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**Animal Health and Well Being**

# Effects of yeast-based pre- and probiotics in lactation diets of sows on litter performance and antimicrobial resistance of fecal *Escherichia coli* of sows

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## Abstract

A total of 80 sows (Line 241; DNA, Columbus, NE) 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 day 110 of gestation and allotted to 1 of 2 dietary treatments. Dietary treatments consisted of a standard lactation diet with or without yeast-based pre- and probiotics (0.10% Actisaf Sc 47 HR+ and 0.025% SafMannan; Phileo by Lesaffre, Milwaukee, WI). Diets were fed from day 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 with the control diet. There was no evidence ($P > 0.10$) that treatment influenced any other sow or litter performance measurements. Fecal samples were collected upon entry into the farrowing house and at weaning from the first farrowing group (27 sows) to determine the resistance patterns of *E. coli*. *E. coli* was isolated from fecal samples and species confirmed 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 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 with sows fed yeast additives. There were no diet main effects ($P > 0.10$) on the resistance of fecal *E. coli*. There was an increased ($P < 0.02$) toward resistance for 11 of the 14 antimicrobials over time. Fecal *E. coli* were resistant to tetracycline and ceftriaxone at weaning. Fecal *E. coli* were susceptible or intermediate in all sampling days 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 resistance 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.

## Lay summary

Feeding sows live yeast and yeast extracts from day 110 of gestation through lactation tended to increase lactation feed intake but did not affect any other sow or litter performance criteria. Live yeast and yeast extracts in the diet had minimal effect on the antimicrobial resistance of fecal *E. coli* isolates. Regardless of the diet, fecal *E. coli* isolates were susceptible to 11 of the 14 antimicrobials when sows entered the farrowing house. But most of the antimicrobials were classified as intermediate or with a tendency toward resistance at weaning even though none of these antibiotics were used during the lactation period. Our findings agree with other cross-sectional studies on AMR where high AMR gene levels reported among young pigs were attributed to sow population.

**Key words:** antimicrobial resistance, litter performance, live yeast, sows, 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

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## Introduction

Supplementing yeast-based prebiotics and probiotics (*Saccharomyces cerevisiae*) 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 yeast through gestation and lactation has shown to increase average daily gain (ADG), body weight (BW), and improve digestibility of gross energy of the offspring in the nursery phase (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, *S. cerevisiae* strain NCYC Sc 47, and a 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 resistance 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 (IACUC # 4506.6). 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 day 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 1).

From day 110 until farrowing (approximately day 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).

**Table 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 an 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.

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Sow BW was measured at entry into the farrowing house (day 110 of gestation), 24 h after farrowing, and at weaning. Sow back fat (BF) depth (measured 7 cm from the midline at the last rib) was measured (Renco Lean Meter, S. E. C. Repro Inc., Golden Valley, MN) 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 days 2, 10, and at weaning. Pre-weaning mortality was calculated as the total mortality (day 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 1).

## Fecal sample collection

Fecal samples were collected from the first farrowing group (27 sows) to determine the antimicrobial susceptibility and 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 zipper storage bags 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 subjected to polymerase chain reaction (PCR) for *uidA* and *clpB* genes for species confirmation. The conformed *E. coli* isolates were stored in cryo-protect beads (Cryocare, Key Scientific Products, Round Rock, TX) at −80 °C.

## *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 day 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 antibiotics. The antimicrobials tested 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 2) 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 BW, 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 $P \leq 0.05$.

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**Table 2.** 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.

## Results

### Sow and litter performance

Inclusion of yeast additives from day 110 of gestation through weaning resulted in no statistical difference ($P > 0.10$) for sow BW or BW change throughout lactation (Table 3). 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 4) 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 day 2 post-farrowing, day 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 resistance to cefoxitin (Table 5). 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 other interactions observed ($P > 0.10$). There was no evidence ($P > 0.10$) that the dietary inclusion of yeast additives influenced the resistance of fecal *E. coli* isolates compared to the control diet for any of the 14 antimicrobials evaluated (Table 6).

**Table 3.** 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 day 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).

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**Table 4.** 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* | | | | |
| Day 2 | 14.2 | 14.3 | 0.60 | 0.836 |
| Day 10 | 13.3 | 13.9 | 0.59 | 0.448 |
| Wean | 12.9 | 13.5 | 0.58 | 0.498 |
| Litter weight, kg | | | | |
| Day 2 | 23.23 | 23.42 | 0.530 | 0.797 |
| Day 10 | 46.33 | 46.19 | 1.739 | 0.946 |
| Wean | 71.35 | 72.67 | 1.961 | 0.635 |
| Mean piglet BW, kg | | | | |
| Day 2 | 1.65 | 1.64 | 0.033 | 0.849 |
| Day 10 | 3.48 | 3.34 | 0.118 | 0.312 |
| Wean | 5.51 | 5.41 | 0.119 | 0.579 |
| Litter ADG Day 2 to wean, kg/day | 2.59 | 2.64 | 0.973 | 0.741 |
| Piglet ADG Day 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 day 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).

Most fecal *E. coli* isolates from sows fed either dietary treatment were 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). Fecal *E. coli* was susceptible to ceftriaxone at entry into the farrowing house but resistant at weaning. The remaining 12 antimicrobials were 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 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,) 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) 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 (Bajagai et al., 2016). While prebiotics have similar modes of action as probiotics, they differ in the sense that prebiotics are not live microbes. Instead, prebiotics serve as substrates that can selectively stimulate beneficial gut microorganisms (Menegat et al., 2019; Chance et al., 2021).

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 day 90 of gestation through lactation. However, many studies that evaluated the inclusion of yeast additives in sow diets reported no statistical impact on sow feed intake during lactation (Kim et al., 2010; Chen et al., 2020; Gao et al., 2021). 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 day 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 day 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

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**Table 5.** 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]

| Item | Control | Yeast[2] | *P* | | |
|---|---|---|---|---|---|
| | (*n* = 14) | (*n* = 13) | 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 day 110 of gestation until weaning with 13 or 14 sows per treatment. Fecal samples were collected upon entry into the farrowing house (approximately day 110 of gestion) and prior to weaning (approximately day 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 day 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.

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**Table 6.** 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* = |
|---|---|---|---|---|---|---|
| | (*n* = 14) | (*n* = 13) | | | | |
| 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 day 110 of gestation until weaning with 13 or 14 sows per treatment. Fecal samples were collected upon entry into the farrowing house (approximately day 110 of gestation) and prior to weaning (approximately day 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 day 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.

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 resistance of gut bacteria in swine when fed pre- or probiotics. To our knowledge, this is one of the few studies reporting the resistance 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. In the present study, an interaction revealed increased resistance to cefoxitin over time when sows were fed yeast additives. Although no antibiotics were administered during lactation, MIC values to 11 of the 14 antimicrobials tested increased over time regardless of dietary treatment. Several studies have reported 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., 2015). 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., unpublished data). The environmental conditions such as farrowing room, diet, weaning, and time of entry into farrowing house does have an impact on AMR. 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 day 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 AMR 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 with 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. Yeast-based pre- or probiotics used in the study did not appear to have any significant impact on prevalence of AMR bacteria in the gut of swine. Possibly, inclusion of antibiotics to exert selection pressure may have provided a better model to evaluate effects of probiotics on AMR in gut bacteria.

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## Conflict of Interest Statement

The authors declare no conflict of interest; however, Joseph A. Loughmiller and Brian Hotze are employees of Phileo by Lesaffre, the company who provided partial financial support for this project.

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