



Phytobiotics and Organic Acids As Potential Alternatives to the Use of Antibiotics in Nursery Pig Diets

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ABSTRACT : Two experiments were conducted to determine the effect of phytobiotics and organic acids on growth performance of nursery pigs as an alternative to antibiotics. Phytobiotics refer bioactive compounds from plant materials including essential oils and herbal extracts. In Exp. 1, 144 pigs, weaned at 23.4 ± 0.3 d age, were allotted to three dietary treatments. Treatment diets were: 1) NC (no antibiotics and no phytobiotics); 2) PC (NC+carbadox, 50 mg/kg); and 3) PB (NC+phytobiotics; 0.1% PEP1000-1[®]). Each treatment had six replicates with eight pigs per pen. Pigs were fed the experimental diets for 5 wks in 3 phases (phase 1 for 2 wk; phase 2 for 2 wk; phase 3 for 1 wk). In Exp. 2, 192 pigs, weaned at 19.2 ± 0.3 d age, were allotted to three dietary treatments: 1) NC; 2) PC; and 3) PBO (NC+phytobiotics; 0.2% or 0.1% PEP1000-1[®] and organic acids; 0.4% or 0.2% Biotronic[®] for the phase 1 and 2, respectively) with eight replicates per treatment and eight pigs per pen. Pigs were fed the assigned diets for 5 wks in 2 phases (phase 1 for 2 wk; phase 2 for 3 wk). Body weights were measured at the beginning of the experiment and at the end of each week in both Exp. 1 and 2. Feed intake was measured at the end of each week in both Exp. 1 and 2. Diarrhea score was measured daily during the entire period for Exp. 1 and during the phase 1 for Exp. 2. In Exp. 1, the PC had a higher ($p < 0.05$) overall ADG than the NC, but the overall ADG of the PB did not differ ($p > 0.05$) from the NC or the PC. In Exp. 2, the overall ADG did not differ ($p > 0.05$) among all the treatments during the entire experimental period. The overall ADFI and the overall gain:feed ratio did not differ ($p > 0.05$) among all the treatments during the entire experimental period in both Exp. 1 and 2. The PC had a higher ($p < 0.05$) overall diarrhea score (harder stools) than the NC and the PB in Exp. 1, and a higher ($p < 0.05$) overall diarrhea score than the NC in Exp. 2. The overall diarrhea score of the PB and the PBO did not differ ($p > 0.05$) from the NC or the PC in Exp. 1 and 2. Results from this study show that the growth of pigs fed the diets with phytobiotics or the combination of phytobiotics and organic acids did not differ from those both with antibiotics and without antibiotics when tested in an environmentally controlled research facility. Further experiments are required to study the growth performance in disease challenged conditions. (**Key Words :** Diarrhea Score, Growth Performance, Organic Acids, Phytobiotics, Nursery Pigs)

INTRODUCTION

Newly weaned piglets are highly susceptible to various stressors such as bacterial diseases (Walsh et al., 2003) resulting in decreased growth and even death (Canibe et al., 2001). Cromwell (2001) reviewed that supplementing antibiotics in swine diets at sub-therapeutic levels have shown to increase the growth rate by average 16% in weanling pigs, 11% in growing pigs, and 4% in growing-finishing pigs. However, due to the developing antibiotic resistance, the use of all antibiotics has been banned in EU (Simon, 2005). Thus, current research involving feed

additives in swine diets is focused on searching for alternatives to antibiotics that would have at least similar effects of antibiotics without causing bacterial resistance (Doyle, 2001). Organic acids (Mroz, 2005), essential oils (Rota, 2004), enzymes such as β -mannanase (Jackson et al., 2003; Kim et al., 2003), probiotics (Scott and Paula, 2002), prebiotics (Christian et al., 2005), and highly available minerals (Doyle, 2001; Hollis et al., 2005) have been tested as alternatives to antibiotics.

