The Effect of Feeding Fermented Liquid Whey + Dextrose Inoculated with Specific Lactic Acid Bacteria of Pig Origin to Weanling Pigs Challenged with *Escherichia coli* O149:K91:F4*

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**CLINICAL RELEVANCE**

The objective of this study was to determine the efficacy of using fermented liquid whey inoculated with specific lactic acid bacteria of pig origin to reduce the severity and progression of postweaning enterotoxigenic *Escherichia coli* diarrhea in weanling pigs challenged with *E. coli* O149:K91:F4. Based on two trials, it was determined that feeding inoculated fermented whey in a liquid diet did not affect growth performance or the severity or duration of postweaning diarrhea compared with a conventional dry feed containing an antibiotic. Because this study is one of very few examining the use of liquid feed and co-products inoculated with probiotics to control postweaning *E. coli* diarrhea, more studies are needed to confirm these results.

**INTRODUCTION**

Postweaning diarrhea caused by enterotoxigenic *Escherichia coli* (PWECD) that express K88 (F4) fimbriae is a major cause of disease and mortality in newly weaned pigs. For several decades, orally administered antibiotics have been used to prevent or treat diarrhea in postweanling pigs; however, studies have shown that *E. coli* isolates from pigs with PWECD and edema disease are resistant to a wide range of antimicrobial agents.\(^1\)\(^-\)\(^3\) This raises concern about the continued effectiveness of antibiotics in the treatment of this disease. In addition,
The potential removal of antibiotics from farm animal feeds as a result of public concern over antimicrobial resistance has directed research toward finding alternative methods to sustain the growth and health of the animals under commercial production conditions.\textsuperscript{4–7}

The commensal gut microflora contributes to the resistance of pathogens colonizing the gut of a healthy animal, but disruption of the microflora at weaning as a result of dietary changes\textsuperscript{8} and stress originating from competition and aggression from other pigs in the group\textsuperscript{9} contributes to an animal’s susceptibility to intestinal infection at this time.\textsuperscript{8} Because intestinal microflora can be manipulated by supplementing feed with probiotics or prebiotics,\textsuperscript{4,5,10} the use of such nonantimicrobial methods may be beneficial for the prevention or treatment of gastrointestinal (GI) diseases (e.g., \textit{E. coli}, \textit{Salmonella}, \textit{Clostridium}, and \textit{Campylobacter} infections).\textsuperscript{11,12}

In general, the effects on growth performance and pig health associated with adding probiotics to dry diets has been inconsistent.\textsuperscript{13,14} However, an approach that is attracting interest is the use of therapeutic fermented liquid feed produced by using specific strains of lactic acid bacteria (LAB) that have probiotic properties and can tolerate the acidic conditions in the stomach and bile acids in the small intestine.\textsuperscript{15,16} Fermented feed containing high levels of LAB reduces gastric pH, decreases microbial activity in the small intestine, and reduces the number of enterobacteria throughout the GI tract.\textsuperscript{17,18}

It has been hypothesized that using carbohydrate-rich feedstuffs for fermentation is a more favorable approach than using fermented compound diets.\textsuperscript{19} In the case of newly weaned pigs, feeding fermented complete compound diets has a negative effect on the feed:gain (F:G) ratio compared with feeding nonfermented diets; this can be attributed to a decrease of synthetic lysine and the breakdown of protein to amines and ammonia, which negatively influence animal performance.\textsuperscript{20,21} However, there are no reports of studies that have tested the effect of feeding diets in which only the carbohydrate portion of the feed has been fermented to newly weaned pigs with serious health conditions such as PWED.

The objective of this study was to determine the efficacy of feeding a diet containing liquid whey + dextrose inoculated with specific LAB of pig origin to reduce the severity and progression of PWED and the effect on growth performance in \textit{E. coli} O149:K91:F4 challenged pigs.

