Effects of Spray-Dried *Lactococcus*-Based Fermentation Products on Growth Performance of Nursery Pigs

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Summary

A total of 720 barrows (initially 13.0 lb; Line 200 × 400; DNA, Columbus, NE) from 2 study groups (360 pigs per study group), were used in a 42-d growth trial to test the effects of spray-dried *Lactococcus*-based fermentation products on nursery pig performance. There were 24 replications per treatment and 5 pigs per pen. For both experiments, pens of pigs were randomly allotted to 1 of 6 dietary treatments in a completely randomized design. There were six treatment diets fed in 3 phases. The positive control diet included zinc oxide (phase 1), zinc oxide + chlortetracycline (CTC; phase 2) while the negative control diet did not include zinc oxide or CTC. Treatment diets included the negative control + 1 of 4 fermentation products (C, D, E, or F) added at 5% of the diet. Phase 3 diets contained a common control diet fed to all pigs plus treatment diets (C, D, E, and F). Phase 1 and 2 diets were fed in pelleted form and phase 3 in mash form. From d 0 to 20, there was an overall treatment effect ($P = 0.001$) where pigs fed the positive control had increased ($P < 0.05$) d 20 weight, average daily gain (ADG), average daily intake (ADFI), and improved feed efficiency (F/G) compared to those fed the negative control and negative control + fermentation product. From d 20 to 42, there was an overall treatment effect ($P = 0.003$) for F/G where pigs fed the negative control had improved ($P < 0.05$) F/G compared to those fed additive D, E, and F. Overall, there was a treatment effect ($P = 0.03$) for pigs fed the positive control having improved ($P < 0.05$) ADG and F/G compared to the negative control and negative control + fermentation product. In addition, pigs fed the negative control had improved ($P < 0.05$) F/G compared to those fed additive D, E, and F. In conclusion, pigs fed the positive control (zinc + CTC) diet had improved performance compared to pigs fed the negative control with or without fermentation product.

Introduction

In-feed antibiotics have been fed to nursery pigs to maintain herd health and improve growth performance. There has been extensive research conducted to find alternatives

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to these antibiotics. The most well-known products used are nutraceuticals, such as heavy metals zinc (Zn) and copper (Cu). Zinc, commonly fed in the form zinc oxide (ZnO), alone or in combination with antibiotics such as chlortetracycline (CTC) have consistently provided improvements in nursery pig performance. Other products currently being tested as antibiotic replacements are probiotics, prebiotics, enzymes, plant extracts, and fermentation products. Intrexon Corporation (Germantown, MD) has developed a series of fermentation products for potential use in swine diets. The fermentation produces protein-based therapeutic agents from the safe, food-grade bacteria (*Lactococcus spp*) to provide novel feed additives. However, their effect on growth performance has not been determined. Therefore, the objective of this study was to evaluate fermentation products for use as antibiotic and ZnO replacements on growth performance of nursery pigs.

Procedures

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 (Manhattan, KS). Pigs were housed in 4 × 4 ft pens containing a three-hole dry self-feeder and one-cup waterer to provide *ad libitum* access to feed and water.

A total of 720 barrows (initially 13.0 lb; Line 200 × 400; DNA, Columbus, NE) from 2 study groups (360 pigs per study group), were used in a 42-d growth trial. Pigs were weaned at approximately 21-d of age and transported to the facility. Upon arrival, pigs were weighed and assigned to pens in a completely randomized design with 5 pigs per pen and 24 pens per treatment (12 per group). Pens of pigs were assigned to 1 of 6 dietary treatments. Treatment diets were fed in three phases (phase 1 = d 0 to 6, phase 2 = d 6 to 21, and phase 3 = d 21 to 42) with dietary phases formulated for 12 to 15, 15 to 25, and 25 to 50 lb weight ranges (Table 1). Phases 1 and 2 were fed in pelleted form and phase 3 was fed in mash form. The phase 1 positive control included zinc oxide (3,000 ppm), not included in the negative control. The phase 2 positive control include chlortetracycline (CTC; 400 g/ton) and zinc oxide (1,500 ppm), not included in the negative control. For treatment diets C, D, E, and F, spray-dried *Lactococcus*-based fermentation products (Intrexon Corporation, Germantown, MD) replaced 5% of corn in the phase 1, 2, and 3 diets. Pens of pigs were weighed, and feed disappearance calculated on d 0, 6, 14, 21, 28, 35, and 42 of the experiment to determine ADG, ADFI, and F/G.

Diet samples were collected at the mill and from feeders and subsampled for analysis. Subsamples were analyzed for dry matter, crude protein, crude fiber, fat, ash, and nitrogen free extract (Ward Laboratories, Inc., Kearney, NE).

