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KANSAS STATE UNIVERSITY SWINE INDUSTRY DAY 2021

Evaluation of a Dried Fermentation Product Administered Through Drinking Water on Nursery Pig Growth Performance, Fecal Consistency, and Antibiotic Injections

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Summary

A total of 350 barrows (DNA Line 200 × 400; initially 13.5 ± 0.02 lb) were used in a 42-d study to evaluate the effects of a dried fermentation product administered through drinking water on nursery pig growth performance, antibiotic injection frequency, fecal consistency, and fecal *E. coli* presence. Upon arrival to the nursery research facility, pigs were randomly assigned to pens (5 pigs per pen) and pens were allotted to 1 of 2 water treatments with 35 pens per treatment. Water treatments were provided with or without a fermentation product administered through the water lines at a 1:128 dilution rate from d 0 to 14 after weaning. From d 0 to 14, 14 to 42, and for the overall experiment, there was no evidence ($P > 0.10$) for differences observed for any growth performance criteria. There was evidence ($P < 0.05$) for day effect on diarrhea presence. Diarrhea presence increased on d 4 and 6, then decreased to low levels. There was no evidence for the fermentation product to influence diarrhea incidence. For antibiotic injections, there was no evidence ($P > 0.10$) for differences observed between treatments. Mortalities were low, with no evidence ($P > 0.10$) for differences observed between treatments for removals or mortalities. For fecal dry matter on d 7 and 14, there was no evidence ($P > 0.10$) for differences observed between treatments. In summary, under these experimental conditions, administering a dried fermentation product for the first 14 d in the nursery through the drinking water did not improve growth performance, fecal dry matter, diarrhea presence, antibiotic injections, or removals and mortalities in nursery pigs. Further evaluation of the dried fermentation product in commercial facilities with greater diarrhea and mortality is needed.

Introduction

Post-weaning diarrhea (PWD) associated with diet change, stress, and environmental bacteria continues to be an issue for the swine industry. Stressors associated with weaning pigs amplify the impact of PWD and increase the prevalence of toxigenic

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bacteria to impact the host. Enteric infections caused by *Escherichia coli,* including subclinical infections, particularly in nursery pigs, are of significant economic importance to the swine industry.[3] The economic impact is due to decreased weight gain and feed efficiency, costs associated with treatment and prevention, and mortality.[4] Enteric colibacillosis in nursery pigs includes three diseases caused by different pathotypes of *E. coli*, neonatal enteritis, post-weaning diarrhea, and edema diseases. The pathotypes involved in enteric colibacillosis in animals and humans are enterotoxigenic (ETEC), enteropathogenic (EPEC), enteroaggregative (EAEC), and Shiga toxigenic (STEC) and hybrid pathotypes (STEC/ETEC and EAEC/STEC).

The major virulence factors carried by the pathotypes responsible for the enteric infections include heat labile (ELT) and heat stable enterotoxins (EST), enteroaggregative heat stable toxin (EAST), fimbriae (F4, F5, F6, F18, and F41) (mainly by ETEC and EPEC), hemolysin (mainly by EPEC), Shiga toxins 1 and 2 and intimin (a protein that mediates adhesion to enterocytes), and enterohemolysin (mainly by STEC).

There have been various studies about feeding probiotics to balance the gut microflora and alleviate the stressors associated with PWD and ETEC. In a more recent technology, probiotic bacteria produce bioactive molecules through fermentation, which when delivered, act through inhibition of quorum sensing (QS) signals. Inhibition of these signals reduces the induction of virulence genes and reduces the ability of the pathogens to cause infection. Importantly, these signals do not affect the bacterial populations through growth inhibition or bactericidal activity. Rather, the fermentation products affect pathogenicity by changing the gene expression profile, reducing genes associated with QS signals, toxins, and adhesion.[5] A recent publication has looked at the *in vivo* effects of these bioactive molecules produced by *Lactobacillus acidophilus.*[6] Researchers observed that after an *E. coli* ETEC challenge in swine, there were improvements in fecal scores when the bioactive compounds were administrated, in comparison to a control. Therefore, the objective of this study was to investigate the effects of a dried fermentation product produced by lactic acid bacteria fermentation and provided through the drinking water to affect growth performance, antibiotic injection frequency, and fecal consistency. We also tested for fecal presence of genes that encode for major virulence factors associated with enteric colibacillosis, and identified pathotypes of *E. coli* in nursery pigs.

