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Effect of Enterotoxigenic Escherichia coli F18 Inoculum Concentration on Clinical Response Following Orogastric Challenge in Nursery Pigs

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Summary

A total of 45 weanling pigs (241 × 600 DNA; initially 15.4 ± 0.47 lb) were used in a 14-d study to evaluate the effect of Enterotoxigenic Escherichia coli (ETEC) F18 inoculum concentration on growth performance and fecal characteristics of nursery pigs. Pigs were group-housed for a 7-d acclimation period before being individually housed for inoculation (d 0 of the study). From d −7 to 14, pigs were fed a common nursery diet without pharmacological levels of Zn or Cu. Pigs were randomly allotted to one of three inoculum concentrations (109, 108, or 107 cfu/mL) in a completely randomized design with 15 replications per treatment. Each pig received 6 mL of the respective inoculum concentration followed by 6 mL of sterile phosphate-buffered saline via orogastric gavage using a 10 Fr feeding tube. The isolate used was an F18 fimbrial type with genes for heat-labile enterotoxin (LT), heat-stable toxin A (STa), heat-stable toxin B (STb), and Shiga toxin type 2e (Stx2e) as determined via PCR and was derived from a field clinical case submission. Pig weights were measured on d 0, 7, and 14 to determine ADG, ADFI, and feed efficiency, and fecal samples were collected on d 0, 3, 7, 10, and 14 to measure fecal dry matter (DM) by drying at 131°F for 48 h. All animals were euthanized on d 14 post-challenge at the conclusion of the experiment. Bodyweight at inoculation was used as a covariate for all statistical analyses. No differences were observed for growth performance during period 1 (d 0 to 7), period 2 (d 7 to 14), or overall (d 0 to 14). Growth rates were very low for d 0 to 7 for all treatment groups and improved from d 7 to 14 for the pigs remaining. A total of five pigs died or were euthanized for welfare reasons during the experiment (P = 0.888), with 2, 0, and 3 pigs resulting in mortality from treatments 109, 108, and 107 cfu/mL, respectively. For fecal DM, there was no evidence of a treatment × day interaction (P = 0.387) or treatment effect (P = 0.146), although a significant time effect (P < 0.0001) was observed. Mean fecal DM was 15.7, 11.4, 12.5, 19.3, and 15.8%, for d 0, 3, 7, 10, and 14, respectively. Averaged across all sample collection days, fecal DM was 15.8, 15.8, and 13.3% for

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treatments 109, 108, and 107 cfu/mL, respectively, although no significant means separation was observed. In conclusion, the ETEC F18 inoculum concentrations used in this study resulted in similar growth performance and clinical outcome.

Introduction

Enterotoxigenic Escherichia coli (ETEC) is a primary cause of post-weaning diarrhea (PWD) in nursery pigs, leading to reduced growth performance and increased morbidity and mortality. In the swine industry, E. coli infections not only impact animal health and welfare but also pose potential food safety concerns for pork consumers. Traditionally, antibiotics and pharmacological levels of Zn have been used to mitigate the negative effects of ETEC; however, investigation of alternative strategies to control PWD is ongoing. To effectively evaluate such interventions, it is necessary to use a reliable challenge model that replicates ETEC infection while minimizing excessive mortality to maintain adequate statistical power. Dahmer et al.[3] summarized methodologies used in ETEC challenge studies with weanling pigs and reported that the average inoculum dosage of F18 ETEC across experiments was approximately 3 × 1010 cfu/mL. Previous studies conducted at the Kansas State University Enteric Center have used an inoculum concentration of 109 cfu/mL, which resulted in an average mortality rate of 23.9% before the conclusion of the trial. Losses due to mortality or euthanasia reduce the number of observations available to detect treatment differences, thereby limiting the ability to interpret results. Both the dose and timing of inoculation are crucial parameters that influence the severity and consistency of the infection model. It was hypothesized that using a lower inoculum concentration may reduce the severity of infection and associated mortality while still eliciting a consistent clinical response. Therefore, the objective of this experiment was to evaluate the effects of varying ETEC F18 inoculum concentrations on growth performance, mortality, and fecal characteristics of nursery pigs.

Procedures

The protocol used in this experiment was approved by the Kansas State University Institutional Animal Care and Use Committee and Institutional Biosafety Committee. This study was conducted at the K-State Swine Enteric Health Research Center facility in Manhattan, KS. Each pen had metal tri-bar flooring, a stainless-steel self-feeder, and nipple waterer to provide ad libitum access to feed and water. Pigs were placed in solid-sided pens to prevent cross-contamination between adjacent pens.