Data were analyzed using the PROC-GLIMMIX procedure of SAS (SAS 9.4 Institute, Inc., Cary, NC) with pen serving as the experimental unit and barn within group as the blocking factor. Results were considered significant at $P \leq 0.05$, and marginally significant at $P \leq 0.10$.

Diet Manufacture
Feed was manufactured in accordance with current good manufacturing practices (cGMPs) at the Kansas State University O.H. Kruse Feed Technology Innovation Center (Manhattan, KS). Whole grain ingredients were ground with a three-high roller mill to a target particle size of 600 μm (RMS Model 924). All ingredients were weighed on certified scales, lot numbers recorded, and amounts verified by the feed manufacturing investigator. Diets were steam conditioned (10-in width × 55-in length Wenger twin staff pre-conditioner, Model 150) to a target conditioning temperature of 130°F for approximately 30 sec and pelleted on a 1-ton 30-horsepower pellet mill (1012-2 HD Master Model, California Pellet Mill) equipped with a 3/16 in × 1 1/4 in pellet die (L:D 6.67). The feeder was set at a constant rate to achieve approximately 1-ton per hour. Cool pellet samples were taken at the die, and pellets were cooled in an experimental counter flow cooler for 15 minutes. Pellets were then sifted to remove fines to ensure no effect of pellet quality on pig performance.

Hot pellet temperatures, production rates, and pellet samples were collected consecutively throughout the treatment run. Three samples from each replicate (treatment run) were collected to provide 9 pelleted samples per treatment (3 samples/treatment/run).

Pellet Durability
A sample of cool pellets was taken, and the fines sifted off by using the corresponding sieve.[4] Sifted pellets were split using a riffle divider and 100 g used for analysis. The 100-g sample was placed into the hopper of the Holmen 100 and the desired run time selected (60 sec). The fines were removed as the sample was run. Once completed, the sample was removed from the hopper and weighed. The pellet durability index (PDI) was calculated by dividing this final sample weight by the 100-g initial sample weight.

Results and Discussion
Chemical analysis of manufactured diets resulted in values consistent with formulation (Tables 1 and 2). Target processing parameters were maintained at a conditioning temperature of 130°F and production rate of 30 lb/min. For phase 1 diets, PDI for all diets were considered good quality with PDI > 88% (Table 3). For phase 2 diets, PDI for the positive control and negative control diets were 60.9 and 45.2%, respectively, and diets with additives were ≥ 93.5%. Differences in PDI were alleviated by sifting pellets post-pelleting to remove excessive fines. This was confirmed by samples collected at the feeder having ≤ 5.7% fines for all treatments (phase 1 and 2).

From d 0 to 20, there was an overall treatment effect ($P = 0.001$) for d 20 body weight (BW), ADG, ADFI, and F/G (Table 4). Pigs fed the positive control diets had increased ($P < 0.05$) d 20 BW, ADG, ADFI, and F/G compared to those fed the negative control and negative control + fermentation product. There was no evidence of difference between d 20 BW, ADG, ADFI, or F/G for pigs any of the negative control + fermentation product compared to those fed the negative control.

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[4] Schofield, Eileen K, and American Feed Industry Association. *Feed Manufacturing Technology V*. (pg. 631). American Feed Industry Association, 2005.

From d 20 to 42, pigs previously fed the positive and negative control were fed a common diet, while pigs fed a treatment were continually fed the feed additive. There was no evidence of differences for ADG and ADFI among the six dietary treatments. There was an overall treatment effect ($P = 0.03$) for F/G. Pigs fed the negative control had improved ($P < 0.05$) F/G compared to those fed additive D, E, and F, and pigs fed the positive control and additive C had intermediate F/G. There was a tendency for increased ($P = 0.078$) d 42 BW in pigs fed the positive control compared to pigs fed the negative control and negative control + fermentation product.

Overall, there was no evidence for differences in ADFI of pigs fed dietary treatments. However, there was an overall treatment effect ($P = 0.032$) for ADG and F/G. Pigs fed the positive control diet had increased ($P < 0.05$) ADG and improved ($P < 0.05$) F/G compared to those fed the negative control and negative control + fermentation product. In addition, pigs fed the negative control had improved ($P < 0.05$) F/G compared to those fed additive D, E, and F.

In conclusion, pigs fed the positive control (zinc + CTC) diet had improved growth performance compared to pigs fed the negative control with or without fermentation product. Adding fermentation products to the negative control diet did not improve nursery pig performance.

