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Effects of Organic Acids and Monoglycerides Supplementation on Growth Performance, Fecal Dry Matter, and Intestinal Morphology of Weanling Pigs Experimentally Infected with Enterotoxigenic *Escherichia coli*

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Summary

A total of 90 weanling pigs (241 × 600 DNA; initially 13.9 ± 0.46 lb) were used in a 10-d study using two groups of pigs (45 pigs/group) to evaluate the effects of organic acids and monoglycerides on growth performance and fecal dry matter (DM) in enterotoxigenic *Escherichia coli* F18 (ETEC)-challenged nursery pigs. Within group, pigs were randomly allotted to one of five dietary treatments in a completely randomized design. There were 18 pigs per dietary treatment, and diets were fed in two phases with phase 1 fed during a 7-d adaptation period before inoculation and phase 2 fed during the 10-d post-inoculation period. Pigs were group-housed for the 7-d acclimation period, then moved to individual pens and inoculated (d 0). Diets were corn-soybean meal-based without pharmacological levels of Zn or Cu and arranged in a one-way treatment structure. Treatment diets were formulated as a basal phase 1 and phase 2 feed and then mixed in smaller batches to include 0.5% of one of the following additives: ground corn (control), ForBen1 (formic/benzoic blend), Acitra G20C, Monoglyceride Mix A (mix of mono-, tri-butyrin and C8C10 monoglycerides), and Monoglyceride Mix B (mix of mono-, tri-butyrin and C8-C12 monoglycerides). There were no effects of dietary treatment on growth performance throughout any period of the study. However, pigs fed Acitra G20C had a numerically increased ADG and ADFI from d 5 to 10 of the experiment and the overall period. Pigs fed Monoglyceride Mix A had the numerically poorest performance across all periods. A main effect of timepoint on fecal DM

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was observed where DM was improved ($P < 0.05$) on d 0 and 10 compared with d 3, 5, and 7. No interactive effects of dietary treatment and timepoint were observed for fecal DM. No significant treatment effects were observed for villus height, crypt depth, or villus height:crypt depth ratio in the duodenum, jejunum, or ileum. However, a tendency was observed for ileal crypt depth ($P = 0.068$) where pigs fed Monoglyceride Mix A had numerically the smallest crypt depth. Additionally, a tendency for duodenal villus height:crypt depth ratio was observed ($P = 0.072$) where pigs fed Monoglyceride Mix B had numerically the greatest ratio. In conclusion, dietary treatment did not have a significant effect on growth performance, fecal DM, or intestinal morphology of ETEC F18-challenged nursery pigs.

Introduction

In the swine industry, Escherichia coli poses many threats to both animal health and food safety for pork consumers. As it relates to the weanling pig, enterotoxigenic E. coli (ETEC) is a primary cause of post-weaning diarrhea (PWD), which can result in reduced growth performance and increased morbidity and mortality. Historically, ETEC infections have been treated with the use of antibiotics or heavy metals such as zinc oxide. However, due to consumer and regulatory pressures to practice more prudent antibiotic stewardship and environmental concerns, the swine industry needs alternative interventions. Many management and nutritional strategies have been investigated to combat PWD caused by ETEC, including delayed weaning, decreased dietary crude protein, acidifiers, added fiber fractions, and more. However, the efficacy of these practices is quite variable. Dietary acidifiers and monoglycerides are known for their antimicrobial properties, which can lower intestinal pH to inhibit bacterial colonization and proliferation as well as disrupt cell membranes causing lysis. Several studies have shown variable results of acidifiers and/or monoglycerides on growth performance in health-challenged vs. non-health-challenged pigs. Whitney et al.[5] observed overall improved average daily gain in nursery pigs when supplementing diets with the combination of an acidifier and monoglyceride. However, Kim et al.[6] observed no effect of acidifiers and/or monoglyceride supplementation on growth performance throughout the overall nursery period. Park et al.[7] evaluated the effects of an organic acid blend, monoglyceride blend, and a combination blend in nursery pigs experimentally infected with ETEC F18 and observed no changes in growth performance. However, a reduction in overall diarrhea frequency was observed for all dietary treatments compared with pigs fed a control diet. The effects of these supplements remain uncertain in health-challenged conditions. Therefore, the objective of this experiment was to evaluate the effects of added organic acid and monoglycerides on growth performance and diarrhea of nursery pigs experimentally challenged with enterotoxigenic Escherichia coli F18.