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[3] Moxley, R. A., G. E. Duhamel. 1999. Comparative pathology of bacterial enteric diseases of swine. Adv. Exp. Med. Bio. 473:83-101. doi:10.1007/978-1-4615-4143-1_7.

[4] García-Meniño, I., V. García, A. Mora, D. Díaz-Jiménez, S. C. Flament-Simon, M. P. Alonso, J. E. Blanco., M. Blanco, and J. Blanco. 2018. Swine enteric colibacillosis in Spain: pathogenic potential of mcr-1ST10 and ST131 *E. coli* isolates. Front. Microbiol. 9:2659. doi:10.3389%2Ffmicb.2018.02659.

[5] Zeinhom, M., A. M. Tellez. V. Delcenserie, A. M. El-Kholy, S. H. El-Shinawy, and M. W. Griffiths. 2012. Yogurt containing bioactive molecules produced by *Lactobacillus acidophilus* La-5 exerts a protective effect against enterohemorrhagic *Escherichia coli* in mice. J. Food. Prot. 10:1796-1805. doi:10.4315/0362-028X.JFP-11-508.

[6] Nordeste, R., A. Tessema, S. Sharma, Z. Kovac, C. Wang, R. Morales, and M. W. Griffiths. 2017. Molecules produced by probiotics prevent enteric colibacillosis in pigs. BMC Vet. Res. 13:335. doi:10.118/s12917-017-1246-6.

Materials and Methods

The Kansas State University Institutional Animal Care and Use Committee approved the protocol used in this experiment. The study was conducted at the Kansas State University Segregated Early Weaning Facility in Manhattan, KS. The facility has two identical barns that are completely enclosed, environmentally controlled, and mechanically ventilated. Each pen contains a 4-hole, dry self-feeder and a cup waterer to provide *ad libitum* access to feed and water. Barns were designed so that either water treatment could be applied to each pen. Pens (4 × 4 ft) had metal tri-bar floors and allowed approximately 2.7 ft^2/pig.

A total of 350 barrows (DNA Line 200 × 400; initially 13.5 ± 0.02 lb) were used in a 42-d study with 5 pigs per pen and 35 pens per treatment. Pigs were randomly assigned to pens and then pens were allotted to 1 of 2 water treatments. Water treatments were provided by a dilution rating of 1:128 of the test product from weaning until d 14. The test product was formulated to be provided at 24 mg/kg BW. Water usage was measured with a water meter for pigs on each treatment within each barn. Product usage was measured by weighing stock solution on a daily basis to determine disappearance. The same common diets were fed to both treatments. Common diets were fed in 3 phases, with 4 lb of phase 1 and 15 lb of phase 2 diet provided per pig, and with phase 3 diet fed until d 42. Diets were manufactured and delivered by Hubbard Feeds in Beloit, KS. Pig weights and feed disappearances were measured on d 0, 7, 14, 21, 28, and 42 of the experiment to determine ADG, ADFI, and F/G.

Fecal scores

From d 0 to 14 of the experiment, fecal scores were assigned to each pen every other day by the same two observers. Fecal scores were assigned based on a 1 to 5 numerical scale, with 1 = fully formed feces; 2 = moist, firm feces; 3 = mild diarrhea; 4 = severe diarrhea; and 5 = watery diarrhea. When the fecal scores between the two observers was not identical, the average of the two was used for analysis. For data analysis, fecal scores were further assigned as either diarrhea present within the pen or not. If the fecal score was a 1, 2, or no diarrhea was observed, the pen was defined as not having diarrhea. If the fecal score was a 3, 4, or 5 the pen was categorized as having diarrhea.