Animal Selection Criteria

Selected piglets came from sows not previously vaccinated for E. coli F18. Piglets were confirmed for genetic susceptibility to the F18 strain of ETEC by sequencing the (1,2) fucosyltransferase-1 (FUT1) gene via PCR at the Iowa State University Veterinary Diagnostic Laboratory. To identify susceptible piglets, an initial group of 96 piglets with similar body weights and an equal distribution of litters were selected. A tail sample was collected at litter processing from the selected piglets. Samples were frozen and shipped on dry ice to the ISU VDL for analysis. After identifying susceptible piglets

[3] Dahmer, P. L., J. M. DeRouchey, J. T. Gebhardt, C. B. Paulk, and C. K. Jones. 2023. Summary of methodology used in enterotoxigenic Escherichia coli (ETEC) challenge experiments in weanling pigs and quantitative assessment of observed variability. Transl. Anim. Sci. 7(1):txad083. doi:10.1093/tas/txad083.

from the initial group, 45 pigs with similar body weights, evenly distributed across litters, were randomly chosen for use in this experiment.

Animals and treatments

A total of 45 weanling pigs (241 × 600 DNA; initially 15.4 ± 0.47 lb) were used in a 14-d study to evaluate the effects of varying ETEC F18 inoculum concentrations on growth performance and fecal characteristics of nursery pigs. Pigs were group-housed for a 7-d adaptation period with five pigs per pen. From d −7 to 14, pigs were fed a common nursery diet without pharmacological levels of Zn or Cu. On the day of inoculation (d 0), pigs were moved to individual pens and randomly allotted to one of three inoculum concentrations (109, 108, or 107 cfu/mL) in a completely randomized design with 15 replications per treatment. Pig weights and feed disappearance were measured on d 0, 7, and 14 to determine ADG, ADFI, and feed efficiency.

Bacterial Strains and Culture Preparations

The ETEC F18 strain was obtained and cultured at the Kansas State University College of Veterinary Medicine. The presence and expression of virulence genes associated with heat-labile enterotoxin (LT), heat-stable toxin A (STa), heat-stable toxin B (STb), and Shiga toxin type 2e (Stx2e) were verified in the ETEC F18 via PCR at the Kansas State University Veterinary Diagnostic Laboratory. The final concentrations of ETEC F18 inoculum were targeted to administer a total of 6 × 109, 108, and 107 CFUs to the challenged pigs.

Inoculation Methods

On d 0, all pigs were inoculated with 6 mL of solution containing approximately either 109, 108, or 107 CFU/mL of ETEC F18. The inoculum was administered via orogastric gavage with a rubber 10 Fr catheter. Briefly, the pig was manually restrained in an upright position with an open mouth and the head elevated compared with the abdomen. The catheter was gently maneuvered into the caudal aspect of the oropharynx. A swallow reflex was stimulated and when visually observed, the tube was slowly introduced into the esophagus. Once the inoculum was dispensed, the catheter was kinked during the removal process to ensure no residual material was deposited in the oropharynx during the removal process.

Fecal Dry Matter and Scoring

On d 0, 3, 7, 10, and 14, fecal samples were collected from each pig to determine fecal dry matter (DM) and fecal score. Using a cotton-tipped applicator to stimulate defecation, fecal samples were collected into plastic bags and frozen at −4°F until fecal DM analysis. Fecal scoring of the bags was conducted by two independent scorers blinded to treatments on d 0, 3, 7, 10, and 14 using a numerical scale where 0 = hard, pellet-like feces; 1 = a firm, formed stool; 2 = a soft, moist stool that retains shape; 3 = a soft, unformed stool; and 4 = a watery, liquid stool. Fecal scores ≥ 3 were considered diarrhea. Fecal samples were dried at 131°F in a forced-air oven for 48 h, and the ratio of dried-to-wet fecal weight determined the percentage fecal DM.

Statistical analysis

Experimental data were analyzed using the GLIMMIX procedure of SAS OnDemand for Academics (SAS Institute Inc., Cary, NC). Individual pig served as the experimental unit, with inoculum as a fixed effect and initial BW as a covariate. Fecal scores were

summarized using the FREQ procedure and reported as the percentage of observations within each score category by treatment. When treatment was a significant source of variation, differences were determined by pairwise comparison using the Tukey-Kramer multiplicity adjustment to control for Type I Error. Results were considered significant at P 0.05 and were considered marginally significant at P 0.10.