[5] Whitney, M. H., and R. Wolfenden. 2025. 105 Effect of supplementing organic acid and/or monoglyceride blends on growth performance and economics in nursery pigs compared to benzoic acid. *J. Anim. Sci.* 103(Suppl. 1):125–126. (Abstr.) doi:10.1093/jas/skaf102.134

[6] Kim, T. H., J. C. Woodworth, M. D. Tokach, J. M. DeRouchey, R. D. Goodband, K. N. Gaffield, J. T. Gebhardt, Y. Chen, and M. Whitney. 2025. Effects of diet and water monoglyceride and acid supplementation on nursery pig performance. *Kansas Agricultural Experiment Station Research Reports* 11(7). doi:10.4148/2378-5977.8761.

[7] Park, S., S. Sun, S. Wongchanla, et al. 2025. Dietary supplementation of a blend of organic acids and monoglycerides alleviated diarrhea and systemic inflammation of weaned pigs experimentally infected with enterotoxigenic *Escherichia coli* F18. *J. Anim. Sci. Biotechnol.* 16:11. doi:10.1186/s40104-024-01148-8.

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 ETEC 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 (VDL). To identify susceptible piglets, an initial group of 104 piglets with similar body weights, age, and an equal distribution of litters were selected within each group of pigs used within the experiment. 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 fully susceptible piglets, 45 pigs with similar body weights, evenly distributed across litters, were randomly chosen to be used within each group of the experiment (90 pigs total).

Animals and diets

A total of 90 weanling pigs (241 × 600 DNA; initially 13.9 ± 0.46 lb) were used in a 10-d study using two groups of pigs (45 pigs/group). Within group, pigs were randomly allotted to one of five dietary treatments in a completely randomized design and grouped with four or five pigs per pen for a 7-d adaptation period. There were 18 pigs per dietary treatment, and diets were fed in two phases with phase 1 fed during the 7-d adaptation period before inoculation and phase 2 fed during the 10-d experiment after inoculation (Table 1). Pigs were group-housed for the 7-d acclimation period, then moved to individual pens and inoculated on d 0. Diets were corn-soybean meal-based without pharmacological levels of Zn or Cu and arranged in a one-way treatment structure. Treatment diets were formulated as a basal phase 1 and phase 2 feed and then mixed in smaller batches to include 0.5% of the following: ground corn (control), ForBen1 (formic/benzoic blend), Acitra G20C, Monoglyceride Mix A (mix of mono-, tri-butyrin and C8C10 monoglycerides), and Monoglyceride Mix B (mix of mono-, tri-butyrin and C8-C12 monoglycerides). Treatment diets were manufactured at the Kansas State University O.H. Kruse Feed Technology Innovation Center in Manhattan, KS, and fed in meal form for phase 1 and phase 2. Pig weights and feed disappearance were measured on d 0, 5, and 10 to determine ADG, ADFI, and feed efficiency (G:F).

Bacterial strains, culture preparations, and inoculation methods

The ETEC F18 strain was obtained and cultured at the Kansas State University College of Veterinary Medicine. 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. On d 0, all pigs were inoculated with 6 mL of solution containing approximately 107 CFU/mL of ETEC F18. The inoculum was administered via orogastric gavage with a rubber 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

On d 0, 3, 5, 7, and 10, fecal samples were collected from each pig to determine fecal dry matter. Using a cotton-tipped applicator to stimulate defecation, fecal samples were collected into plastic bags and frozen at -4°F until fecal dry matter analysis. For dry matter analysis, samples were placed in a drying oven set at 131°F for 48 h.

