADFI, g	383	408	8.0	0.031	400	391	8.0	0.479
G:F, g/kg	695	711	7.6	0.153	703	703	7.6	0.974
Phase 3 common diet (d 24 to 45)								
ADG, g	708	738	6.8	0.003	735	710	6.8	0.011
ADFI, g	1,072	1,103	11.5	0.057	1,104	1,072	11.5	0.060
G:F, g/kg	661	669	3.7	0.123	667	663	3.7	0.446
Overall (d 0 to 45)								
ADG, g	471	496	5.2	0.001	490	477	5.2	0.079
ADFI, g	703	729	8.7	0.037	725	708	8.7	0.163
G:F, g/kg	671	681	3.6	0.040	677	675	3.6	0.599

[1] A total of 340 pigs (initial BW of 5.1 ± 0.03 kg) were used in a 45-d nursery trial with 5 pigs per pen and 34 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 additives) and nursery pig treatment (control or yeast additives).

[2] Sow treatment consisted of providing a control diet or a yeast-based pre- & probiotic diet supplemented with ActiSaf Sc 47 HR+ at 0.10% and SafMannan at 0.025% (Phileo by Lesaffre, Milwaukee, WI) from d 110 of gestation until weaning.

[3] Nursery treatment consisted of providing a control diet or a yeast-based pre- & 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).

Table 3-4. Exp. 1– Interactive effects of yeast-fed sows and yeast-fed nursery pigs over time on antimicrobial susceptibilities of nursery pig fecal *Escherichia coli* according to National Antimicrobial Resistance Monitoring System (CLSI, 2018) established breakpoints[1,2]

	Sow treatment[2]/Nursery treatment[3]										
	Control		Yeast		*P* =						
Item	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							
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							

Continued

Table 3-4. (cont.)

	Sow treatment[2]/Nursery treatment[3]										
	Control		Yeast		*P* =						
Item	Control	Yeast	Control	Yeast	Sow	Nursery	Day	Sow × Nursery	Sow × Day	Nursery × Day	Sow × Nursery × Day
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							
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 1:19 ratio[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							

[1] A total of 340 pigs (initially 5.1 ± 0.03 kg) were used in a 45-d nursery trial with 5 pigs per pen and 17 pens per treatment. Pigs were weaned at approximately19 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 MIC ± SEM.

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

[3] Sow treatment consisted of providing a control diet or a yeast-based pre- & probiotic diet supplemented with ActiSaf Sc 47 HR+ at 0.10% and SafMannan at 0.025% (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.

[4] Nursery treatment consisted of providing a control diet or a yeast-based pre- & 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).

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

[6] A three-way interaction of sow treatment × nursery treatment × day was observed ($P = 0.010$). On d 24 ($P = 0.081$) and on d 45 ($P = 0.044$), pigs that were fed yeast in the nursery and came from the yeast sow group having 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.

[7] A three-way interaction of sow treatment × nursery treatment × day was observed ($P = 0.045$). MIC values of fecal *E.coli* isolated from pigs of the yeast sow and yeast nursery treatment being 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.

[8] A three-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.

[9] A three-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 3-5. Exp. 1– Main effects of yeast-fed sows, yeast-fed nursery pigs, and sampling time on antimicrobial susceptibilities of nursery pig fecal *Escherichia coli* according to National Antimicrobial Main Resistance Monitoring System (CLSI, 2018) established breakpoints[1,2]

	Sow treatment[3]			Nursery treatment[4]			Day			
Item	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

[1] A total of 340 pigs (initially 5.1 ± 0.03 kg BW) were used in a 45-d nursery trial with 5 pigs per pen and 34 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 additives) and nursery pig treatment (control or yeast additives). Data reported as geometric mean of MIC ± SEM.

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

[3] Sow treatment consisted of providing a control diet or a yeast-based pre- & probiotic diet supplemented with ActiSaf Sc 47 HR+ at 0.10% and SafMannan at 0.025%
(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.

[4] Nursery treatment consisted of providing a control diet or a yeast-based pre- & 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).

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

[a,b] Superscripts signify a statistical difference of $P < 0.05$.

Table 3-6. Exp. 2– Interactive effects of yeast-fed sows and DFM-fed nursery pigs on growth performance of nursery pigs[1]

	Sow treatment[2]/Nursery treatment									
	Control			Yeast				*P* =		
Item	Control	DFM 1[3]	DFM 2[4]	Control	DFM 1	DFM 2	SEM	Sow	Nursery	Sow × Nursery
BW, kg										
d 0	5.91	5.90	5.90	5.64	5.65	5.61	0.032	< 0.001	0.738	0.722
d 10	6.52	6.77	6.73	6.66	6.64	6.64	0.112	0.753	0.498	0.399
d 24	12.90	13.34	13.37	13.11	13.42	13.40	0.254	0.591	0.206	0.930
d 38	20.99	21.62	21.89	20.98	21.79	21.95	0.371	0.800	0.028	0.969
Phase 1 (d 0 to 10)										
ADG, g	60	82	82	101	99	101	10.7	0.003	0.508	0.428
ADFI, g	114	138	130	142	156	156	9.6	0.002	0.103	0.850
G:F, g/kg	491	596	623	697	624	644	47.5	0.023	0.680	0.081
Phase 2 (d 10 to 24)										
ADG, g	441	469	464	454	457	470	12.7	0.815	0.235	0.559
ADFI, g	560	611	598	584	589	610	17.4	0.738	0.117	0.333
G:F, g/kg	788	468	777	778	777	771	10.8	0.750	0.548	0.530
Phase 3 (d 24 to 38)										
ADG, g	578	592	608	562	598	599	13.6	0.553	0.033	0.685
ADFI, g	895	896	908	856	891	878	18.6	0.090	0.533	0.613
G:F, g/kg	645	660	671	656	672	684	11.4	0.179	0.064	0.992
Overall (d 0 to 38)										
ADG, g	383	412	411	399	408	411	10.5	0.596	0.094	0.599
ADFI, g	555	591	581	565	577	576	13.7	0.811	0.730	0.632
G:F, g/kg	690	697	707	706	708	714	8.4	0.069	0.307	0.809
Removals, %	7.4	1.7	5.6	2.1	7.4	10.0	3.87	0.625	0.402	0.179

