The Effects of Supplementing Weanling Pig Diets with Organic and Inorganic Acids on Growth Performance and Microbial Shedding

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Introduction

Newly weaned pigs are very susceptible to developing digestive disturbances and gastro-intestinal diseases, resulting in stresses on the pig and consequently an inability to meet their growth potential. The continuous use of antibiotics at low levels at this crucial time has been recognized to improve feed intake, growth performance and feed efficiency.

However, growing public concern relating to food safety and antibiotic resistance is forcing the swine industry to explore alternatives to the sub-therapeutic use of antibiotics. The objective of this study was to investigate the use of dietary organic and inorganic acids on the growth performance of nursery pigs and microbial shedding.

Materials and Methods

One hundred and eighty pigs were weaned at an average of 18.2 days of age and were assigned to one of five dietary treatments. Pigs were allotted based on genetics, sex and initial body weight. Treatments were as follows: A) Basal diet, no antibiotics (negative control); B) Diet A plus Carbadox at 45.4g/ton (50 ppm; positive control); C) .4% Organic acid blend, added to diet A by substituting for corn; D) .2% Inorganic acid based blend, added to Diet A by substituting for corn; E) Combination of .4% organic and .2% inorganic acid based blends, added to Diet A by substituting for corn.

Pigs were weighed and feed intake recorded weekly during the five-week trial. Pigs were housed at six pigs per pen (3.0 sq. ft./pig) and six pens per treatment. All pigs had unlimited access to feed and water through a five-hole self feeder and a single nipple waterer in each pen. Phase 1 diets were fed from day 0 to 7, Phase 2 diets were fed from day 7 to 21, and Phase 3 diets were fed from day 21 to 35. All diets were formulated to meet or exceed the nutrient requirements for their phase of growth based on NRC (1998). Experimental treatments were continued during all three phases (Tables 1, 2 and 3).

Three pigs per pen had fresh feces samples collected at days 6, 20 and 34 of the study for measurement of pH. These three samples were then pooled on an equal weight basis to form a pen composite that was plated for *E. coli* and salmonella.

The procedures used for plating *E. coli* and salmonella were as follows:

A 1 g sub-sample of each pen composite fecal sample was mixed into 9 mL of peptone broth, serially diluted and used to inoculate MacConkey agar plates for *E. coli* isolation. The *E. coli* plates were inoculated at 3 dilutions and in triplicate at each dilution.

Samples taken following Phase 1 diets were serially diluted and plated at 10^5, 10^6 and 10^7 cfu/µg . Samples taken following Phase 2 and Phase 3 were serially diluted and plated at 10^4, 10^5 and 10^6 cfu/µg. This change in dilution rate was as a result of a fall in bacteria numbers. The MacConkey agar plates were then incubated for 24 h at 98.6 °F, and following removal from the incubator *E. coli* colonies were immediately counted.

For salmonella, the serially diluted peptone broth tubes were then incubated overnight at 98.6 °F, and 1 mL was then transferred to 9 mL tetrathionate broth and incubated for 48 h at 107.6 °F. From these tubes 1 mL was used to inoculate Pappaport Vassiliadis broth and incubated for 48 h at 107.6°F. The Pappaport broth was then used to inoculate XLT4 plates for salmonella isolation. Salmonella was then presumptively positively identified using LIA and TSI agar tubes.

Results

Growth performance. At the beginning of the study, there were no significant differences in initial body weights of pigs between treatments (Table 5). During the Phase 1 time period (d 0 to d 7), the ADG of pigs fed the negative control tended to be higher than the ADG of those pigs fed carbadox and combination treatments (.298 vs .233 and .230 lb/d, respectively; $P<.10$).

During Phase 1, pigs fed the negative control had a significantly higher ADFI ($P<.05$) than those fed the antibiotic diet, the organic acid blend diet, and the combination acid diet. While the ADFI for the organic acid blend diet and the inorganic acid based blend diet were not different from each other, the inorganic acid fed pigs did have greater ADFI ($P<.05$) than the antibiotic fed pigs. During Phase 1, pigs fed the organic acid blend diet tended to convert feed more efficiently than the pigs fed the combination acid diet (.750 vs. .642; $P<.10$). At the end of the Phase 1 time period (d 7), pigs fed the negative control diet tended to be heavier than the pigs fed the combination acid diet (15.35 vs. 14.91 lbs; $P< .10$).