Results and Discussion

Growth Performance

No differences were observed for growth performance during period 1 (d 0 to 7), period 2 (d 7 to 14), or overall (d 0 to 14). Growth rates were low for d 0 to 7 for all treatment groups and improved from d 7 to 14.

Fecal Dry Matter

For fecal DM, there was no evidence of a treatment × day interaction or treatment effect, although a significant time effect ($P < 0.0001$) was observed. Mean fecal DM was 15.7, 11.4, 12.5, 19.3, and 15.8% for d 0, 3, 7, 10, and 14, respectively. Averaged across all sample collection days, fecal DM was 15.8, 15.8, and 13.3% for treatments 109, 108, and 107 cfu/mL, respectively, although no significant means separation was observed. On d 7, pigs inoculated with 108 cfu/mL tended ($P = 0.077$) to have the highest fecal DM, whereas those inoculated with 107 cfu/mL had the lowest, with those inoculated with 109 cfu/mL intermediate.

Fecal Scoring

Fecal scores were summarized by treatment as the percentage of observations within each score category and reported using descriptive statistics, where a score of 0 indicated hard feces and 4 indicated diarrhea. No day × treatment interaction was observed. However, a day effect ($P < 0.0001$) was observed, with the greatest frequency of diarrhea occurring on d 3 across all treatments and decreasing thereafter. A treatment effect was also observed ($P = 0.031$) where pigs inoculated with 107 cfu/mL exhibited a greater frequency of diarrhea on d 7, 10, and 14 compared with those receiving higher inoculum concentrations. On d 10, pigs inoculated with 108 cfu/mL had the lowest frequency of diarrhea among all treatments.

In conclusion, the ETEC F18 inoculum concentrations used in this study resulted in similar growth performance and clinical outcome.

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

Ingredient, %	
Corn	53.02
Soybean meal, 47% CP	18.32
Whey powder	16.50
Specialty soy protein concentrate[2]	7.50
Soybean oil	1.00
Calcium carbonate	0.50
Monocalcium P, 21% P	1.13
Salt	0.65
L-Lys-HCl	0.38
DL-Met	0.20
L-Thr	0.17
L-Trp	0.04
L-Val	0.09
Trace mineral premix	0.15
Vitamin premix	0.25
Choline chloride	0.05
Phytase[3]	0.06
Total	100
Calculated analysis	
SID AA, %	
Lys, %	1.35
Ile:Lys	59
Leu:Lys	118
Met:Lys	36
Met and Cys:Lys	58
Thr:Lys	64
Trp:Lys	20.1
Val:Lys	70
His:Lys	35
NE, kcal/lb	1,145
SID Lys:NE, g/Mcal	5.35
CP, %	20.8
Ca, %	0.69
STTD P, %	0.46
Ca:P	1.00

[1] Common diet was fed from d −7 to 14.

[2] PurePro Soy; Bunge; Chesterfield, MO.

[3] HiPhorius 2400 (DSM-Firmenich; Parsippany, NJ) provided at an estimated release of 0.12% STTD P with 681 FTU/lb.

Table 2. Effects of Enterotoxigenic *Escherichia coli* F18 inoculum dose on nursery pigs[1]

	Concentration, cfu/mL				
	10^9	10^8	10^7	SEM	*P* =
BW, lb					
d 0	15.4	15.4	15.4	0.47	0.999
d 7	17.8	17.1	16.7	0.67	0.523
d 14	25.4	25.0	25.5	0.89	0.893
Period 1 (d 0 to 7)					
ADG, lb	0.16	0.24	0.05	0.123	0.546
ADFI, lb	0.47	0.48	0.46	0.046	0.939
G:F	0.25	0.12	-0.08	0.308	0.740
Period 2 (d 7 to 14)					
ADG, lb	1.09	1.13	1.23	0.077	0.423
ADFI, lb	1.21	1.18	1.14	0.100	0.901
G:F	0.94	1.00	1.01	0.068	0.740
Overall (d 0 to 14)					
ADG, lb	0.72	0.69	0.64	0.076	0.800
ADFI, lb	0.85	0.83	0.81	0.069	0.903
G:F	0.83	0.81	0.85	0.037	0.749
Fecal DM, %					
d 0	15.7	16.2	15.3	1.80	0.947
d 3	14.3	10.8	9.3	2.01	0.173
d 7	12.9	15.4	9.3	2.01	0.077
d 10	19.4	21.8	16.6	1.93	0.147
d 14	16.7	14.9	15.8	2.01	0.797

[1] A total of 45 pigs (initially 15.4 ± 0.47 lb) were used in a 14-d growth study with 1 pig per pen and 15 replicates per treatment. Each pig was inoculated with 6 mL of inoculum at the respective concentration, followed by 6 mL of saline.