\section*{MATERIAL AND METHODS}

\section*{Experimental Design}

In two trials, a total of 48 pigs were systematically assigned to three rooms (eight pigs each per trial) based on weight and litter. Each room was then randomly assigned to one of three treatment diets:
• **Group 1**: Nonfermented liquid whey + dextrose without an LAB inoculum + dry feed without an antibiotic (NFLWD [control group])
• **Group 2**: Fermented liquid whey + dextrose with an LAB inoculum + dry feed without an antibiotic (FLWD)
• **Group 3**: Dry feed with an antibiotic (0.1% lincomycin) (DFA)

In each trial, 24 weaned pigs were transported from the Arkell Swine Research Centre (Guelph, Ontario, Canada) to the Ontario Veterinary College isolation unit on the main campus of the University of Guelph. The day of arrival was defined as day 1, and pigs were followed for 12 days. All pigs were challenged by gavage on day 5 with an oral dose (5 ml × 10⁹ CFU/ml) of *E. coli* O149:K91:F4 (JG 280 strain; donated by Dr. Carlton Gyles, Department of Pathobiology, University of Guelph). The study was approved by the University of Guelph Animal Care Committee.

**Animals and Housing**
A total of 48 Yorkshire piglets with an average body weight of 7.5 ± 0.2 kg in the first trial and 7.9 ± 0.2 kg in the second trial were used. Before weaning, piglets had free access to water but did not receive creep feed. Each room had a single pen with heating lamps, a solid cement floor, and feeders divided to create four eating places. The pigs were given ad libitum access to water supplied via a nipple drinker. The rooms were cleaned and disinfected with a quaternary amine-based product (Ascend, Ecoblab, St. Paul, MN) before and after each trial. The interval between trials was 16 days.

**Experimental Diets and Feeding**
From day 1 to day 2, all pigs were fed a dry feed without an antibiotic. On day 3, the pigs were introduced to their treatment diets, which were fed until the end of the trial (day 12). The NFLWD diet (Group 1) was prepared just before feeding the pigs by mixing the whey + dextrose (1:1) with water at a ratio of one part of whey + dextrose:three parts water (1:3) and then adding the dry feed without antibiotic at a ratio of (1:1). The FLWD diet (Group 2) was prepared just before feeding the pigs by first stirring the fermented liquid whey + dextrose for 5 minutes and then adding the dry feed without antibiotic at a ratio of (1:1). Piglets in Groups 1 and 2 received their daily feed in two similar portions at 0900 and 1700 to ensure the feed was as fresh as possible; uneaten feed was collected before the next feeding. The pigs in Group 3 received their daily dry feed allowance once a day at 0900; the feed was remixed by hand at 1700, and uneaten feed was collected before the next feeding. Feed intake was calculated based on an average consumption of 400 g/pig/day for the first week and 500 g/pig/day for the second week. ²²,²³

**Preparation of the Lactic Acid Bacteria Inoculum**
Three isolates of *Lactobacillus plantarum* (23E13, 98L11, and 2P22) were used to inoculate the liquid whey + dextrose. These strains were isolated in our laboratory from either nursing or weanling pigs and proved to be tolerant to pH 4 and 0.3% bile acids and to inhibit different pathogenic strains of *E. coli* in vitro.²⁴

To prepare the inoculum for the liquid whey + dextrose, each *L. plantarum* strain was streaked onto de Man, Rogosa, and Sharpe (MRS) agar plates and incubated anaerobically at 37°C for 48 hours. A McFarland 3 suspension (approximately 9 × 10⁸ CFU/ml) was prepared in sterile phosphate-buffered saline (PBS; pH 7.2) for each of the *Lactobacillus* strains. Three milliliters of each suspension was used to inoculate three tubes containing 47 ml of sterile MRS.
broth. The broths were incubated aerobically for 24 hours at 37°C. All the LAB inocula were used to inoculate 200 ml of sterile MRS broth, which was then incubated for 24 hours at 37°C; the resultant culture was used to inoculate the liquid whey + dextrose.