Table 1. Diet composition (as-fed basis)[1,2,3]

Ingredient, %	Phase 1[4]	Phase 2[5]	Phase 3
Corn	43.93	54.68	63.05
Soybean meal	18.08	29.36	32.55
Fish meal	6.00	4.00	---
Dried whey	12.50	5.00	---
Whey permeate[6]	7.50	1.75	---
Enzymatically treated soybean meal[7]	6.00	---	---
Choice white grease	3.00	2.00	1.00
Monocalcium P, 21% P	0.80	0.88	0.80
Limestone	0.43	0.60	0.95
Sodium chloride	0.35	0.55	0.55
L-Lysine-HCL	0.40	0.35	0.35
DL- Methionine	0.19	0.15	0.13
L-Threonine	0.20	0.16	0.13
L-Tryptophan	0.05	0.03	0.02
L-Valine	0.12	0.06	0.04
Choline chloride, 60%	0.04	---	---
Phytase[8]	0.03	0.03	0.03
Trace mineral premix	0.15	0.15	0.15
Vitamin premix	0.25	0.25	0.25
Total	100	100	100

continued

Table 1. Diet composition (as-fed basis)[1,2,3]

Ingredient, %	Phase 1[4]	Phase 2[5]	Phase 3
Calculated analysis			
Standard ileal digestible (SID) AA,[9] %			
Lysine (Lys)	1.40	1.35	1.25
Isoleucine:lysine	57	59	61
Leucine:lysine	110	118	125
Methionine:lysine	37	35	33
Methionine and cysteine: lysine	56	56	56
Threonine:lysine	63	63	62
Tryptophan:lysine	19.5	19.5	19.6
Valine:lysine	69	69	69
Histidine:lysine	34	37	40
Total Lys, %	1.56	1.51	1.40
ME, kcal/lb	1,587	1,540	1,508
NE, kcal/lb	1,196	1,145	1,115
SID Lys:NE, g/Mcal	5.31	5.35	5.08
CP, %	21.8	22.3	21.3
Ca, %	0.80	0.75	0.67
P, %	0.75	0.70	0.57
Available P, %	0.63	0.53	0.37
Lactose, %	15.0	5.0	---

[1]Experimental diets were fed in 3 phases formulated for 12 to 15, 15 to 25, and 25 to 50 lb body weight ranges.
[2]Experimental fermentation products were included at 5% to the negative control diets at the expense of corn.
[3]Treatment diets included the negative control plus the experimental product.
[4]The phase 1 positive control diet included zinc oxide (3,000-ppm) at 0.41% not included in the negative control.
[5]The phase 2 positive control included chlortetracycline (CTC; 400-g/ton) and zinc oxide (1,500 ppm), at 0.4% and 0.2%, respectively, not included in the negative control.
[6]DairyLac80 (International Ingredients, St. Louis, MO).
[7]HP300 (Hamlet Protein, Findlay, OH).
[8]HiPhos 2700 (DNA Nutritional Products, Inc., Parsippany, NJ), providing 367.9 phytase units (FTU)/lb and an estimated release of 0.12%.
[9]AA = amino acids. ME = metabolizable energy. NE = net energy.

Table 2. Chemical analysis of experimental diets (as-fed basis)[1]

	Control		Fermentation product[2]			
Item, %	Positive	Negative	C	D	E	F
Phase 1 diets						
Dry matter	90.48	90.48	90.62	90.07	90.53	90.30
Crude protein	21.60	21.55	21.50	21.70	21.55	21.30
Crude fiber	1.25	1.30	1.50	1.40	1.40	1.30
Fat (oil)	5.10	5.35	5.00	4.95	5.20	5.35
Ash	6.54	6.18	6.88	6.98	6.72	6.97
Nitrogen free extract (NFE)	55.95	56.15	55.70	55.05	55.65	55.30
Phase 2 diets						
Dry matter	89.75	89.19	89.47	88.77	89.40	89.58
Crude protein	22.60	22.20	21.70	21.80	21.25	21.65
Crude fiber	1.75	1.70	1.65	1.70	2.10	1.95
Fat (oil)	5.20	4.95	4.80	4.90	4.75	5.05
Ash	6.11	5.66	6.28	6.08	6.13	6.32
Nitrogen free extract (NFE)	54.10	54.65	55.05	54.30	55.15	54.60
Phase 3 diets						
Dry matter	88.76	88.38	88.78	88.44	88.62	88.52
Crude protein	21.60	21.33	21.75	21.60	21.65	21.40
Crude fiber	2.60	2.47	2.18	2.23	2.35	2.05
Fat (oil)	4.33	3.93	3.83	3.90	3.78	3.63
Ash	4.95	4.68	5.08	5.05	4.99	5.05
Nitrogen free extract (NFE)	55.30	55.97	55.93	55.68	55.83	56.38

[1]Three samples were collected for each diet at each phase. Samples were composited for one representative sample for analysis (Ward Laboratories, Inc., Kearney, NE).
[2]Experimental products (Intrexon Corporation, Germantown, MD).