Phytobiotics is a term applied for essential oils and extracts derived from certain plants such as cinnamon, oregano, and thyme etc. (Lee et al., 2004b). The essential oils are volatile oils that are natural vegetable products typically extracted by steam distillation, or by enzymatic hydrolysis followed by steam distillation (Zhang et al., 2005), which include thymol, carvacrol, and cinnamaldehyde. The essential oils can be used as

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Received March 6, 2006; Accepted May 30, 2006

Table 1. Ingredients and chemical composition of the diets for the Experiment 1 (as-fed basis)

Ingredient (%)	Phase 1	Phase 2	Phase 3
Corn grain	27.00	42.05	61.10
Soybean meal (dehulled)	22.00	26.00	32.70
Fish meal (Menhaden)	3.55	0.00	0.00
Dried whey	35.00	20.00	0.00
Salt	0.45	0.35	0.25
Vitamin-mineral premix ^a	4.00	3.00	2.00
Restaurant grease	2.00	2.00	1.00
Dicalcium phosphate	0.25	0.55	1.15
Limestone	0.75	1.05	0.80
Plasma protein ^b	4.00	4.00	-
Supplement ^c	1.00	1.00	1.00
Total	100.00	100.00	100.00
Chemical composition:			
Dry matter (%)	91.7	91.0	90.0
ME (Mcal/kg)	3.3	3.3	3.3
Crude protein (%) ^d	22.7	21.6	20.6
Lysine (%)	1.51	1.36	1.15
Cystine+methionine (%)	0.80	0.75	0.68
Tryptophan (%)	0.30	0.28	0.25
Threonine (%)	1.08	0.97	0.78
Calcium (%)	0.91	0.80	0.71
Available phosphorus (%)	0.55	0.40	0.32
Total phosphorus (%)	0.70	0.60	0.59

^a Vitamin-mineral premix contained the amounts per kilogram of premix: Vit. A, 503,742 IU; Vit. D₃, 54,996 IU; Vit. E, 4,125 IU; Vit. B₁₂, 3.6 mg; Choline, 110,470 mg; Riboflavin, 915.4 mg; Niacin, 3,657.4 mg; D-pantothenic acid, 2,926.8 mg; Menadione, 182.5 mg; Folic acid, 110.1 mg; Thiamine, 365.2 mg; Pyridoxine, 365.6 mg; D-biotin, 18.3 mg; Ca, 3.17 mg; P, 0.15 mg; K, 0.18 mg; Mg, 3.11 mg; Na, 0.02 mg; Cl, 1.68 mg; Fe, 5,000 mg; Mn, 3,340 mg; Se, 15 mg; S, 4,160 mg; Zn, 6,920 mg; Cu, 632 mg/kg; I, 47.8 mg.

^b APC920 (APC, Boone, IA).

^c For the NC, supplement was 1% corn; for the PC, supplement was diluted form of carbadox 50 mg/kg with corn to bring it to 1%; for the PB, supplement was PEP1000-1[®] (Biomim Inc., San Antonio, TX) 0.1% with corn to bring it to 1%.

^d Analyzed values averaged 23.1, 21.9, and 20.4% for phase 1, 2, and 3, respectively.

antimicrobial feed additives (Chang et al., 2001), and as flavor enhancers (Lee et al., 2004a). Organic acids such as formic, acetic, and citric acids can play a profound role as antimicrobials (Mroz, 2005), and as growth promoters (Canibe et al., 2001). According to Langhout (2000), the organic acids are active in the crop and gizzard of the chicken, whereas the essential oils will be active in the distal portions of the gut. Thus, a combination of the essential oils and organic acids could be beneficial for improving digestion throughout the digestive tract.

The objective of this study was to evaluate the growth performance of the nursery pigs by supplementing phytobiotics and organic acids as alternatives to antibiotics in their diets. This study was conducted in an environmentally controlled research facility without disease challenges.

MATERIALS AND METHODS

Test materials

The phytobiotics (PEP1000-1[®], Biomim Inc., San Antonio, TX) used in this study were composed of essential oils including anis oil, citrus oil, oregano oil, and natural flavors. Organic acids (Biotronic[®], Biomim Inc.) used in this study were composed of selected organic and inorganic acids including phosphoric acid and lactic acid.