Preparation of the Fermented Liquid Whey + Dextrose
The fermented liquid whey + dextrose was prepared in 20-L plastic storage jugs; 2.5 kg each of nonhygroscopic whey powder (obtained from Pestell Minerals and Ingredients, New Hamburg, Ontario, Canada) and dextrose (Cerelose, Corn Products International, Westchester, IL) were mixed with 15 L of double distilled water (1:3 ratio of liquid whey + dextrose:water) for 15 minutes. Each jug of liquid whey + dextrose was inoculated with 200 ml of the LAB-inoculated MRS broth. The amount of LAB added to the liquid whey + dextrose was 9.7 × 10^8 CFU/ml in Trial 1 and 8.6 × 10^8 CFU/ml in Trial 2. After the inoculum was added, the jugs were mixed for 10 minutes. The mixture was incubated at 37°C for 4 days for fermentation and then stored in a walk-in refrigeractor at 4°C. During fermentation, 20-ml samples were collected from the jugs daily to determine the pH and LAB counts; immediately before samples were collected, the mixture was stirred for at least 5 minutes. The pH of the mixture was measured daily using a pH meter (Accumet AR15, Fisher Scientific, Pittsburgh, PA). The number of CFU per milliliter of mixture was determined as follows: Serial 10-fold dilutions of the liquid whey + dextrose were performed in PBS (pH 7.4). Aliquots of the serial dilutions, from 10^6 to 10^0, were inoculated onto MRS plates and incubated aerobically at 37°C for 48 hours for LAB counting.

Preparation of the E. coli O149:K91:F4 Challenge Inoculum
Five milliliters of brain–heart infusion broth were inoculated with E. coli O149:K91:F4 (JG280 strain) and incubated for 24 hours at 37°C. The JG280 strain is a hemolytic urease-positive E. coli of serotype O149:H10:F4ac with genes for LT, STa, STb, and EAST-1 enterotoxins. It was isolated from a field case of PWEC D and has been used extensively as an experimental challenge organism to induce diarrhea in weaned pigs.24,25 Fifty microliters of the culture was used to inoculate 100 ml of sterile brain–heart infusion broth. The broth culture was incubated overnight at 37°C on a shaker at 200 rpm. The amount of inoculum given by gavage to each pig was 5 ml × 10^9 CFU/ml. A 2-ml sample from the inoculum was taken for colony counting on blood agar plates. The average dose of the E. coli inoculum was 2.06 × 10^9 CFU for Trial 1 and 3.3 × 10^9 CFU for Trial 2.

Sampling and Measurements
The weight of each pig was determined on days 1, 5 (challenge day), and 12. Average daily gain (ADG) was calculated before and after the challenge with E. coli. Average daily feed intake (ADFI) and the F:G ratio were calculated only after challenge. ADFI/pen was calcu-
lated as feed offered minus feed remaining in the feeder. Feed intake based on dry matter (DM) content for the group fed FLWD (Group 2) was calculated by multiplying the amount of liquid feed offered every day by the average DM of the liquid whey + dextrose mixture on day 5 of fermentation (i.e., 16%). In the NFLWD group (Group 1), feed intake was calculated by multiplying the amount of liquid feed offered every day by the average DM of the liquid whey + dextrose mixture on day 1 (or at the moment of preparation before fermentation [20%]). Since no samples of the leftover feed were taken, the DM of the feed not consumed by the pigs in Groups 1 and 2 was calculated assuming a ratio of 1:1 of liquid:dry feed left in the feeder and multiplying by the same proportions of DM. The amount of feed left in the feeders was minimal. The DM of the dry feed was calculated by multiplying the amount of dry feed offered by a constant of 88%. The F:G ratio was calculated as ADFI on a DM basis divided by the ADG and expressed as kg of feed/kg of weight gain.

Fecal consistency was evaluated for each pig daily from day 0 to day 12 by the same person who administered the diets. The following criteria were used:

- **0** = Firm, dry feces
- **1** = Soft, pasty feces
- **2** = Yellowish fluid feces
- **3** = Clear, water-like feces

Scores of 2 and 3 were considered to represent diarrhea. The observer was not blinded to the treatments but waited for each pig to defecate before determining the diarrhea score for each pig.