Table 3. Feed processing and pellet quality of phase 1 and 2[1,2]

	Control		Fermentation product[2]			
Item, %	Positive	Negative	C	D	E	F
Phase 1 diets[3]						
Hot pellet temperature, °F	148.1	153.0	149.2	147.5	149.0	147.7
Fines, %[4]	4.1	5.7	3.7	3.0	4.9	3.9
Pellet durability index (PDI), %	88.8	87.5	97.0	97.2	97.1	97.1
Phase 2 diets[5]						
Hot pellet temperature, °F	149.7	148.3	149.1	147.6	149.3	149.9
Fines, %	3.6	3.7	0.5	0.6	0.4	0.5
Pellet durability index (PDI), %	60.9	45.2	95.4	93.4	95.5	93.5

[1]Diets were steam conditioned (10-in width × 55-in length Wenger twin staff pre-conditioner, Model 150) to the target conditioning temperature of 130°F for approximately 30 sec and pelleted on a 1-ton 30-horsepower pellet mill (1012-2 HD Master Model, California Pellet Mill) equipped with a 3/16 in × 1 1/4 in pellet die (L:D 6.67).
[2]For phase 1 and 2 respectively, average conditioning temperatures were 130.6 and 130.5°F and average production rates were 32.5 and 33.1 lb/min.
[3]For group 1 and 2 respectively, average mash temperatures were 62.5 and 73.2°F, average indoor temperatures were 77.2 and 77.7°F, and average relative humidity was 14.1 and 43.8% for phase 1.
[4]Fines were collected at the feeder.
[5]For group 1 and 2 respectively, average mash temperatures were 62.0 and 69.2°F, average indoor temperatures were 77.0 and 76.2°F, and average relative humidity was 13.6 and 37.4% for phase 2.

Table 4. Effect of spray-dried *Lactococcus*-based fermentation products on growth performance of nursery pigs[1]

	Control		Fermentation product[4]					Probability,
Item	Positive[2]	Negative[3]	C	D	E	F	SEM	$P<$
Body weight								
d 0	13.0	13.0	13.0	13.0	13.0	13.0	0.264	0.999
d 6	14.0[a]	13.6[b]	13.6[b]	13.7[b]	13.7[b]	13.7[b]	0.422	0.048
d 20	24.9[a]	22.3[b]	22.5[b]	22.9[b]	22.9[b]	22.6[b]	0.885	0.001
d 42	52.5[x]	50.2[y]	50.3[y]	50.8[y]	50.1[y]	50.2[y]	1.533	0.078
d 0 to 20								
ADG, lb[5]	0.59[a]	0.45[b]	0.47[b]	0.49[b]	0.49[b]	0.47[b]	0.031	0.001
ADFI, lb[5]	0.68[a]	0.58[b]	0.58[b]	0.61[b]	0.60[b]	0.60[b]	0.019	0.001
F/G[5]	1.15[a]	1.26[b]	1.26[b]	1.26[b]	1.25[b]	1.29[b]	0.045	0.001
d 20 to 42								
ADG, lb	1.25	1.27	1.26	1.26	1.22	1.25	0.033	0.676
ADFI, lb	1.90	1.89	1.92	1.94	1.89	1.92	0.052	0.798
F/G	1.52[ab]	1.50[a]	1.53[ab]	1.54[b]	1.55[b]	1.54[b]	0.012	0.034
d 0 to 42								
ADG, lb	0.94[a]	0.87[b]	0.88[b]	0.89[b]	0.87[b]	0.88[b]	0.030	0.032
ADFI, lb	1.32	1.25	1.28	1.30	1.27	1.29	0.033	0.261
F/G	1.41[a]	1.44[b]	1.46[bc]	1.46[c]	1.46[c]	1.47[c]	0.014	0.001

[1]A total of 720 barrows (DNA 400 × 200; initially 13.5 lb) were used in a 42-d experiment with 5 pigs per pen and 24 pens per treatment.

[2]The positive control diet included zinc oxide (phase 1, 3,000 ppm; and phase 2, 1,500 ppm) and CTC (phase 2, 400 g/ton or 440 ppm), which were not included in the negative control or fermentation product diets.

[3]The negative control diet was the same as the positive control without added zinc oxide or CTC.

[4]Treatment diets consisted of the negative control plus a 5% spray-dried *Lactococcus*-based fermentation product inclusion in place of corn.

[5]ADG = average daily gain. ADFI = average daily feed intake. F/G = feed efficiency.

[a,b,c]Within a row, means without a common superscript differ ($P < 0.05$).

[x,y]Within a row, means without a common superscript differ ($P < 0.10$).