Fecal collection

On d 7 and 14 of the experiment, feces were collected from 3 piglets per pen. Fecal samples were sub-divided with some of the feces used for *E. coli* gene typing at the Kansas State University Preharvest Food Safety Laboratory. The remaining fecal sample was dried at 130°F for 48 h to determine fecal dry matter (DM).

Detection of major virulence genes of E. coli pathotypes

Approximately 1 g of feces was suspended in *E. coli* broth (Difco, BD, Waltham, MA; Paddock et al., 2012), vortexed for 1 min, and incubated at 104°F (40°C) for 6 h. After incubation, 1 mL was pipetted into a 2-mL centrifuge tube, boiled for 10 min, centrifuged at 9,400 × g for 5 min, and DNA in the supernatant was purified using a GeneClean Turbo Kit (MP Biomedicals, Solon, OH). The purified DNA was subjected to a multiplex PCR assay to detect genes that encode for 11 major virulence factors associated with intestinal *E. coli* pathotypes in swine: *estA*, *estB*, *elt*, *hlyA*, *bfpA*, *aggA*, *astA*, *stx1*, *stx2*, *eae*, and *ehxA*.

Isolation and identification of E. coli pathotypes by culture method

Enriched fecal samples were spot inoculated with a sterile cotton swab onto MacConkey agar (MAC; Remel, Lenexa, KS) and then sterile loops were used to streak from the swabbed area for isolation of *E. coli*. Also, samples were diluted (1 in 100 dilution) in EC broth, and 25 µl of the diluted inoculum were spread-plated onto MAC plates. Inoculated plates were incubated at 37°C for 18-24 h. A total of 10 putative colonies presumptive for *E. coli* (pink, round, smooth colonies) for each sample were streaked onto BAP plates and incubated at 37°C for 18-24 h. The colonies were tested for spot indole production and confirmed as *E. coli* by PCR assay for *clpB/uidA/ybbW* genes, which were encoded for beta-glucuronidase, caseinolytic peptidase B, and putative allantoin receptor, respectively.[7] The 10 colonies obtained for each sample were pooled in 50 µl of distilled water, boiled for 10 min, and centrifuged at 2,200 × g for 2 min. The boiled lysate was subjected to the 11-plex PCR assay to detect virulence genes associated with swine enteric colibacillosis. If pooled lysates were positive for any of the 11 virulence genes, then each of the 10 colonies was tested individually by the 11-plex PCR assay to identify virulence gene-positive *E. coli*. All virulence gene-positive isolates were stored at -80°C in cryogenic beads.

Pigs were removed for welfare concerns when they were observed to continually lose weight or were unthrifty. Pigs that required antibiotic, received penicillin G or enrofloxacin (Baytril 100; Bayer HealthCare LLC, Shawnee Mission, KS) for lack of thriftiness (gaunt), *S. suis*, lameness, or joint infections.

Statistical analysis

Growth performance, antibiotic injection frequency, and fecal dry matter data were analyzed as a completely randomized design with pen serving as the experimental unit. Treatment was included in the statistical model as a fixed effect and barn was incorporated in the model as a random effect. Fecal dry matter data were analyzed using a repeated measures analysis. Data were analyzed using R Studio (Version 3.5.2, R Core Team, Vienna, Austria). Results were considered significant at $P \leq 0.05$ and marginally significant at $0.05 < P \leq 0.10$.

Fecal score data were analyzed using a logistic regression model fit with the GLIMMIX procedure of SAS (v. 9.4, SAS Inst. Inc., Cary, NC) using a logit link function. Treatment, day of evaluation, and the associated interaction were considered fixed effects, and pen nested within treatment and the cross product of pen, treatment, and day were considered random effects. Data were analyzed as repeated measures over time and reported as percentage of pens having diarrhea.

Results and Discussion

The actual concentration of test product delivered was greater than the targeted level of 24 mg/kg BW due to the water medicator dosing at a higher rate than expected, likely because of low water intake immediately after weaning (Table 1). Water leakage during one day in one barn resulted in very high water and product usage. Thus, a portion of

[7] Walker, D. I., J. McQuillan, M. Taiwo, R. Parks, C. A. Stenton, H. Morgan, M. C. Mowlem, D. N. Lees. 2017. A highly specific Escherichia coli qPCR and its comparison with existing methods for environmental waters. Water Res. 126:101-110. doi:10.1016/j.watres.2017.08.032.

this day was removed from the analysis. The calculated product disappearance, which includes water that may be wasted by the animals, averaged 35.7 mg/kg.