Rectal swabs were taken from each pig on days 1, 5 (immediately before challenge), 6, 8, 10, and 12 to check for the presence of *E. coli* O149:K91:F4. The samples were coded only with the pig number and sent to Gallant Custom Laboratories (Cambridge, Ontario, Canada). The slide agglutination test for F4 and O149:K91 antigens was performed for each hemolytic isolate using standard techniques.26

### pH Measurement of Intestinal Contents

All the pigs were euthanized on day 12 with an IV injection of 3 ml (340 mg/ml) of sodium pentobarbital (Euthansol, Schering-Plough Canada, Kirkland, Quebec). For each pig, necropsy was performed immediately after euthanasia and approximately 14 to 16 hours after feeding. The pH of digesta from the stomach, jejunum, and cecum was measured immediately after dissection (Accumet Research AR15, Fisher Scientific).

### Statistical Analysis

The associations between treatment and ADG, ADFI, F:G ratio, and the pH of intestinal contents were analyzed with a mixed model procedure (SAS version 8.0, SAS Institute, Cary, NC). ADG analysis was conducted at the individual level while controlling for time, the fixed effects of treatment (room), the random effect of trial, and the interaction of treatment and trial. ADFI and F:G ratio were calculated from day 5 to day 12, and the room (treatment) was the experimental unit. The models used for the statistical analysis of ADFI and F:G ratio had the random effects of treatment (room), the fixed effect of trial, and the interactions of treatment and trial. Following the detection of a significant interaction effect of trial and treatment, an analysis of variance was performed within trial to determine an effect of treatment on the pig performance parameters. A Bonferroni multiple comparison test was performed post hoc to identify the treatment groups that differed significantly. The Mantel–Haenszel χ² test (controlling for time) was used to analyze the results of the swabs positive to *E. coli* O149:K91:F4 and the number of pigs with diarrhea within trials. The analysis was performed
Correlations between the score of diarrhea and cultural status of the *E. coli* swabs were calculated using Spearman’s correlation test. A simple χ² test to determine the effect of treatment was applied for mortality. The correlation between diarrhea scores and *E. coli*-positive swabs and mortality were analyzed using Stata (Intercooled Stata 8 for XP 2003, Stata Corporation, College Station, TX). A *P* value less than .05 was considered significant; *P* values between .06 and .10 were considered numerically reportable as potential trends.

**RESULTS**

The pH of the liquid whey + dextrose decreased from 5.5 on day 1 to 4.0 by day 4 of fermentation. The log₁₀ LAB count of the liquid mixture verified the growth of probiotic strains, rising from 7.4 on day 1 to 9.0 on day 4 of fermentation. The pH of the feed and the log₁₀ LAB counts were correlated in Trial 1 (*r* = −0.92) and Trial 2 (*r* = −0.79).

The means ± SE for ADG, ADFI, and F:G ratio by treatment and trial are presented in Table 1. No differences in body weight were observed among the three treatment groups at the start of the trials. The mixed model for ADG showed that the interaction of trial, treatment, and time tended to be significant (*P* = .06). Analysis within trial and before and after challenge was performed. Pigs in Group 2 gained more weight in Trial 1 from day 1 to day 5 (challenge) compared with

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**TABLE 1. Average Daily Gain (ADG), Average Daily Feed Intake (ADFI), and Feed:Gain (F:G) Ratio of Pigs Fed a Nonfermented Liquid Whey + Dextrose Diet Not Inoculated with Lactic Acid Bacteria (NFLWD [Control]), a Fermented Liquid Whey + Dextrose Diet Inoculated with Lactic Acid Bacteria (FLWD), and a Conventional Dry Feed Diet with Antibiotic (DFA) in Trials 1 and 2**

