Kansas Agricultural Experiment Station Research Reports

Volume 7
Issue 11 *Swine Day*

Article 9

2021

The Effect of Live Yeast and Yeast Extracts Included in Lactation Diets on Antimicrobial Susceptibility of Fecal Escherichia coli in Sows

Jenna A. Chance
Kansas State University, jchance10@ksu.edu

Jordan T. Gebhardt
Department of Diagnostic Medicine/Pathology, College of Veterinary Medicine, Kansas State University, jgebhardt@k-state.edu

Joel M. DeRouchey
Kansas State University, jderouch@ksu.edu

Recommended Citation

Chance, Jenna A.; Gebhardt, Jordan T.; DeRouchey, Joel M.; Amachawadi, Raghavendra G.; Ishengoma, Victor; Nagaraja, T. G.; Tokach, Mike D.; Woodworth, Jason C.; Goodband, Robert D.; Kang, Qing; Loughmiller, Joseph A.; and Hotze, Brian (2021) "The Effect of Live Yeast and Yeast Extracts Included in Lactation Diets on Antimicrobial Susceptibility of Fecal Escherichia coli in Sows," *Kansas Agricultural Experiment Station Research Reports*: Vol. 7: Iss. 11. https://doi.org/10.4148/2378-5977.8173

The Effect of Live Yeast and Yeast Extracts Included in Lactation Diets on Antimicrobial Susceptibility of Fecal Escherichia coli in Sows

Authors

Jenna A. Chance, Jordan T. Gebhardt, Joel M. DeRouchey, Raghavendra G. Amachawadi, Victor Ishengoma, T. G. Nagaraja, Mike D. Tokach, Jason C. Woodworth, Robert D. Goodband, Qing Kang, Joseph A. Loughmiller, and Brian Hotze

K-STATE Research and Extension

SWINE DAY 2021

The Effect of Live Yeast and Yeast Extracts Included in Lactation Diets on Antimicrobial Susceptibility of Fecal *Escherichia coli* in Sows

Jenna A. Chance, Jordan T. Gebhardt,[1] Joel M. DeRouchey, Raghavendra G. Amachawadi,[2] Victor Ishengoma,[2] T.G. Nagaraja,[1] Mike D. Tokach, Jason C. Woodworth, Robert D. Goodband, Qing Kang,[3] Joseph A. Loughmiller,[4] and Brian Hotze[4]

Summary

A total of 27 sows (Line 241; DNA Genetics) were used in a study to evaluate the effect of feeding live yeast and yeast extracts to lactating sows on antimicrobial susceptibilities of fecal *E. coli*. Sows were blocked by BW and parity on d 110 of gestation and allotted to 1 of 2 dietary treatments. Dietary treatments consisted of a standard corn-soybean meal 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). Sow fecal samples were collected to determine the antimicrobial susceptibility of *E. coli* upon entry into the farrowing house and at weaning for each treatment. The *E. coli* was isolated from fecal samples, and species confirmation was accomplished 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 (CLSI, 2018). An interaction ($P = 0.026$) of diet × sampling day was observed for cefoxitin where fecal *E. coli* isolates showed no significant 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 significant diet main effects ($P > 0.10$) on the antimicrobial resistance (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* isolates were resistant to tetracycline and ceftriaxone at weaning. All other isolates were considered susceptible or intermediate across sampling day. In conclusion, feeding live yeast and yeast extracts did not influence either sow or litter performance measurements or the AMR of fecal *E. coli*

[1] Department of Diagnostic Medicine/Pathology, College of Veterinary Medicine, Kansas State University.
[2] Department of Clinical Sciences, College of Veterinary Medicine, Kansas State University.
[3] Department of Statistics, College of Arts and Sciences, Kansas State University.
[4] Phileo by Lesaffre, Milwaukee, WI.

during lactation except for cefoxitin, which had a higher MIC at the end of lactation when live yeast and yeast extracts were present in the diet.

Introduction

Supplementing live yeast and yeast extracts in sow diets has been researched due to the potential for a healthier/heavier piglet which may be more equipped to handle weaning stress, leading to improved nursery performance. This report is a companion to another research report where we evaluated the effects of a live yeast and a yeast extract on sow and litter performance.[5]

While there are many studies exploring the effects of feeding live yeast to sows and its influence on litter performance in the farrowing house, to our knowledge there is little-to-no data related to the impacts of feeding live yeast and yeast extracts on the antimicrobial resistance 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 *Saccharomyces cerevisiae* on the antimicrobial susceptibility of sow fecal *E. coli.*

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 27 mixed-parity sows (DNA 241, DNA Genetics) were used in one batch farrowing group with 13 or 14 sows per treatment at the Kansas State University Swine Teaching and Research Center in Manhattan, KS. Sows were blocked by farrowing group, BW, and parity on d 110 of gestation and randomized to treatments. Details as to sow allotment, experimental design, and diet preparation and analysis can be found in Chance et al.[5]

Briefly, dietary treatments consisted of a standard corn-soybean meal 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 *Saccharomyces 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 *Saccharomyces cerevisiae* (SafMannan). From d 110 until farrowing (approximately d 115), sows were fed approximately 6 lb of their respective treatment diets, then sows were allowed *ad libitum* access to feed post-farrowing until weaning.

Fecal collection

Fecal samples were collected from each sow 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). Samples were stored in a clean, single-use zipper storage bag and kept on ice until delivered to the laboratory for bacterial isolation and further characterization.