Animal harvesting and tissue collection

On d 10 of the experiment, all remaining pigs were euthanized via captive bolt and opened viscerally for necropsy. For necropsy, the duodenum, jejunum, and ileum were identified, removed from the mesentery, and three 2-cm-long segments from each section, plus an additional 4-cm-long segment from the jejunum were cut and fixed in 10% formalin for histological preparation at the Kansas State Veterinary Diagnostic Laboratory. Briefly, tissue samples were sectioned at 4 µm and stained with hematoxylin and eosin (H&E) using the Leica ST5010 Autostainer XL (Leica Biosystems, Deer Park, IL). Slides were digitized by optical scanning using a brightfield Leica Aperio AT2 scanner at 400× magnification (Leica Biosystems) and resulting scanned slides were used for evaluation. To assess villus height, crypt depth, and the villus height to crypt depth ratio, representative villi and their respective crypts (n = 10) were measured and recorded, and the average of these measurements was used for statistical analysis.

Statistical analysis

Experimental data for growth performance and intestinal morphology were analyzed using the GLIMMIX procedure of SAS OnDemand for Academics (SAS Institute Inc., Cary, NC). Individual pig served as the experimental unit, with dietary treatment as a fixed effect and group included as a random intercept. Data points with studentized residuals greater than 4 were evaluated for data entry errors, and if no error was identified, these data points were removed from data analysis. 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. Fecal DM data were analyzed using repeated measures with treatment, timepoint, and the interaction of treatment and timepoint evaluated with group included as a random effect. Results were considered significant at P 0.05 and were considered marginally significant at P 0.10.

Results and Discussion

Growth performance

At weaning, pigs were allotted to dietary treatment for a 7-d adaptation period with an average weight of 13.0 lb. On inoculation day (d 0), BW across treatments did not differ (13.9 lb).

From d 0 to 5, all treatments lost weight and had a negative ADG and G:F, while ADFI remained positive but was low. No treatment differences were observed for d 0 to 5. From d 5 to 10, ADG, ADFI, and G:F were positive except for the G:F response of

Monoglyceride Mix B, which remained negative. Pigs fed Acitra G20C had a numerically increased ADG, ADFI, and G:F compared with other treatments. No treatment differences were observed for d 5 to 10. In the overall period (d 0 to 10), Monoglyceride Mix A had the numerically lowest ADG, ADFI, and G:F compared with all other treatments. Pigs fed Acitra G20C continued to have a numerically increased ADG and ADFI for d 0 to 10. Overall, no dietary treatment differences were observed during the experiment.

Fecal dry matter

No interactive effect of treatment and timepoint was observed for fecal DM during the experiment. Pigs fed Monoglyceride Mix A had the numerically lowest fecal DM on all timepoints compared with other treatments. A main effect of timepoint on fecal DM was observed ($P < 0.001$) throughout the experiment where fecal DM on d 3, 5, and 7 was reduced compared with the day of inoculation (d 0), and fecal DM increased on d 10; however, it was still lower ($P < 0.05$) compared with d 0.

Intestinal morphology

No significant treatment effects were observed for villus height, crypt depth, or villus height:crypt depth ratio in the duodenum, jejunum, or ileum. A tendency for a treatment effect ($P = 0.068$) was observed for ileal crypt depth where pigs fed Monoglyceride Mix A had numerically the smallest crypt depth, with ForBen1 having numerically the greatest crypt depth. Additionally, a tendency for duodenal villus height:crypt depth ratio was observed ($P = 0.072$) where pigs fed Monoglyceride Mix B had numerically the greatest ratio.

In conclusion, the data generated in this study did not demonstrate benefits of supplementing organic acids or monoglycerides in the diet for growth performance, fecal dry matter, or intestinal morphology of ETEC-challenged pigs.