<table>
<thead>
<tr>
<th></th>
<th>Group 1 (NFLWD)</th>
<th>Group 2 (FLWD)</th>
<th>Group 3 (DFA)</th>
<th><em>P</em> value</th>
</tr>
</thead>
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<tr>
<td><strong>TRIAL 1</strong></td>
<td></td>
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<tr>
<td><strong>ADG</strong></td>
<td></td>
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<td></td>
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<tr>
<td>Day 0 to day 5 (challenge)</td>
<td>0.112 ± 0.03&lt;sup&gt;a&lt;/sup&gt;,&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.153 ± 0.02&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.042 ± 0.03&lt;sup&gt;b&lt;/sup&gt;</td>
<td>.03</td>
</tr>
<tr>
<td>Day 5 (challenge) to day 12</td>
<td>0.046 ± 0.13</td>
<td>0.111 ± 0.06</td>
<td>0.146 ± 0.07</td>
<td>.74</td>
</tr>
<tr>
<td><strong>ADFI</strong> (dry matter basis) day 5 (challenge) to day 12</td>
<td>0.288 ± 0.03</td>
<td>0.248 ± 0.02</td>
<td>0.255 ± 0.04</td>
<td>.65</td>
</tr>
<tr>
<td><strong>F:G ratio</strong> day 5 (challenge) to day 12</td>
<td>6.9 ± 0.63&lt;sup&gt;d&lt;/sup&gt;</td>
<td>2.42 ± 0.23&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.97 ± 0.33&lt;sup&gt;b&lt;/sup&gt;</td>
<td>&lt;.001</td>
</tr>
<tr>
<td><strong>TRIAL 2</strong></td>
<td></td>
<td></td>
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<tr>
<td><strong>ADG</strong></td>
<td></td>
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<td></td>
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</tr>
<tr>
<td>Day 0 to day 5 (challenge)</td>
<td>0.082 ± 0.03</td>
<td>0.167 ± 0.03</td>
<td>0.146 ± 0.02</td>
<td>.11</td>
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<tr>
<td>Day 5 (challenge) to day 12</td>
<td>0.258 ± 0.02</td>
<td>0.264 ± 0.03</td>
<td>0.248 ± 0.02</td>
<td>.93</td>
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<tr>
<td><strong>ADFI</strong> (dry matter basis) day 5 (challenge) to day 12</td>
<td>0.320 ± 0.03</td>
<td>0.310 ± 0.02</td>
<td>0.371 ± 0.03</td>
<td>.33</td>
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<tr>
<td><strong>F:G ratio</strong> day 5 (challenge) to day 12</td>
<td>1.34 ± 0.15</td>
<td>1.24 ± 0.09</td>
<td>1.65 ± 0.11</td>
<td>.07</td>
</tr>
</tbody>
</table>

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*All values are listed as ±SE.
<sup>a,b</sup>Values with different superscripts differ significantly (*P* < .05).
the pigs in Group 3 \( (P = .03) \). No significant difference was observed in ADG between treatment groups from day 5 to day 12 in Trial 1; after challenge on day 5, however, weight gain decreased in pigs in Groups 1 and 2 by 62.5% and 38%, respectively, while weight gain in the Group 3 pigs increased by 71.2%. Posttreatment ADFI did not differ by treatment, but a difference was observed between trials \( (P = .03) \). The F:G ratio was influenced by treatment \( (P < .004) \), and the interaction of treatment and trial was significant \( (P < .001) \). Analysis within trial was performed, and treatment was significant in Trial 1 \( (P < .001) \), in which the F:G ratio of Group 1 \((6.9 \text{ kg feed/kg weight gained})\) was higher \( (P < .001) \) than in the other two groups. In Trial 2, the F:G ratio of Group 3 tended to be worse (i.e., higher) than that of Group 2 \( (P = .09) \).

The number of swabs positive for \textit{E. coli} O149:K91:F4 between trials and the number of pigs with diarrhea are presented in Table 2. In Trial 1, some swabs were positive for \textit{Proteus} spp, and the overgrowth of these bacteria made it difficult to assess how many pigs were positive for \textit{E. coli}. Two, nine, and one \textit{Proteus}-positive swabs were found in Groups 1, 2, and 3, respectively. For the Mantel–Haenszel \( \chi^2 \) test, these \textit{Proteus}-positive swabs were considered as missing values. In addition, the pigs in Trial 1 were not infected with a field strain of \textit{E. coli} O149:K91:F4 on day 1, but some pigs in all treatment groups were infected on day 5 at the time of the challenge. In Trial 2, pigs were positive for a field strain of \textit{E. coli} O149:K91:F4 on days 1 and 5. Because of the variation between trials, analysis was performed within trial. In Trial 1, 18, 17, and 13 \textit{E. coli}–positive swabs were identified from Groups 1, 2 and 3, respectively. On day 10, Group 1 had six \textit{E. coli}–positive swabs versus zero \textit{E. coli}–positive swabs for Group 3 \( (P < .001) \). Group 2 had a total of four \textit{Proteus}-positive swabs on day 10. In Trial 2, no significant difference in the number of \textit{E. coli}–positive swabs was observed over time; 14, 9, and 13 \textit{E. coli}–positive swabs were isolated from Groups 1, 2, and 3, respectively.