[1] A total of 330 pigs (initially 5.8 ± 0.03 kg BW) were used in a 38-d nursery trial with 6 pigs per pen and 8 to 10 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 × 3 factorial with main effects of sow treatment (control or yeast additives) and nursery pig treatment (control, DFM 1, or DFM 2).

[2] Sow treatment consisted of providing a control diet or a yeast-based pre- & probiotic diet supplemented with Actisaf Sc 47 HR+ at 0.10% and SafMannan at 0.025% (Phileo by Lesaffre, Milwaukee, WI) from d 110 of gestation until weaning.

[3] DFM 1 was a yeast-extract blend with SafMannan (0.05% in phases 1, 2, and 3) and NucleoSaf (0.05% in phase 1, 0.025% in phase 2 and 0% in phase 3); Phileo by Lesaffre, Milwaukee, WI.

[4] DFM 2 was a *Bacillus* spp. and yeast-extract blend with MicroSaf (0.10% in phases 1, 2, and 3) and NucleoSaf (0.05% in phase 1, 0.025% in phase 2 and 0% in phase 3); Phileo by Lesaffre, Milwaukee, WI.

Table 3-7. Exp. 2– Main effects of yeast-fed sows and DFM-fed nursery pigs on growth performance of nursery pigs[1]

	Sow treatment[2]				Nursery treatment				
Item	Control	Yeast	SEM	*P* =	Control	DFM 1[3]	DFM 2[4]	SEM	*P* =
BW, kg									
d 0	5.90	5.64	0.017	< 0.001	5.78	5.77	5.76	0.022	0.738
d 10	6.67	6.65	0.061	0.753	6.59	6.71	6.69	0.077	0.498
d 24	13.21	13.31	0.139	0.591	13.01	13.38	13.39	0.174	0.206
d 38	21.50	21.57	0.203	0.800	20.98[b]	21.71[ab]	21.92[a]	0.255	0.028
Phase 1 (d 0 to 10)									
ADG, g	75	100	5.9	0.003	81	90	92	7.4	0.508
ADFI, g	127	151	5.2	0.002	128	147	143	6.6	0.103
G:F, g/kg	570	655	26.0	0.023	594	610	634	32.7	0.680
Phase 2 (d 10 to 24)									
ADG, g	458	460	7.0	0.815	447	463	467	8.7	0.235
ADFI, g	590	594	9.5	0.738	572	600	604	11.9	0.117
G:F, g/kg	778	775	5.9	0.772	783	773	774	7.5	0.547
Phase 3 (d 24 to 38)									
ADG, g	592	586	7.4	0.553	570[b]	595[ab]	604[a]	9.3	0.033
ADFI, g	900	875	10.2	0.090	876	893	893	12.8	0.533
G:F, g/kg	659	670	6.3	0.191	651[b]	666[ab]	677[a]	7.9	0.057
Overall (d 0 to 38)									
ADG, g	402	406	5.8	0.596	391	410	411	7.2	0.094
ADFI, g	575	573	7.5	0.811	560	584	579	9.4	0.173
G:F, g/kg	698	709	4.6	0.080	698	703	711	5.7	0.276
Removals, %	4.1	5.4	2.08	0.625	4.0	3.6	7.5	2.54	0.402

[1] A total of 330 pigs (initially 5.8 ± 0.03 kg BW) were used in a 38-d nursery trial with 6 pigs per pen and 16 to 20 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 × 3 factorial with main effects of sow treatment (control or yeast additives) and nursery pig treatment (control, DFM 1, or DFM 2).

[2] Sow treatment consisted of providing a control diet or a yeast-based pre- & probiotic diet supplemented with Actisaf Sc 47 HR+ at 0.10% and SafMannan at 0.025% (Phileo by Lesaffre, Milwaukee, WI) from d 110 of gestation until weaning.

[3] DFM 1 was a yeast-extract blend with SafMannan (0.05% in phases 1, 2, and 3) and NucleoSaf (0.05% in phase 1, 0.025% in phase 2 and 0% in phase 3); Phileo by Lesaffre, Milwaukee, WI.

[4] DFM 2 was a *Bacillus* spp. and yeast-extract blend with MicroSaf (0.10% in phases 1, 2, and 3) and NucleoSaf (0.05% in phase 1, 0.025% in phase 2 and 0% in phase 3); Phileo by Lesaffre, Milwaukee, WI.

[a,b] Superscripts signify a statistical difference of $P < 0.05$.

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