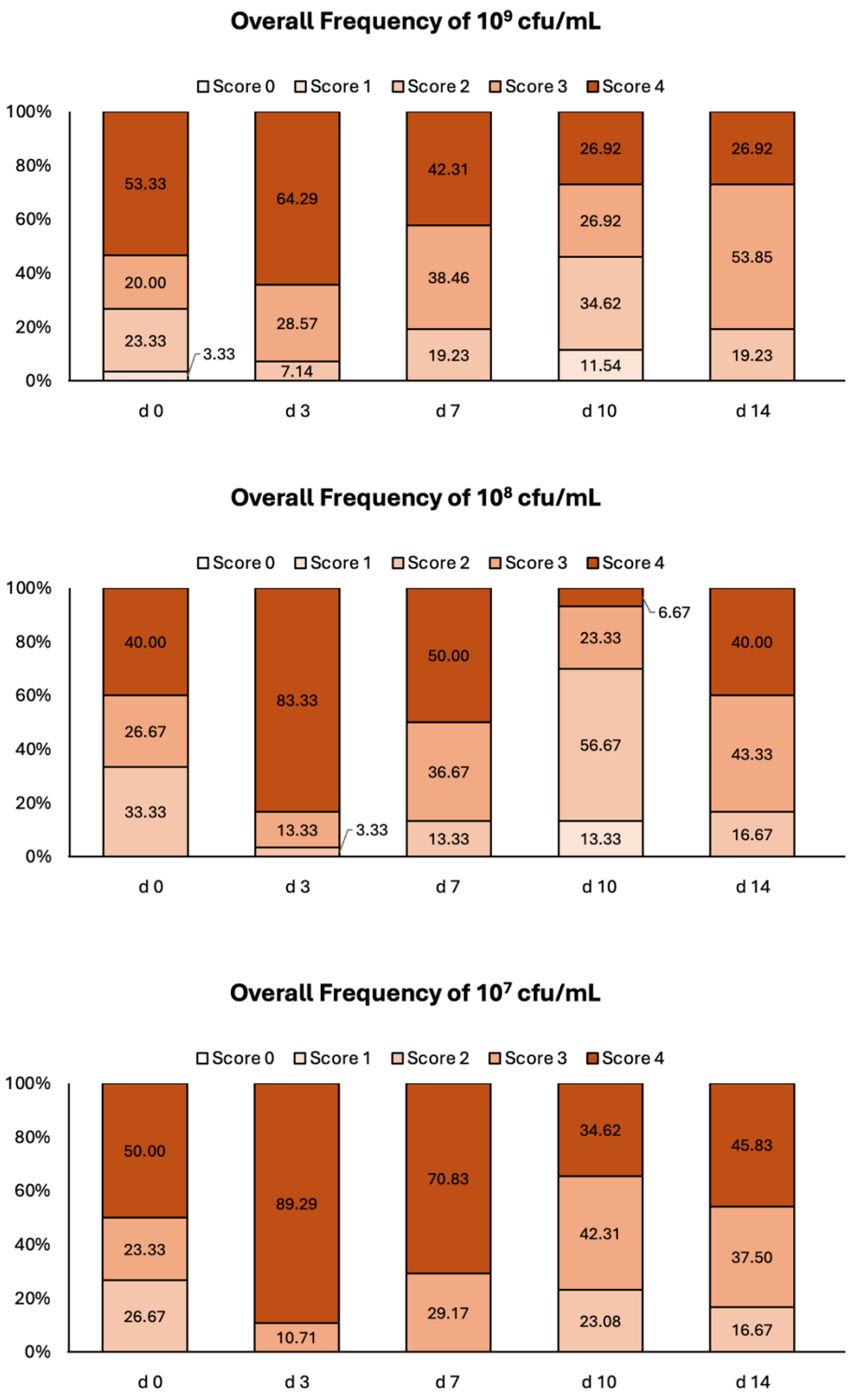


Figure 1. Fecal scores are presented on a 4-point scale: 0 = hard, pellet-like lumps; 1 = firm, formed feces; 2 = normal feces; 3 = mild looseness; and 4 = diarrhea scored by two observers. Each pig was inoculated with 6 mL of inoculum at the respective concentration, followed by 6 mL of saline. Day × treatment ($P = 0.382$). Day ($P < 0.0001$). Treatment ($P = 0.031$).