The presence of diarrhea varied over time. Significant differences were observed in Trial 1 on day 7 \( (P = .003) \), day 8 \( (P = .06) \), and day 9 \( (P = .05) \). In general, more occurrences of diarrhea were observed in Group 1 than in Groups 2 and 3. No association between positive \textit{E. coli} swabs and diarrhea scores was found in either trial.

No differences were observed in mortality among the treatment groups. Two Group 1 pigs and one Group 3 pig died, but no mortalities were observed in Group 2. All mortalities occurred in Trial 1 and were associated with diarrhea problems.

The average pH values of the stomach and jejunum contents of the pigs were not significantly different among the treatments or between trials; however, the pH of the jejunum contents was numerically higher in Group 2 than in the other two groups in both trials (Figure 1). The pH value of the cecum contents was significantly different among treatments \( (P = .04) \); pigs in Group 3 had a lower cecum pH value than did pigs in Group 1 or Group 2 \( (P = .03 \text{ and } .02, \text{ respectively}) \).
TABLE 2. Number of Pigs with Diarrhea and Pigs Positive for *E. coli* O149:K91:F4 and *Proteus* spp by Trial and Day Among Pigs Fed a Nonfermented Liquid Whey + Dextrose Diet Not Inoculated with Lactic Acid Bacteria (NFLWD [Control]), a Fermented Liquid Whey + Dextrose Diet Inoculated with Lactic Acid Bacteria (FLWD), and a Conventional Dry Feed Diet with Antibiotic (DFA)

<table>
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<tr>
<th>Day</th>
<th>No. of Pigs</th>
<th>Trial 1</th>
<th></th>
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<tr>
<td></td>
<td></td>
<td>Group 1 (NFLWD)</td>
<td>Group 2 (FLWD)</td>
<td>Group 3 (DFA)</td>
<td>Group 1 (NFLWD)</td>
<td>Group 2 (FLWD)</td>
<td>Group 3 (DFA)</td>
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</tr>
<tr>
<td>1</td>
<td>With diarrhea</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>3</td>
<td>0</td>
<td>4</td>
<td></td>
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<tr>
<td></td>
<td>Positive for <em>E. coli</em></td>
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<td>0</td>
<td>0</td>
<td>3</td>
<td>2</td>
<td>4</td>
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<td></td>
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<tr>
<td>2</td>
<td>With diarrhea</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>3</td>
<td>1</td>
<td>3</td>
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<td>3</td>
<td>With diarrhea</td>
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<td>0</td>
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<tr>
<td>4</td>
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<td>4</td>
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</tr>
<tr>
<td></td>
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<td>6*</td>
<td>3*</td>
<td>0*</td>
<td>1</td>
<td>1</td>
<td>1</td>
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<tr>
<td></td>
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<tr>
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<td>With diarrhea</td>
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<td>2</td>
<td>1</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>12</td>
<td>With diarrhea</td>
<td>1</td>
<td>0</td>
<td>0</td>
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<td>1</td>
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<tr>
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<td>0</td>
<td>1</td>
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<tr>
<td></td>
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*Day of challenge.

*Values with different superscripts differ significantly (*P* < .05).
DISCUSSION

In this study, three LAB strains were used as inoculants to ferment liquid whey + dextrose. The addition of LAB at 1% controlled fermentation of the liquid co-product as expected based on previous studies. LAB counts of 9 log_{10} CFU/g were reached on day 3 of fermentation and were associated with a decrease in pH (to less than 4). These parameters are considered the optimum values for fermentation of liquid feed and have been associated with the production of lactic acid, yeasts, hydrogen peroxide, and bacteriocins. These LAB metabolites have the potential to reduce enterobacteria counts in the feed (to less than 3.2 log_{10} CFU/g) and, when fed to pigs, can reduce the number of coliform bacteria in the GI tract.