[5] Chance, J. A., J. T. Gebhardt, J. M. DeRouchey, M. D. Tokach, J. C. Woodworth, R. D. Goodband, and J. A. Loughmiller. 2021. The Effect of Live Yeast and Yeast Extracts Included in Lactation Diets on Sow and Litter Performance. *Kansas Experimental Station Research Reports:* Vol. 7, Issue 11.

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 98.6°F for 24 h. An indole test was done and indole-positive isolates were stored in cryo-protect beads (Cryocare, Key Scientific Products, Round Rock, TX) at −112°F. Species confirmation of *E. coli* was accomplished by polymerase chain reaction (PCR) detection of *uidA* and *clpB* genes.

Antimicrobial susceptibility testing of E. coli isolates

Antimicrobial susceptibility testing was conducted on *E. coli* isolates recovered upon entry into the farrowing house (approximately d 110 of gestation) and at weaning (approximately 18 d post-farrowing). The microbroth dilution method as outlined by the Clinical and Laboratory Standards Institute (CLSI, 2018)[6] was used to determine the minimal inhibitory concentrations (MIC) of antibiotics. The antimicrobials evaluated included: amoxicillin/clavulanic acid 2:1 ratio, ampicillin, azithromycin, cefoxitin, ceftiofur, ceftriaxone, chloramphenicol, ciprofloxacin, gentamicin, nalidixic acid, streptomycin, sulfisoxazole, tetracycline, and trimethoprim/sulfamethoxazole. Each isolate, stored in cryo-protect beads, was streaked onto a blood agar plate and incubated at 98.6°F for 24 h. Individual colonies were suspended in demineralized water (Trek Diagnostic Systems, Cleveland, OH) and turbidity was adjusted to 0.5 McFarland turbidity standards. Then, 10 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 98.6°F for 18 h and bacterial growth was assessed using Sensititre ARIS and Vizion systems (Trek Diagnostic Systems). Clinical and Laboratory Standards Institute[6] (Table 1) guidelines were used to classify each isolate as susceptible, intermediate, or resistant according to the breakpoints established for each antimicrobial. The MIC values greater than the susceptible breakpoint but lower than the resistant breakpoint were considered intermediate.

Statistical analysis

The MIC data of each antimicrobial were analyzed using a linear mixed model. Fixed effects of the model included 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)

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

PROC MIXED with option DDFM=KR in the MODEL statement. Differences between treatments were considered significant at $P \leq 0.05$ and marginally significant at $0.05 < P \leq 0.10$.

Results and Discussion

An interaction ($P = 0.026$) of diet × sampling day was observed for the antimicrobial cefoxitin (Table 2). It was observed that fecal *E. coli* isolates from sows fed the control diet had lower ($P = 0.035$) MIC values for cefoxitin 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 3).

Fecal *E. coli* isolates from feces of sows fed either dietary treatment were resistant to tetracycline antibiotic. The *E. coli* isolates were considered intermediate to tetracycline when fecal samples were collected at entry into the farrowing house; however, MIC values increased ($P < 0.001$) by the end of weaning with isolates being classified as resistant. Interestingly, this effect carried over into the nursery.[7] All nursery pig 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 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 sampling days.

E. coli isolated from sow feces had increased ($P < 0.02$) MIC values for amoxicillin/clavulanic acid 2:1 ratio, 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 2:1 ratio, ampicillin, cefoxitin, ceftiofur, ceftriaxone, and streptomycin upon entry into the farrowing house but showed inclination toward resistance over time at weaning. In contrast, fecal *E. coli* isolates were susceptible at both time points for azithromycin, ciprofloxacin, nalidixic acid, streptomycin, and trimethoprim/sulfamethoxazole. Chloramphenicol, gentamicin, and sulfisoxazole were the only antimicrobials that statistically ($P > 0.10$) maintained *E. coli*'s MIC values as susceptible at both time points.

In conclusion, feeding live yeast and yeast extracts from d 110 of gestation through weaning lactation 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 diet, 11 of the 14 antimicrobials had increased AMR at weaning compared to entry into the farrowing house,

[7] Chance, J. A., J. T. Gebhardt, J. M. DeRouchey, R. G. Amachawadi, V. Ishengoma, T. G. Nagaraja, M. D. Tokach, J. C. Woodworth, R. D. Goodband, Qing Kang, and J. A. Loughmiller. 2021. The Effect of Live Yeast and Yeast Extracts on Growth Performance and Antimicrobial Susceptibilities of Fecal *Escherichia coli* of Nursery Pigs Weaned from Sows Fed Diets with or without Yeast Additives. *Kansas Agricultural Experiment Station Research Reports:* Vol. 7, Issue 11.

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

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

[1] Breakpoints established by Clinical and Laboratory Standards Institute (Clinical and Laboratory Standards Institute (CLSI). 2018. Performance standards for antimicrobial disk and dilution susceptibility tests for bacteria isolated from animals. Approved standard, 5th ed. CLSI supplement VET08. CLSI, Wayne, PA.) which are categorized as susceptible (treatable), intermediate (possibly treatable with higher doses), and resistant (not treatable). The 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 2. 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			

continued

Table 2. 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**
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. 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 were reported as geometric mean of minimum inhibitory concentration (MIC) ± standard error of the mean.

[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 treatment differences ($P = 0.237$) observed at the entry into the farrowing house.

Table 3. Main effects of including live yeast and a yeast extract in lactation diets 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. 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 were reported as geometric mean of minimum inhibitory concentration (MIC) ± standard error of the mean.

[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.