Experiment 1

A total of 144 nursery pigs, weaned at 23.4±0.3 d age, with an initial BW of 7.9±0.3 kg were randomly allotted to one of three dietary treatment groups. The pigs in the negative control treatment group (NC) were fed a diet without any supplemental phytobiotics or carbadox; the pigs in the positive control treatment group (PC) were fed a diet supplemented with carbadox at 50 mg/kg; and the pigs in the test treatment group (PB) were fed a diet supplemented with phytobiotics (PEP1000-1[®]) at 0.1%. Each treatment had six replicates with eight pigs (4 gilts and 4 barrows) per pen. Each pen (1.5×2.1 m) had slatted plastic floor. The room temperature was kept at 30°C during the first week of the study and reduced to 25°C by the end of the study. The photoperiod consisted of 10 h of artificial light and 14 h of darkness. The pigs were fed the assigned experimental diets (Table 1) for 5 wks based on a three phase feeding program (phase 1 for 2 wk; phase 2 for 2 wk; phase 3 for 1 wk). The phase 1 diet contained 3.29 Mcal/kg ME and 1.51% total lysine; the phase 2 diet contained 3.33 Mcal/kg ME and 1.36% total lysine; and the phase 3 diet had 3.32 Mcal/kg ME and 1.15% total lysine. All the pigs had free access to feed and water during the entire experimental period. The body weights were measured at the beginning of the experiment and at the end of each phase. Feed intake was measured at the end of each phase. Incidence of diarrhea was measured on a daily basis during the whole experimental period based on a five point scale wherein 1 = watery, 3 = normal, and 5 = hard as previously described by Quigley (2004).

Experiment 2

A total of 192 pigs, weaned at 19.2±0.3 d age, with a mean initial body weight of 6.3±0.3 kg were randomly allotted to one of three dietary treatment groups. The pigs in the negative control treatment group (NC) were fed a diet without any supplemental phytobiotics or organic acids or carbadox; the pigs in the positive control treatment group (PC) were fed a diet supplemented with carbadox at 50 mg/kg; and the pigs in test treatment group (PBO) were fed a diet supplemented with a combination of phytobiotics (PEP1000-1[®]) and organic acids (Biotronic[®]) at 0.4% and

Table 2. Ingredients and chemical composition of the diets for the Experiment 2 (as-fed basis)

Ingredients (%)	Phase 1	Phase 2
Corn, yellow	29.35	59.65
Soybean meal (48%)	25.00	35.00
Dried whey	30.00	0.00
Fish meal (Menhaden)	3.00	0.00
Plasma protein ^a	3.00	0.00
Zinc oxide	0.25	0.00
Salt	0.40	0.25
Vitamin-mineral premix ^b	4.00	1.00
Dicalcium phosphate	0.80	1.40
Limestone	0.60	0.70
Restaurant grease	2.60	1.00
Supplement ^c	1.00	1.00
Total	100.00	100.00
Calculated composition:		
Dry matter (%)	91.52	89.83
ME (Mcal/kg)	3.24	3.34
Crude protein (%)	22.59	21.66
Lysine (%)	1.47	1.21
Cystine+methionine (%)	0.78	0.71
Tryptophan (%)	0.30	0.26
Threonine (%)	1.04	0.82
Calcium (%)	0.93	0.74
Available phosphorus (%)	0.59	0.37
Total phosphorus (%)	0.76	0.65

^aAPC920 (APC, Boone, IA).

^bVitamin-mineral premix contained the amounts per kilogram of premix: Vit. A, 503,742 IU; Vit. D₃, 54,996 IU; Vit. E, 4,125 IU; Vit. B₁₂, 3.6 mg; Choline, 110,470 mg; Riboflavin, 915.4 mg; Niacin, 3,657.4 mg; D-pantothenic acid, 2,926.8 mg; Menadione, 182.5 mg; Folic acid, 110.1 mg; Thiamine, 365.2 mg; Pyridoxine, 365.6 mg; D-biotin, 18.3 mg; Ca, 3.17 mg; P, 0.15 mg; K, 0.18 mg; Mg, 3.11 mg; Na, 0.02 mg; Cl, 1.68 mg; Fe, 5,000 mg; Mn, 3,340 mg; Se, 15 mg; S, 4,160 mg; Zn, 6,920 mg; Cu, 632 mg/kg; I, 47.8 mg.