Acknowledgments

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

Item	Phase 1	Phase 2
Ingredient, %		
Corn	42.85	54.99
Soybean meal, 46.5% CP	20.90	23.57
Milk, whey powder	15.00	12.50
Milk, whey permeate	9.00	---
Access starter protein[2]	6.25	3.75
Soybean oil	2.00	1.00
Calcium carbonate	0.40	0.58
Monocalcium P, 21.5% P	1.10	0.95
Salt	0.47	0.56
L-Lys-HCl	0.45	0.49
DL-Met	0.28	0.26
L-Thr	0.20	0.22
L-Trp	0.04	0.06
L-Val	0.11	0.13
Trace mineral premix	0.15	0.15
Vitamin premix	0.25	0.25
Phytase[3]	0.06	0.06
Test ingredient[4]	0.50	0.50
Total	100	100
Calculated analysis		
SID AA, %		
Lys, %	1.35	1.35
Ile:Lys	59	57
Leu:Lys	113	114
Met:Lys	41	39
Met and Cys:Lys	58	58
Thr:Lys	65	65
Trp:Lys	20.9	21.2
Val:Lys	70	70
His:Lys	34	34
Net energy, kcal/lb	1,175	1,140
SID Lys:net energy, g/Mcal	5.21	5.37
CP, %	20.8	20.8
Ca, %	0.67	0.68
STTD P, %	0.46	0.40
Ca:P	1.02	1.10

[1] Phase 1 diets were fed from d -7 to 0. Phase 2 diets were fed from d 0 to 10.

[2] Prairie Aquatech, Brookings, SD.

[3] HiPhorius 2,400 (DSM-Firmenich, Parsippany, NJ) provided at 1,500 FYT/kg and provided an estimated release of 0.12% STTD P.

[4] Test ingredient was added at 0.5% and included either ForBen1, Acitra G20C, Monoglyceride Mix A (mix of mono-, tri-butyrin and C8C10 monoglycerides) and Monoglyceride Mix B (mix of mono-, tri-butyrin and C8-C12 monoglycerides) or ground corn for the control treatment.

Table 2. Effect of organic acid and monoglyceride supplementation on growth performance and fecal dry matter of ETEC F18-challenged nursery pigs1

Items	Control	For-Ben1	Acitra G20C	Mono-glycer-ide mix A	Mono-glycer-ide mix B	SEM	*P* =
BW, lb							
d -7	13.03	13.03	13.03	13.01	13.00	---	---
d 0	13.73	14.07	13.97	13.92	13.94	0.460	0.986
d 5	13.16	14.07	13.90	13.22	13.55	0.540	0.656
d 10	16.74	17.30	17.84	15.77	16.55	1.096	0.642
d 0 to 5							
ADG, lb	-0.13	-0.12	-0.08	-0.22	-0.07	0.071	0.600
ADFI, lb	0.34	0.36	0.32	0.30	0.28	0.117	0.874
G:F	-0.80	-0.73	-0.55	-1.27	-0.29	0.386	0.213
d 5 to 10							
ADG, lb	0.60	0.44	0.68	0.07	0.41	0.175	0.126
ADFI, lb	0.47	0.44	0.55	0.33	0.53	0.082	0.261
G:F	0.73	0.05	0.88	0.88	-0.0032	0.838	0.857
Overall (d 0 to 10)							
ADG, lb	0.24	0.19	0.33	0.002	0.19	0.099	0.237
ADFI, lb	0.40	0.40	0.44	0.33	0.39	0.056	0.537
G:F	0.65	0.04	0.58	-0.31	0.26	0.348	0.125
Fecal DM[2], %							
d 0	23.45	20.29	23.12	16.73	20.39	2.280	0.179
d 3	13.97	12.71	11.36	9.07	11.64	2.596	0.689
d 5	10.28	8.70	13.29	8.38	10.48	2.309	0.447
d 7	11.64	11.65	12.94	10.75	12.14	2.523	0.976
d 10	16.74	18.07	15.81	14.71	19.95	2.324	0.365

[1] A total of 90 pigs were used in two 10-d growth studies with 1 pig per pen and 18 replicates per treatment. Pigs were weaned at approximately 19 d of age and assigned to treatment diets for 7 d following weaning. On d 0 (7 d post-weaning), pigs were placed in individual pens and inoculated with 6 mL of solution containing approximately 107 CFU/mL of ETEC F18 via orogastric gavage.

[2] Treatment × time interaction, P = 0.883. Treatment, P = 0.238. Time, P < 0.001.