During Phase 2 (d 7 to d 21), pigs fed the diet containing carbadox tended to have higher ADG than pigs fed the combination acid diet (.586 vs .470 lbs/d; $P<.10$). The ADFI for the pigs fed the combination acid diet was significantly ($P<.05$) lower than the ADFI for all the other treatments, with the exception of the inorganic acid based blend diet which tended ($P<.10$) also to be higher than the combination diet. There were no differences among treatments for feed efficiency or d 21 bodyweights during Phase 2.

There were no significant differences between dietary treatments in relation to ADG or ADFI during the third phase of the study (d 21 to d 35). Feed conversion efficiency tended ($P<.10$) to be higher in pigs fed the carbadox diet compared with pigs fed the negative control diet and the inorganic acid based blend diet. There were no significant differences in body weight at d 35 among the different dietary treatments.

Overall, pigs fed the carbadox diet had numerically higher ADG; however, there were no statistical differences in ADG across treatments for the entire 35-d study. Over the five week trial period, the ADFI for pigs fed the combination acid diet tended to be lower ($P<.10$) than the ADFI of pigs fed all other dietary treatments. For the entire 35-d study, the feed conversion efficiency of pigs fed the negative control tended to be lower ($P<.10$) than the feed conversion efficiency of pigs fed the carbadox diet, with all acid treatments being intermediate. Final weights of the pigs after the five-week study indicate that pigs fed the diet containing carbadox gained the most weight, and were approximately 1 lb heavier than the negative control and 3 lbs heavier than the pigs fed the combination acid diet.

Bacteria Counts. E. coli and salmonella colonies shed were determined at three separate time points during the study (Table 6): at the end of the first week (d 6), at the end of the third week (d 20), and at the end of week five (d 34). In addition, pre-weaning samples were taken to provide background levels of microbial populations (Table 4). E. coli counts on day 6 indicate that pigs fed the negative control diet had significantly lower E. coli counts than pigs fed any of the other dietary treatments ($P<.05$). The presence of an unidentified bacteria was documented at this time point also; however, this bacteria was only present in pigs that were being fed the negative control diet.

The presence or absence of salmonella was a concern in this study, and therefore the quantity being shed was not determined. There was no significant effect of dietary treatment on the presence of salmonella shedding (P>.10). There was no salmonella present in any pigs fed either the inorganic acid based blend diet or the combination acid diet on day 6 of the study. However, it was noted that 33.3% of the pigs fed the organic acid blend diet were positive for salmonella and 16.7% of the pigs fed the negative control diet and the carbadox diet, were positive for salmonella on day 6 of the study.

Samples taken on day 20 of the study indicated that pigs fed the negative control diet now had numerically higher *E. coli* colonies being shed than pigs on any of the other dietary treatments; however, there were no statistical differences among treatments at this time. The unidentified bacteria were present in all the acid diets at this time point, but not in the negative or positive controls. However, there were no statistical differences among treatments. On day 20 of the study 16.7% of the pigs fed the antibiotic diet and the organic acid blend diets were found to be positive for salmonella.

At d 34, the *E. coli* counts for pigs being fed the carbadox diet was significantly higher than pigs fed the combination acid diet (P<.05). Also, pigs fed the combination acid diet and the organic acid blend diet tended to have lower *E. coli* fecal shedding than pigs fed the positive and negative control diets with the inorganic acid based blend diet being intermediate (P<.10). At this time point, the unidentified bacteria was present in pigs on all dietary treatments with the exception of the negative control diet (P<.10). Counts of these particular bacteria tended to be numerically lower in pigs fed the organic acid blend diet. On day 34 of the study, 16.7% of the pigs fed the antibiotic diet and the organic acid blend diet were found to be positive for salmonella, which coincides with that which was found on day 20 of the study.