During the experimental period, there was no evidence ($P > 0.10$) for differences in growth performance (Table 2). There was no evidence for differences in the percentage of pigs in a pen injected with antibiotic between the control group and those receiving the fermentation product (Table 3). During the common period, there was no evidence ($P > 0.10$) for differences in growth performance; however, there was a tendency ($P < 0.10$) for pigs that were previously provided the fermentation product through the water to have more injections per pen and greater injection percentage compared to the control treatment. For the overall experiment, there was no evidence ($P > 0.10$) for differences in growth performance or injection criteria. There was no evidence for differences in removals, mortalities, or total removals between treatments due to the size of the study. Further research with greater pig numbers is warranted. For fecal dry matter, there was no evidence for differences between treatments.

Average fecal scores by treatment for each day are presented in Table 4. For diarrhea presence, there was no evidence ($P > 0.10$) for a treatment × day interaction or a treatment effect (Table 4). There was evidence ($P < 0.05$) for a day difference in both the control and the fermentation product treatments. From d 0 to 6, diarrhea presence increased, then decreased from d 6 to 12 and increased again to d 14. The increased diarrhea presence from d 0 to 6 is likely associated with the pigs adapting from a milk to a solid diet after weaning. The reason for the increase in diarrhea presence from d 12 to 14 is unknown.

For detection of virulence genes of *E. coli* pathotypes, it was clear that *E. coli* was present within the population of both the control and the dried fermentation product, and that this prevalence was higher on d 14. For detection of virulence genes of *E. coli* pathotypes on d 7, the majority of the samples submitted were positive for *hlyA* gene, 11 and 9 for fecal bacteria samples from pigs provided with the control and the dried fermentation product, respectively. Other genes present on d 7 in both treatment groups were *exhA*, *eae* (3 samples each, respectively), and 1 sample positive for *astA* gene in the control group. For the ETEC and EPEC genes on d 7, only 1 sample was positive for ETEC in the control. For the fimbriae genes, 1 sample tested positive for detection of F18 gene. The gene detection was increased on d 14. For the enteropathogenic strains *elt*, *estA*, *estB,* and *astA*, the number of positive samples increased but not greater than 50%. Positive samples were analyzed for the control and the dried fermentation product on d 14; *elt* – 5 and 7, *estA* – 6 and 7, *estB* – 6 and 9, and *astA* – 5 and 7, respectively. The detection of *hlyA* gene on d 14 was similar to that on d 7 with 10 and 11 positive samples for the control and the dried fermentation product, respectively. The positive samples of *ehxA* and *eae* increased numerically for both treatment groups (6 samples each, respectively). The number of tested positive samples for fimbriae genes F4 and F18 as well as ETEC and EPEC increased on d 14. The number of positive F4 fimbriae genes were 5 and 7 for control and treatment, respectively, and F18 had 1 positive sample from each treatment. Positive ETEC gene samples were 3 for the control and 7 for treatment, with 3 and 2 EPEC positive samples for the control and the treatment group respectively.

The detection of 1 of the 5 fimbriae genes with the ETEC and EPEC genes was tested. There were no positive samples on d 7 for ETEC + fimbriae genes or EPEC + fimbriae genes. On d 14, however, the number of tested positive samples for ETEC + fimbriae were 3 and 5 for the control and the treatment group respectively, and 2 positive samples were detected for EPEC + fimbriae in both treatments.