To the best of our knowledge, this study is the first to investigate the effect of feeding fermented whey + dextrose on the growth performance of weaned pigs challenged with a specific pathogenic bacteria. We encountered difficulty in summarizing the data between trials because of the variability observed in the parameters recorded in this study. The variability in results may be partially explained by the difference in the initial E. coli O149:K91:F4 status of the pigs in each trial. In the first trial, pigs were not infected until the day of challenge; in the second trial, pigs were infected with a field E. coli O149:K91:F4 strain before the trial started. Pigs were obtained from a farm where PWECD had been previously diagnosed, but the animals were clinically normal at the start of the two trials. PWECD is sporadic by nature; thus, at the time when pigs are selected for a trial, it is difficult to predict whether clinical disease will occur spontaneously. Despite the different E. coli status of the pigs in the two trials, we decided that reporting the data from each trial separately would be informative in terms of the effect of fermented and nonfermented liquid diets in pigs that were either naive or previously sensitized with this pathogen.

In the first trial, clinical signs were more severe and mortalities occurred in Groups 1 and 3; all the Group 2 pigs survived, but the severity of their illness is reflected by their very poor weight gain. Pigs fed FLWD had an F:G ratio similar to that of pigs fed DFA and a better F:G ratio than pigs fed NFLWD.

In the second trial, in which the pigs were previously sensitized to the bacteria, clinical signs were less severe and similar growth performance was observed for pigs fed FLWD and those fed DFA. Based on a limited number of studies, pigs fed fermented co-products have shown favorable ADG and F:G ratios compared with pigs fed nonfermented liquid diets and similar growth performance when compared with pigs fed dry diets with antibiotics.

At first glance, an F:G ratio as high as 6.9 (which was noted in Trial 1 in pigs fed NFLWD) would appear to be a calculation error or the result of a great deal of feed wastage. Because the experimental pens had cement floors, spilled feed would have been noticeable, and thus we are confident that this unusually high F:G ratio is not a result of feed wastage.

This group of pigs suffered severe diarrhea and gained almost no weight over the week of monitoring, although they did continue to

No association between positive E. coli swabs and diarrhea scores was found in either trial.
consume some feed. Dehydration undoubtedly accounted for some of this very low weight gain and contributed to this very poor F:G ratio. The pigs in Group 2 were affected to a lesser extent, but their high F:G ratio is also likely distorted by dehydration. Overall, the severity of clinical disease and the short monitoring period make the interpretation of this parameter difficult.

Clinical disease was less marked in the second trial. Because these pigs were positive for a field E. coli O149:K91:F4 on day 1, we speculate that they may have developed some immunity against the E. coli used in the experimental challenge. It is possible to effectively prime the intestinal immune system by feeding low doses of enterotoxigenic E. coli (ETEC),34 and there is complete cross-protection between E. coli strains with similar fimbrial variants.35 Experiments show that pigs colonized by an F18 ETEC were protected against colonization by a heterologous ETEC sharing the same fimbriae.3 Moreover, the humoral secretory immune system develops early in life, and a protective immune response (mainly of the IgM and IgA class) can be stimulated in young pigs by oral immunization with live E. coli.36,37

In general, the growth performance of pigs fed FLWD and DFA did not differ. The addition of organic acids to pig diets has a positive influence on the appar-

Figure 1. Least squares means (±SE) of pH of the stomach, jejunum, and cecum contents of pigs (n = 8 pigs/treatment/trial) fed a nonfermented liquid whey + dextrose diet not inoculated with lactic acid bacteria (NFLWD; Group 1 [control]), a fermented liquid whey + dextrose diet inoculated with lactic acid bacteria (FLWD; Group 2), and a conventional dry feed diet with antibiotic (DFA; Group 3).
ent total tract digestibility of crude protein and energy.\textsuperscript{38} This might be true for fermented liquid co-product diets as well.\textsuperscript{19,39} Short-chain fatty acids can contribute up to 28% of the total maintenance energy requirements; improve the apparent ileal digestibility of protein and amino acids; provide energy to colonocytes; and stimulate sodium, calcium, and water absorption from the large intestine in vivo, even under diarrheic conditions.\textsuperscript{19,38,40} It has been suggested that the lactic acid produced as a metabolite during fermentation of a lactic acid-producing bacterial culture is the main cause of improvement in performance.\textsuperscript{41}