Fecal pH were also determined at the same three sampling time points during the study as the bacterial data (Table 6). The fecal pH of pigs fed the combination acid diet was significantly higher than pigs fed the organic acid blend diet or the inorganic acid based blend diet (7.01 vs 6.65 and 6.63 ; P<.05) at day 6. Also, the fecal pH of pigs fed the carbadox diet tended to be higher than pigs fed the inorganic acid based blend diet (P<.10) on day 6 of the study.

At the second sampling time point (d 20) pigs fed the carbadox diet had numerically the highest fecal pH and pigs fed the inorganic acid based blend diet had the lowest fecal pH. However, these were not statistically different to the fecal pH of pigs fed the other dietary treatments. By the third sample collection day it was noted that pigs fed the negative control diet had numerically the highest fecal pH value while pigs fed the carbadox diet had numerically the lowest fecal pH value. However, there were no statistical differences between treatments for fecal pH at d 34.

Discussion

The objective of this study was to evaluate the performance of organic and inorganic acid blends and also a combination of these acids as potential alternatives to antibiotic growth promoters in nursery pig diets. While antibiotics help ensure a control of diarrhea outbreaks and mortality caused by bacterial infections in pigs, a risk of developing cross-resistance of pathogens to antibiotics used in human therapy has become more evident (Piva, 1998; Hillman, 2001). Due to this reason the swine industry is now searching for an equally competent, if not better, alternative to antibiotics in relation to growth performance and health in nursery pig diets.

Results from this study demonstrate that the overall growth performance of nursery pigs could be affected by the addition of growth promoting agents to their diets. It has been previously reported that the addition of antibiotic growth promoters to the nursery pig diet has significantly increased the growth performance over that of the control pigs. In this study it was found that pigs fed a diet containing carbadox had the numerically the highest ADG, most favorable feed efficiency and the heaviest weights across treatments. However, during week 1 of the study the very opposite was found in this study, with the pigs being fed the carbadox diet having the poorest growth performance. This may be due to the abrupt change in diet of these nursery pigs and a poor acclimation period post-weaning by these pigs, as the growth performance following this first seven day period was highest for the pigs fed the carbadox diet. The performance of pigs fed the organic and inorganic acid based blend diets were similar to each other and somewhat intermediate between the carbadox-fed pigs and the pigs that received no growth promoting additives in their diets. The pigs fed the combination acid diet had the numerically the poorest growth performance, lower than the negative control. This may have been as a result of the high levels of acid in the diet (0.6%) suppressing feed intake and depressing overall performance.

This study also demonstrated that the amount of *E. coli* being shed differs in relation to the dietary treatment being fed. It was noted that the greatest reduction in *E. coli* shedding from d 6 to d 34 was seen in pigs fed the combination acid diet. However, similar, less dramatic, reductions were also seen in pigs fed the inorganic and the organic acid based blend diets. The amount being shed by the negative control pigs did not differ greatly throughout the entire study. *E. coli* counts for the remaining treatments were higher on day 6 of the study compared to the negative control, but by day 34 the *E. coli* counts for all the acid treatments were lower than for the pigs fed the negative or positive control diets. The presence of unidentified bacteria was also quantified during this study. Our results lead us to believe that there may be a competitive exclusion relationship existing between the *E. coli* and these unidentified bacteria. While the population of *E. coli* undergoes a decrease, the population of the unidentified bacteria increases.

The results of this study suggest that the presence of salmonella is not affected by these dietary treatments. Salmonella was consistently found in pigs fed the carbadox diet and also the organic acid blend diet throughout the study. This is most likely due to initial salmonella infection during the nursing period of lactation and was not eliminated during the post-weaning nursery period.

Overall, a fall in fecal pH took place during the course of the study. However, the fecal pH of pigs fed different dietary treatments were not statistically different from each other.

Implications

The use of organic acid and inorganic acid based blends in nursery pig diets has been demonstrated in this study to have similar performance to the use of carbadox or no antibiotics. This study also shows that a combination acid diet may not be as beneficial, as the high acid content (0.6%) reduced feed intake and therefore growth performance of the nursery pig. The acid diets also reduced *E. coli* shedding compared with the medicated and non-medicated diets, which may have long term pig health benefits.