Table 3. Effect of organic acid and monoglyceride supplementation on intestinal morphology of ETEC F18-challenged nursery pigs[1,2]

Items	Control	ForBen1	Acitra G20C	Monoglyceride mix A	Monoglyceride mix B	SEM	*P* =
Villi height, µm							
Duodenum	278.0	303.4	283.0	259.5	333.3	29.02	0.217
Jejunum	362.7	303.6	381.4	312.7	374.7	33.32	0.236
Ileum	348.1	314.2	336.1	313.0	331.3	37.85	0.851
Crypt depth, µm							
Duodenum	480.5	467.9	452.7	407.3	427.3	31.88	0.390
Jejunum	316.8	324.4	326.0	316.4	338.3	16.53	0.874
Ileum	229.4	261.2	259.4	208.7	247.7	15.23	0.068
Villi height:crypt depth ratio							
Duodenum	0.60	0.68	0.65	0.63	0.83	0.090	0.072
Jejunum	1.17	0.90	1.20	1.07	1.13	0.112	0.190
Ileum	1.54	1.33	1.42	1.55	1.44	0.214	0.866

[1] A total of 90 pigs were used in two 10-d growth studies with 1 pig per pen and 18 replicates per treatment. Pigs were weaned at approximately 19 d of age and assigned to treatment diets for 7 d following weaning. On d 0 (7 d post-weaning), pigs were placed in individual pens and inoculated with 6 mL of solution containing approximately 107 CFU/mL of ETEC F18 via orogastric gavage.

[2] Pigs were euthanized and necropsied on d 10 of the experiment to determine villi height, crypt depth, and villi height:crypt depth ratio.

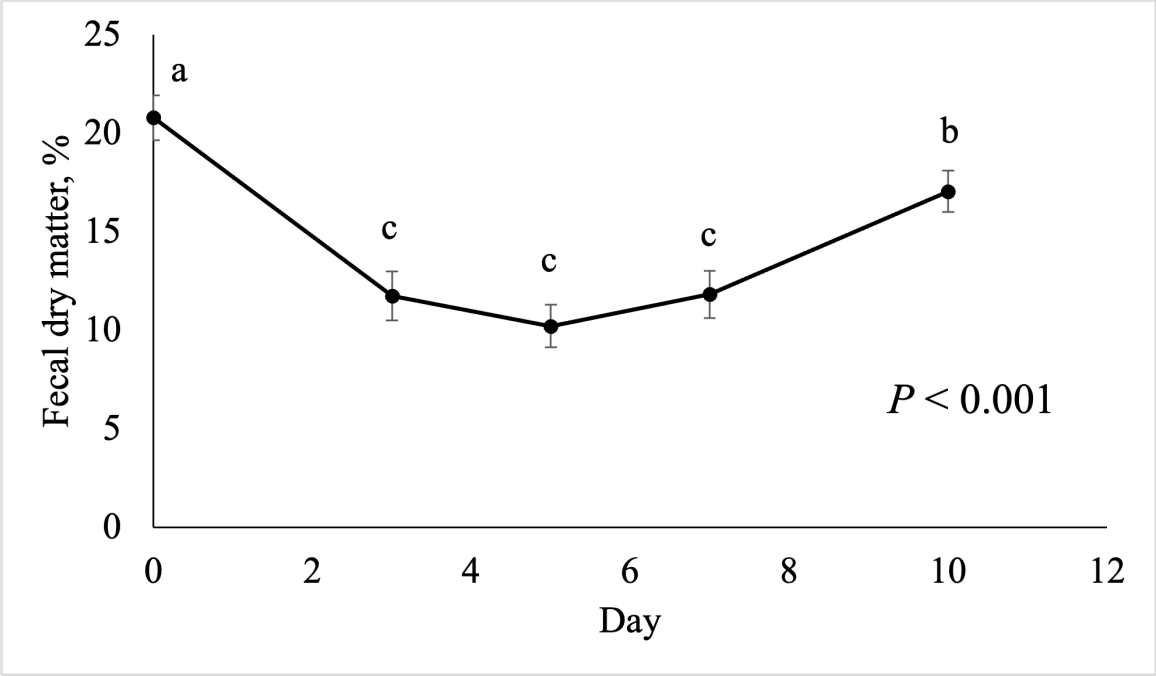


Figure 1. Fecal dry matter over time. Error bars represent ± 1 SEM. Means lacking common superscript differ, $P < 0$.