^cAPC920 (APC, Boone, IA).

^dFor the NC, supplement was corn 1%; for the PC, supplement was diluted form of carbadox 50 mg/kg with corn to bring it to 1%; and for the PBO, supplement was (PEP1000-1[®], Biomin Inc., San Antonio, TX) 0.2%+(Biotronic[®], Biomin Inc., San Antonio, TX) 0.4% for phase 1 and (PEP1000-1[®], Biomin Inc., San Antonio, TX) 0.1%+(Biotronic[®], Biomin Inc., San Antonio, TX) 0.2% for phase 2 with corn to bring it to 1%.

^eAnalyzed values averaged 23.3, and 21.6% for phase 1, and 2, respectively.

0.2% respectively for phase 1, and 0.2% and 0.1% respectively for phase 2. Each treatment had eight replicates with eight pigs (4 gilts and 4 barrows) per pen. The pigs were fed the assigned experimental diets (Table 2) for 5 wks based on a two phase feeding program (phase 1 for 2 wk; phase 2 for 3 wk). The phase 1 diet contained 3.24 Mcal/kg ME and 1.47% total lysine, and the phase 2 diet contained 3.34 Mcal/kg ME and 1.21% total lysine. All the pigs had free access to feed and water during the entire experimental period. Each pen (1.5×2.1 m) had slatted polypropylene plastic panel flooring. The room temperature was kept at 30°C, and the photoperiod consisted of 10 h of artificial light and 14 h of darkness. The body weights were

measured at the beginning of the experiment and at the end of each phase. Feed intake was measured at the end of each phase. Incidence of Diarrhea was measured during phase 1 as described in Experiment 1.

Protocols for Experiment 1 and Experiment 2 were approved by Texas Tech University Animal Care and Use Committee.

Statistical analysis

All data were analyzed as a completely randomized design using the General Linear Model (PROC GLM) procedure in SAS/STAT[®] software (SAS Inst. Inc., Cary, NC). Treatments were the main effect of the model and the pen was the experimental unit. Least square means, probability of difference, and standard errors were used to evaluate the differences among the treatment groups. Mean differences were considered significant at $p < 0.05$.

RESULTS

Experiment 1

In phase 1, the ADG of the PC was higher ($p < 0.05$) than the NC and the PB. However, there was no difference ($p > 0.05$) in the ADG between the PB and the NC. The ADFI of the PC was higher ($p < 0.05$) than the NC and the PB, and there was no difference ($p > 0.05$) in the ADFI between the PB and the NC. There was no difference ($p > 0.05$) between the treatment groups in the gain:feed ratio. The PC showed a higher ($p < 0.05$) diarrhea score than the NC and the PB, and there was no difference ($p > 0.05$) in the diarrhea score between the PB and the NC. In phase 2, there was no difference ($p > 0.05$) in the ADG among the treatment groups. The ADFI was higher ($p < 0.05$) for the PC than the NC. However, the PB did not differ ($p > 0.05$) from the NC and the PC in the ADFI. The gain:feed ratio was lower ($p < 0.05$) for the PC and the PB than the NC. The diarrhea score was higher ($p < 0.05$) for the PC than the NC. However, the PB did not differ ($p > 0.05$) from the NC and the PC in the diarrhea score. In phase 3, the ADG was greater ($p < 0.05$) for the PC than the NC. However, the PB did not differ ($p > 0.05$) from the NC and the PC in the ADG. There was no difference ($p > 0.05$) in the ADFI, gain:feed, and the diarrhea score among the treatment groups. Overall, the ADG was greater ($p < 0.05$) for the PC than the NC. However, ADG of the PB did not differ ($p > 0.05$) from that of the NC and the PC. There was no difference ($p > 0.05$) in ADFI, and gain:feed ratio among the treatment groups. The diarrhea score of the PC was higher ($p < 0.05$) than the PB and the NC. However, there was no difference ($p > 0.05$) in the diarrhea score between the PB and the NC (Table 3).