References

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Table 1. Composition of Phase 1 experimental nursery pig diets

	Neg. Control	Carbadox	Organic acid	Inorganic acid	Combination
Ingredients, %					
Corn	38.40	37.93	37.67	38.03	37.30
SBM, 48%CP	20.30	20.30	20.30	20.3	20.30
Soybean oil	5.00	5.20	5.30	5.15	5.45
Limestone	0.58	0.58	0.58	0.58	0.58
Dical.Phos	0.90	0.90	0.90	0.90	0.90
Vitamin premix[a]	0.25	0.25	0.25	0.25	0.25
TM premix[b]	0.125	0.125	0.125	0.125	0.125
Salt	0.20	0.20	0.20	0.20	0.20
Fishmeal	4.00	4.02	4.03	4.02	4.05
Dried whey	25.00	25.00	25.00	25.00	25.00
Plasma protein	5.00	5.00	5.00	5.00	5.0
Lysine-HCL	0.10	0.10	0.10	0.10	0.10
Methionine	0.10	0.10	0.10	0.10	0.10
Carbadox-10	---	0.25	---	---	---
Organic Acid Blend	---	---	0.40	---	0.40
Inorganic Acid Based Blend	---	---	---	0.20	0.20
Se 600	0.05	0.05	0.05	0.05	0.05
Total	100.00	100.00	100.00	100.00	100.00
Calculated composition					
ME, kcal/lb	1598	1598	1598	1598	1598
Lysine, %	1.55	1.55	1.55	1.55	1.55
Meth., %	0.45	0.45	0.45	0.45	0.45
M + C, %	0.89	0.89	0.89	0.89	0.89
Thre., %	1.01	1.01	1.01	1.01	1.01
Tryp., %	0.29	0.29	0.29	0.29	0.29
Ca, %	0.90	0.90	0.90	0.90	0.90
P, %	0.80	0.80	0.80	0.80	0.80
Analyzed composition					
Carbadox, g/ton	<1.00	44.7	<1.00	<1.00	<1.00

[a] Trace mineral premix provided per lb of diet: Iron, 55 mg; Zinc, 55 mg; Manganese, 6.8 mg; Copper, 5.1 mg; Iodine, .21 mg.

[b] Vitamin premix provided per lb of diet: vitamin A, 2750 IU; vitamin D_3, 275 IU; vitamin E, 20 IU; menadione, .907 mg; vitamin B_{12}, 15.9 µg ; riboflavin, 3.22 mg ; d-pantothenic acid, 9.97 mg; niacin, 14.97 mg.

Table 2. Composition of Phase 2 experimental nursery diets

	Neg. control	Carbadox	Organic acid	Inorganic acid	Combination
Ingredients, %					
Corn	45.34	44.87	44.59	44.96	44.21
SBM, 48%	28.40	28.40	28.40	28.40	28.40
Soybean oil	5.00	5.18	5.31	5.15	5.47
Limestone	0.51	0.51	0.51	0.51	0.51
Dical.Phos	0.80	0.80	0.80	0.80	0.80
Vitamin premix[a]	0.25	0.25	0.25	0.25	0.25
TM premix[b]	0.125	0.125	0.125	0.125	0.125
Salt	0.30	0.30	0.30	0.30	0.30
Fishmeal	4.00	4.04	4.04	4.03	4.06
Dried whey	15.00	15.00	15.00	15.00	15.00
Plasma protein	---	---	---	---	---
Lysine-HCL	0.125	0.125	0.125	0.125	0.125
Methionine	0.07	0.07	0.07	0.07	0.07
Carbadox-10	---	0.25	---	---	---
Organic Acid Blend	---	---	0.40	---	0.40
Inorganic Acid Based Blend	---	---	---	0.20	0.20
Se 600	0.05	0.05	0.05	0.05	0.05
Total	100.00	100.00	100.00	100.00	100.00
Calculated composition					
ME, kcal/lb	1597	1597	1597	1597	1597
Lysine, %	1.40	1.40	1.40	1.40	1.40
Meth., %	0.43	0.43	0.43	0.43	0.43
M + C, %	0.79	0.79	0.79	0.79	0.79
Thre., %	0.90	0.90	0.90	0.90	0.90
Tryp., %	0.27	0.27	0.27	0.27	0.27
Ca, %	0.80	0.80	0.80	0.80	0.80
P, %	0.70	0.70	0.70	0.70	0.70
Analyzed composition					
Carbadox, g/ton	<1.00	46.4	<1.00	<1.00	<1.00

[a] Trace mineral premix provided per lb of diet: Iron, 55 mg; Zinc, 55 mg; Manganese, 6.8 mg; Copper, 5.1 mg; Iodine, .21 mg.