All the tested fecal samples were negative for the presence of *stx*1 and *stx*2, which encode for Shiga toxins 1 and 2, respectively. Shiga toxin 2 is the major virulence factor involved in the edema disease. The absence indicates none of the piglets were shedding STEC in the feces. The *aggA* gene, which encodes for a protein responsible for aggregation of *E. coli* cells, was absent in all the samples indicating that piglets were not shedding EAEC pathotype. However, the *astA* gene that encodes for enteroaggregative heat stable enterotoxin was prevalent in the feces. The absence of *bfpA*, which encodes for a protein, in all samples indicates that none of the piglets carried typical EPEC pathotype. Strains of EPEC produce a characteristic adherence, called local adherence, in which bacterial cells form microcolonies or clusters. This type of adherence is associated with the presence of a plasmid, called EAF (EPEC adherence factor) plasmid, which also has a cluster of genes that encode bundle-forming pili (BFP).[8] Strains of EPEC carrying *bfpA* gene are called typical EPEC. In contrast to typical EPEC, certain strains that carry *eae* gene and one or more of the four enterotoxin genes, but do not have the EAF plasmid encoding *bfpA,* are called atypical EPEC (aEPEC).[9] Of the four genes that encode for enterotoxins, *astA* was the most dominant and the prevalence increased with age. Among the three genes, *elt, estA,* and *estB,* which encode for heat labile, heat stable A, and heat stable B, respectively, and are characteristic of ETEC pathotype and involved in neonatal enteritis and post-weaning diarrhea, heat stable B was the most dominant and the prevalence increased with age. The *eae* gene, which encodes for intimin, a protein that mediates attachment of *E. coli* to enterocytes and is characteristic of both STEC and EPEC, was prevalent in the feces of both groups. The genes *hlyA* and *ehxA,* which encode for two different hemolysins, were prevalent in almost all samples. Hemolysins are produced by a number of *E. coli* pathotypes, including non-pathogenic strains. As expected, the prevalence of any of the 11 virulence genes did not appear to be affected by inclusion of the dried fermentation product in the diet of piglets.

All the strains carried one of the five fimbrial genes tested (F4, F5, F6, F18, and F41). The fimbriae mediate the attachment of *E. coli* to enterocytes before enterotoxins are secreted that induce secretory diarrhea. Again, as would be expected, the dried fermentation product did not appear to have any effect on the fecal prevalence of the ETEC or aEPEC pathotypes; however, the analysis does demonstrate the presence of *E. coli*.

Overall, under the conditions used in this experiment, there was no effect of the fermentation product to improve growth performance, antibiotic injection criteria through d 42, or fecal scores through d 14 of the nursery period. All four genes that code for enterotoxins were found in the feces of nursery pigs. The dominant enterotoxin gene was *astA*, which encodes for enteroaggregative heat stable enterotoxin. The

[8] Nataro, J. P. and J. B. Kaper. 1998. Diarrheagenic *Escherichia coli*. Clin. Microbiol. Rev. 11:142-201. doi:10.1128/cmr.11.1.142.

[9] Chen, H. D. and G. Frankel. 2005. Enteropathogenic *Escherichia coli*: unraveling pathogenesis. FEMS. Microbiol. Rev. 29:83-98. doi:10.1016/j.femsre.2004.07.002.

two pathotypes of *E. coli* detected were ETEC and aEPEC. None of nursery pigs used in the study shedded STEC, EAEC, or any of the hybrid pathotypes in the feces. These results indicate that although virulence genes or pathotypes associated with enteric colibacillosis were present, the dried fermentation product did not influence diarrhea presence or performance. Due to the number of animals per treatment and low mortality percentage in this study, a statistical difference would not be expected. Further research is needed to determine if a dried fermentation product is able to reduce mortality or morbidity in nursery pigs.

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Table 1. Dosage of a dried fermentation product administered through the water to post-weaning piglets

Day	Product usage, mg/kg BW[1]	Percentage of targeted usage[2]
1	43.3	181
2	39.8	166
3	40.5	169
4	37.4	156
5	35.6	148
6	50.3	209
7	45.6	190
8	42.3	176
9	34.6	144
10	32.7	136
11	29.4	122
12	26.1	109
13	21.3	89
14	21.3	89

[1] Dried fermentation product (provided by MicroSintesis, Victoria, Canada) was administered through water lines at a dilution rate of 1:28 and a targeted inclusion rate of 24 mg/kg BW from d 0 to 14 after weaning.
[2] Percentage of targeted dose was calculated based on stock solution water disappearance and water intake for each day.