Experiment 2

In phase 1, phase 2, and overall there was no difference

Table 3. Growth performance and diarrhea score of nursery pigs (Experiment 1)

Treatment	NC ^a	PC ^b	PB ^c	SEM
Average daily gain (kg)				
Phase 1	0.187 ^d	0.255 ^e	0.199 ^d	0.016
Phase 2	0.399	0.411	0.382	0.014
Phase 3	0.516 ^d	0.584 ^e	0.563 ^{de}	0.018
Overall	0.381 ^d	0.429 ^e	0.395 ^{de}	0.012
Average daily feed intake (kg)				
Phase 1	0.258 ^d	0.345 ^e	0.279 ^d	0.022
Phase 2	0.587 ^d	0.700 ^e	0.631 ^{de}	0.023
Phase 3	1.101	1.079	1.055	0.041
Overall	0.679	0.736	0.684	0.023
Gain:feed ratio				
Phase 1	0.720	0.751	0.705	0.018
Phase 2	0.681 ^d	0.587 ^e	0.605 ^e	0.014
Phase 3	0.478	0.545	0.540	0.019
Overall	0.567	0.586	0.580	0.013
Diarrhea score ^f				
Phase 1	1.33 ^d	1.98 ^e	1.46 ^d	0.12
Phase 2	1.75 ^d	2.01 ^e	1.83 ^{de}	0.05
Phase 3	2.20	2.28	2.11	0.04
Overall	1.76 ^d	2.09 ^e	1.80 ^d	0.05

^a Negative control without any supplement (n = 6).

^b Positive control with antibiotics (carbadox, 50 mg/kg) (n = 6).

^c Phytobiotic treatment with phytobiotics (PEP1000-1[®], Biomin Inc, San Antonio, TX, 0.1%) (n = 6).

^{d, e} Means lacking common superscript differ (p<0.05).

^f Score 1: 'watery'; 3: 'normal'; and 5: 'hard'.

(p>0.05) in the ADG, ADFI, and the gain:feed ratio among the treatment groups. The diarrhea score was higher (p<0.05) for the PC than the NC, however, that of the PB was not different (p>0.05) from the NC and the PC (Table 4).

DISCUSSION

There has been growing concerns about finding alternatives to antibiotics, primarily due to the developing antibiotic resistance in several bacterial strains such as *E.coli* (Doyle, 2001), *campylobacter* (Beilei et al., 2003), *enterococci* (Aarestrup et al., 2002), and due to decreased growth performance in animals after antibiotic feed withdrawal (Krause et al., 2005). Essential oils (Lee et al., 2004b) and organic acids (Partanen and Mroz, 1999) have been studied to understand their growth enhancing effects in the nursery pigs and broiler chickens.

In the current study, the growth of the pigs in the PC was better than the NC in the Exp. 1 whereas it was not different in the Exp. 2. The observed growth response in Exp. 1 was most likely due the increased feed intake in the PC than the NC. It has been previously reported that carbadox stimulates growth in the nursery pigs by increasing the nutrient utilization in their gastrointestinal tract (Yen and Pond, 1990; Cromwell, 2001). However, no improvement by the use of carbadox observed in the Exp. 2

Table 4. Growth performance and diarrhea score of nursery pigs (Experiment 2)

Treatment	NC ^a	PC ^b	PBO ^c	SEM
Average daily gain (kg)				
Phase 1	0.158	0.165	0.153	0.007
Phase 2	0.372	0.381	0.364	0.012
Overall	0.286	0.294	0.280	0.008
Average daily feed intake (kg)				
Phase 1	0.225	0.226	0.221	0.006
Phase 2	0.562	0.588	0.588	0.017
Overall	0.427	0.443	0.441	0.012
Gain:feed ratio				
Phase 1	0.696	0.727	0.688	0.019
Phase 2	0.664	0.649	0.620	0.012
Overall	0.671	0.665	0.636	0.011
Diarrhea score ^d				
Phase 1	3.20 ^e	4.00 ^f	3.43 ^{ef}	0.15

^a Negative control without any supplement (n = 8).