[b] Vitamin premix provided per lb of diet: vitamin A, 2750 IU; vitamin D_3, 275 IU; vitamin E, 20 IU; menadione, .907 mg; vitamin B_{12}, 15.9 μg ; riboflavin, 3.22 mg ; d-pantothenic acid, 9.97 mg; niacin, 14.97 mg.

Table 3. Composition of Phase 3 experimental nursery diets

	Neg. control	Carbadox	Organic acid	Inorganic acid	Combination
Ingredients, %					
Corn	59.93	59.45	59.13	59.50	58.79
SBM, 48%	33.65	33.70	33.75	33.70	33.75
Soybean oil	---	---	---	---	---
Swine grease	3.00	3.18	3.3	3.18	3.44
Limestone	0.83	0.83	0.83	0.83	0.83
Dical.Phos	1.62	1.62	1.62	1.62	1.62
Vitamin premix[a]	0.25	0.25	0.25	0.25	0.25
TM premix[b]	0.125	0.125	0.125	0.125	0.125
Salt	0.35	0.35	0.35	0.35	0.35
Fishmeal	---	---	---	---	---
Dried whey	---	---	---	---	---
Plasma protein	---	---	---	---	---
Banmith	0.10	0.10	0.10	0.10	0.10
Lysine-HCL	0.10	0.10	0.10	0.10	0.10
Methionine	---	---	---	---	---
Carbadox-10	---	0.25	---	---	---
Organic Acid Blend	---	---	0.40	---	0.40
Inorganic Acid Based Blend	---	---	---	0.20	0.20
Se 600	0.05	0.05	0.05	0.05	0.05
Total	100.00	100.00	100.00	100.00	100.00
Calculated composition					
ME, kcal/lb	1553	1553	1553	1553	1553
CP, %	21.00	21.00	21.00	21.00	21.00
Lysine, %	1.25	1.25	1.25	1.25	1.25
Meth., %	0.325	0.325	0.325	0.325	0.325
Ca, %	0.80	0.80	0.80	0.80	0.80
P, %	0.70	0.70	0.70	0.70	0.70
Analyzed composition					
Carbadox, g/ton	<1.00	45.0	<1.00	<1.00	<1.00

[a] Trace mineral premix provided per lb of diet: Iron, 55 mg; Zinc, 55 mg; Manganese, 6.8 mg; Copper, 5.1 mg; Iodine, .21 mg.

[b] Vitamin premix provided per lb of diet: vitamin A, 2750 IU; vitamin $D_{3,}$ 275 IU; vitamin E, 20 IU; menadione, .907 mg; vitamin $B_{12,}$15.9 µg ; riboflavin, 3.22 mg ; d-pantothenic acid, 9.97 mg; niacin, 14.97 mg.

Table 4. Bacteria count pre -weaning

No. litters sampled	12
Average *E. coli* count, nlog	19.85
Salmonella presence	1 in 24
Average unknown bacteria count, nlog	19.80[a]

[a] Unknown bacteria only present in 2 of the 12 pens sampled.