Table 2. Effect of a dried fermentation product provided through the drinking water on nursery pig growth performance and fecal consistency[1]

Item	Control	DFP[2]	SEM	*P* =
Initial BW, lb	13.5	13.5	0.02	0.377
d 7	14.4	14.5	0.09	0.606
d 14	17.8	17.7	0.14	0.651
d 21	22.9	23.1	0.18	0.506
d 28	30.7	30.7	0.21	0.837
d 42	47.8	48.4	0.81	0.410
d 0 to 7				
ADG, lb	0.13	0.14	0.012	0.447
ADFI, lb	0.20	0.21	0.008	0.291
F/G	1.83	2.54	0.575	0.388
d 7 to 14				
ADG, lb	0.47	0.45	0.012	0.325
ADFI, lb	0.60	0.61	0.025	0.661
F/G	1.28	1.34	0.050	0.065
Experimental period (d 0 to 14)				
ADG, lb	0.30	0.30	0.009	0.925
ADFI, lb	0.39	0.41	0.012	0.337
F/G	1.35	1.38	0.023	0.305
d 14 to 21				
ADG, lb	0.73	0.76	0.015	0.169
ADFI, lb	1.09	1.10	0.037	0.737
F/G	1.52	1.48	0.067	0.295
d 21 to 28				
ADG, lb	1.11	1.08	0.019	0.237
ADFI, lb	1.58	1.60	0.016	0.340
F/G	1.43	1.50	0.019	0.014
d 28 to 42				
ADG, lb	1.22	1.26	0.049	0.253
ADFI, lb	1.87	1.91	0.064	0.435
F/G	1.54	1.52	0.015	0.347
Common period (d 14 to 42)				
ADG, lb	1.07	1.09	0.022	0.320
ADFI, lb	1.60	1.63	0.043	0.367
F/G	1.50	1.50	0.011	0.847

continued

Table 2. Effect of a dried fermentation product provided through the drinking water on nursery pig growth performance and fecal consistency[1]

Item	Control	DFP[2]	SEM	*P* =
Overall (d 0 to 42)				
ADG, lb	0.80	0.82	0.013	0.300
ADFI, lb	1.19	1.21	0.027	0.220
F/G	1.48	1.48	0.011	0.751
Close-out data[3]				
Gain, lb/pig placed	31.74	33.41	0.751	0.120
Intake, lb/pig placed	48.04	50.15	0.808	0.070
F/G	1.53	1.51	0.029	0.353
Removals and mortalities, %				
Mortality	1.71	0.57	0.981	0.338
Removal	3.43	2.29	1.375	0.524
Total removal	5.14	2.86	1.670	0.282
Fecal DM, %[4]				
d 7	19.75	20.08	1.004	0.770
d 14	20.59	20.47	1.004	0.912

[1] A total of 350 weaned pigs were used in a 42-day study with 5 pigs per pen and 35 pens per treatment.
[2] Dried fermentation product (provided by MicroSintesis, Victoria, Canada) was administered through water lines in a dilution rate of 1:128 from d 0 to 14 after weaning.
[3] Close out data are calculated on a pig placed basis: total gain and intake per pen divided by the number of pigs at the start of the trial.
[4] Fecal samples from the same 3 piglets were pooled and dried for 48 h in 130°F. Treatment × day, $P = 0.775$; treatment, $P = 0.898$; day, $P = 0.432$.

Table 3. Effect of a dried fermentation product provided through the drinking water on individual postweaning antibiotic injection regimens[1]