None of the treatments prevented the shedding of ETEC O149:K91:F4, and variable results were observed between trials as a result of the timing of \textit{E. coli} infection. Because the pigs in Trial 1 were naive to ETEC, it is possible that they became severely affected and shed \textit{E. coli} for at least 5 days after challenge in all treatments; however, pigs receiving DFA appeared to eliminate the bacteria faster than the pigs in the other treatment groups. The reason for this is uncertain because lincomycin, the antibiotic used in this study, has poor efficacy against gram-negative organisms.\textsuperscript{42,43} Fermentation of a complete liquid feed has been shown to decrease enterobacteria counts (CFU/g) in the GI tract of pigs compared with dry feed and/or nonfermented liquid feed\textsuperscript{18,44}; in these studies, however, a decrease of enterobacteria counts was observed only after 14 days of feeding the fermented ration.

In Trial 1, \textit{E. coli} was isolated on days 8 and 10 from all pigs fed NFLWD, and a high number of pigs were infected in the other two groups. It is not possible to conclude that all these pigs were susceptible to F4 \textit{E. coli}. Binding specificity for each F4 phenotype (F4ab, F4ac, and F4ad) has been described.\textsuperscript{45} However, litter was a variable that we controlled for to minimize genetic differences between the groups. It may be that because the challenge dose was excessively high, even pigs without receptors for F4 had difficulty clearing \textit{E. coli} from their intestinal tracts. In future studies, it would be useful to determine the presence of F4 receptors in pigs included in the study.

Pigs in all groups showed clinical signs of diarrhea, but in general, the diarrhea was more severe in pigs fed NFLWD than those fed FLWD or DFA. However, no correlation be-

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\textbf{The variability in results may be partially explained by the difference in the initial \textit{E. coli} O149:K91:F4 status of the pigs in each trial.}
with LAB to newly weaned pigs may have produced diarrhea because of higher feed intake or because of probiotic overdosing. Many factors could be related to the development of diarrhea in this study, and future research should pay attention to the variable causes of diarrhea when testing diets when a fermented co-product is added to the feed.

Several studies have reported that the effect of feeding fermented diets versus nonfermented or dry diets on the pH of different portions of the swine GI tract is more obvious in the stomach. In those studies, the pigs were euthanized 1.5 hours and 3 hours after their last meal, and fermented feed may have been present in the stomach of the pigs when samples were collected. In our study, pigs were last fed about 16 hours before they were euthanized. It has been reported that low gastric pH is a result of lactic acid produced in the fermented feed before ingestion and not the result of hydrochloric acid produced in the stomach.

In our study, a tendency for a higher pH in the contents of the small intestine was observed. It has been reported that the addition of lactic acid to weanling diets increased the volume and protein and bicarbonate content of pancreatic secretions, which can buffer the pH in the first part of the small intestine. The higher pH might also be related to short-chain fatty acids being rapidly absorbed by the mucosal cells. We speculate that by the time the pigs were euthanized, most of the lactic acid ingested in the feed might already have been taken up by intestinal cells, which could explain why no differences between DFA and FLWD were observed.

A lower pH in the contents of the cecum was observed in pigs fed DFA. The potential rates of fermentation increase from the proximal to the distal parts of the GI tract (cecum and colon), especially in pigs fed dry feed. Consequently, high concentrations of acetic, propionic, and butyric acids are found in the cecum and colon of pigs fed dry diets.

**CONCLUSION**

None of the treatment diets tested in this study was able to reduce the severity or progression of diarrhea in pigs challenged with pathogenic *E. coli*. However, growth performance may not be affected in postweaning pigs fed fermented liquid whey inoculated with LAB when compared with pigs receiving a dry feed with an antibiotic under disease conditions. Many factors have to be accounted for when studying the effect of new diets in pigs with *E. coli* diarrhea. A larger trial is needed to specifically assess growth performance, reduction of mortality, and diarrhea in postweaned pigs fed fermented liquid co-product and probiotics.

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