Table 5. The effect of dietary acids on the growth performance of nursery pigs

	Negative control	Carbadox	Organic acid[1]	Inorganic Acid[2]	Combination[3]	SE
Number of pens/trt	6	6	6	6	6	
Number of pigs/pen	6	6	6	6	6	
Initial weight, lbs	13.26	13.31	13.30	13.32	13.31	.034
Phase 1 (d 0 to d 7)						
ADG, lbs	0.298^{a}	0.233^{b}	0.280^{ab}	0.280^{ab}	0.230^{b}	.022
ADFI, lbs	0.450^{xa}	0.333^{zc}	0.372^{yzbc}	0.418^{xyab}	0.356^{yzbc}	.024
G:F	0.672^{ab}	0.702^{ab}	0.750^{a}	0.672^{ab}	0.642^{b}	.039
d 7 weight, lbs	15.35^{a}	14.95^{ab}	15.25^{ab}	15.28^{ab}	14.91^{b}	.159
Phase 2 (d 7 to d 21)						
ADG, lbs	0.545^{ab}	0.586^{a}	0.563^{ab}	0.561^{ab}	0.470^{b}	.043
ADFI, lbs	0.952^{xa}	0.973^{xa}	0.970^{xa}	0.930^{xya}	0.798^{yb}	.046
G:F	0.573	0.605	0.570	0.608	0.592	.028
d 21 weight, lbs	22.9	23.16	23.13	23.14	21.52	.718
Phase 3 (d 21 to d 35)						
ADG, lbs	1.01	1.07	1.05	1.03	0.966	.042
ADFI, lbs	1.80	1.78	1.80	1.86	1.67	.076
G:F	0.562^{b}	0.606^{a}	0.585^{ab}	0.555^{b}	0.580^{ab}	.016
d 35 weight, lbs	37.10	38.15	37.79	37.47	35.03	1.22
Overall (d 0 to d 35)						
ADG, lbs	0.680	0.712	0.700	0.688	0.622	.035
ADFI, lbs	1.19^{b}	1.16^{a}	1.18^{a}	1.19^{a}	1.06^{b}	.047
G:F	0.572^{b}	0.612^{a}	0.592^{ab}	0.578^{ab}	0.586^{ab}	.014

[1] Organic acid blend was included at .4% of the diet, Kemin Americas, Inc.
[2] Inorganic acid based blend was included at .2% of the diet , Kemin Americas, Inc.
[3] Combination of both organic and inorganic acid based blends in the diet at .4% and .2% respectively, Kemin Americas, Inc.
[abc] Values with different superscripts differ by $P<.10$ by means separation using the Duncan multiple range test.
[xyz] Values with different superscripts differ by $P<.05$ by means separation using the Duncan multiple range test.

Table 6. The effect of dietary acids on fecal *E. coli* and salmonella shedding, and fecal pH in nursery pigs

	Negative control	Carbadox	Organic acid[1]	Inorganic acid[2]	Combination[3]	SE
Phase 1, d 6						
E. coli, log_{10}	6.74[y]	8.04[x]	7.79[x]	8.06[x]	7.87[x]	.3101
Unknown bacteria, log_{10}	1.26	0	0	0	0	.5619
Salmonella, % positive	16.7	16.7	33.3	0	0	--
Fecal pH	6.81[xyabc]	6.91[xyab]	6.65[ybc]	6.63[yc]	7.01[xa]	.116
Phase 2, d 20						
E. coli, log_{10}	7.07	6.99	6.79	6.70	6.44	.4127
Unknown bacteria, log_{10}	0	0	.866	2.00	2.21	.9664
Salmonella, % positive	0	16.7	16.7	0	0	--
Fecal pH	6.14	6.24	6.13	6.11	6.22	.093
Phase 3, d 34						
E. coli, log_{10}	6.58[xya]	6.70[xa]	5.72[xyb]	6.02[xyab]	5.62[yb]	.3930
Unknown bacteria, log_{10}	0[yb]	3.82[xa]	2.78[xya]	4.14[xa]	4.99[xa]	1.117
Salmonella, % positive	0	16.7	16.7	0	0	--
Fecal pH	6.45	6.21	6.41	6.36	6.44	.126

[1] Organic acid blend was included at .4% of the diet, Kemin Americas, Inc.
[2] Inorganic acid based blend included at .2% of the diet , Kemin Americas, Inc.
[3] Combination of both organic and inorganic acid based blends in the diet at .4% and .2% respectively, Kemin Americas, Inc.
[abc] Values with different superscripts differ by P<.10 by means separation using the Duncan multiple range test.
[xyz] Values with different superscripts differ by P<.05 by means separation using the Duncan multiple range test.