Item	Control	DFP[2]	SEM	*P* =
Experimental period (d 0 to 14)				
Injections/pen[3]				
Enrofloxacin[4]	0.77	0.57	0.464	0.726
Penicillin[5]	0.37	0.31	0.160	0.788
Total [6]	1.14	0.89	0.697	0.769
Injections, %[7]				
Enrofloxacin	15.43	11.43	0.027	0.274
Penicillin	7.43	6.29	0.020	0.673
Total	20.57	14.86	0.031	0.163
Total injections[8]				
Enrofloxacin	27	19	---	---
Penicillin	13	11	---	---
Total	40	30	---	---
Common period (d 14 to 42)				
Injections/pen				
Enrofloxacin	0.34	0.66	0.138	0.069
Penicillin	0.34	0.66	0.138	0.069
Total	0.60	1.03	0.172	0.051
Injections, %				
Enrofloxacin	7.02	13.14	0.026	0.063
Penicillin	7.02	13.14	0.026	0.063
Total	12.28	19.43	0.030	0.071
Total injections				
Enrofloxacin	12	23	---	---
Penicillin	9	13	---	---
Total	21	36	---	---

continued

Table 3. Effect of a dried fermentation product provided through the drinking water on individual postweaning antibiotic injection regimens[1]

Item	Control	DFP[2]	SEM	*P* =
Overall (d 0 to 42)				
Injections/pen				
Enrofloxacin	1.11	1.23	0.223	0.709
Penicillin	0.63	0.69	0.141	0.769
Total	1.74	1.91	0.700	0.856
Injections, %				
Enrofloxacin	22.29	24.57	0.033	0.614
Penicillin	12.57	13.71	0.026	0.752
Total	26.29	29.71	0.035	0.475
Total injections				
Enrofloxacin	39	42	---	---
Penicillin	22	24	---	---
Total	61	66	---	---

[1] A total of 350 weaned pigs were used in a 42-d study with 5 pigs per pen and 35 pens per treatment.
[2] Dried fermentation product (provided by MicroSintesis, Victoria, Canada) was administered through water lines in a dilution rate of 1:128 from d 0 to 14 after weaning.
[3] Count of pigs per pen that had a treatment regimen started during the period.
[4] Pigs were treated with enrofloxacin (Baytril 100; Bayer HealthCare LLC, Shawnee Mission, KS) for signs of diarrhea, being gaunt, or lack of thriftiness.
[5] Pigs were treated with penicillin G for strep, lame, or joint infections. Pigs previously treated with enrofloxacin were subsequently treated with penicillin if they remained gaunt.
[6] Sum of enrofloxacin and penicillin injections.
[7] Percentage of pigs that had a treatment regimen started.
[8] Total number of pigs that had a treatment regimen started during the period.

Table 4. Effect of a dried fermentation product provided through the drinking water on fecal score and presence of postweaning diarrhea in the pen, %[1]

	Day								
Item	**0**	**2**	**4**	**6**	**8**	**10**	**12**	**14**	***P* =**
Fecal score									
Control	1.71	1.61	2.51	2.70	2.24	2.03	2.23	3.26	
DFP[2]	1.48	1.71	2.74	2.44	2.33	2.01	2.03	2.69	
Diarrhea presence									
Control	2.3 ± 1.48	0.9 ± 0.78	36.6 ± 12.27	52.7 ± 13.1	7.0 ± 3.86	1.0 ± 0.75	0.6 ± 0.54	10.2 ± 5.12	0.343
DFP[2]	0.6 ± 0.54	0.8 ± 0.66	70.8 ± 11.04	39.1 ± 12.65	17.1 ± 7.71	1.0 ± 0.76	2.3 ± 1.49	10.8 ± 5.28	
Day	1.2 ± 0.65^{c}	0.9 ± 0.51^{c}	54.2 ± 9.33^{a}	45.8 ± 9.26^{a}	11.1 ± 3.96^{b}	1.0 ± 0.53^{c}	1.2 ± 0.66^{c}	10.5 ± 3.68^{b}	< 0.001

[1] A total of 350 weaned pigs were used in a 42-day study with 5 pigs per pen and 35 pens per treatment. Two observers individually classified pens of pigs as either having diarrhea present (score > 2) within a pen or no diarrhea (score < 3) or feces observed. There was no main effect of treatment ($P = 0.540$).

[2] Dried fermentation product provided by MicroSintesis (Victoria, Canada) was administered through water lines in a dilution rate of 1:128 from d 0 to 14 after weaning.

[a,b,c] Means with different superscripts differ, $P < 0.05$.