^b Positive control with antibiotics (carbadox, 50 mg/kg) (n = 8).

^c Phytobiotic+organic acid treatment with both phytobiotics (PEP1000 - 1[®], Biomin Inc, San Antonio, TX, at 0.2 and 0.1% for the phase 1 and 2, respectively) and organic acids (Biotronic[®], Biomin Inc., at 0.4 and 0.2% for the phase 1 and 2, respectively) (n = 8).

^d Score 1: 'watery'; 3: 'normal'; and 5: 'hard'.

^{e, f} Means lacking common superscript differ (p<0.05).

could be due to environmental conditions at the research facility where the disease causing and other stress factors were possibly kept low. Numerous studies have also indicated that the growth enhancing effects of the antibiotics in the nursery pigs would be twice greater in farm conditions than in the research facilities with well controlled environments (Cromwell, 2000, 2001). Similarly, Lee et al. (2004b) reported that the supplemental effects of essential oils and antimicrobials on the growth of broiler chickens could be higher when they are raised in disease challenged conditions.

No significant growth responses were reported (Lee et al., 2003; Zhang et al., 2005) in broiler chickens when they were fed the diets supplemented with essential oils alone or with a combination of essential oils and organic acids as compared to those without any antibiotics. Furthermore, growth performances were not different between the nursery pigs fed diets supplemented with either δ -aminolevulinic acid or carbadox and those fed diets without any supplementation (Mateo et al., 2006). van Lunen et al. (2003) had reported no differences in growth performances between the nursery pigs fed diets supplemented with tylosin phosphate and those fed diets without any antibiotics. All these experiments were conducted in an environmentally controlled research facility without any disease challenge. This indicates that nursery environment might play a significant role in the response of pigs towards testing the efficacy of supplemental effects of essential oils and antimicrobials.

A possible interaction between the supplemented

essential oils and the organic acids has been reported by Manzanilla et al. (2004) in the nursery pigs. Partanen and Mroz (1999) had suggested that the decreased gastric emptying rate is a possible mechanism to improve the protein digestion in the gastrointestinal tract, and is associated with the effects of the organic acids to lower the pH in the pyloric region. The receptors for the herbal extracts are present in the same duodenal receptors which cause a reduction in the gastric emptying rate, and therefore, the supplementation of these herbal extracts may prevent the duodenal receptors to reduce the acid induced gastric emptying rate (Manzanilla et al., 2004). Thus, another potential reason for improved growth performance observed in the current study could be due to beneficial interactions between essential oils and organic acids. However, the gastric emptying rate was not measured in this study.

Results from the current study show that the PC had a higher diarrhea score than the NC in both the experiments indicating that the carbadox was effective in reducing the diarrhea score of the nursery pigs. Numerous studies have also shown that essential oils can decrease the pathogenic bacterial load (Dorman and Deans, 2000) in the gastrointestinal tract of the pigs (Manzanilla et al., 2004). The current study shows that there was no significant difference in the diarrhea score between the PB or the PBO and the other treatment groups in both the experiments. A probable explanation for this result would involve insignificant changes of the pH and a less alteration of the microflora concentration in the gastrointestinal tract of the nursery pigs. However, the pH of the gastrointestinal tract and the coliform count from the gut were not measured in the current study. The supplementation of organic acids, especially, lactic acid in the weanling pig diets would decrease the gastric pH and modify their gut microflora (Scipioni et al., 1978; Tsiloyiannis et al., 2001) and thus reduce the incidence of scours (Thomlinson and Lawrence, 1981; Mathew et al., 1991). Even though several studies (Bolduan et al., 1988; Eidelsburger et al., 1992; Radcliffe et al., 1998) have shown a decrease in gastric pH by the supplementation of organic acids in weanling pig diets, many other studies have also shown an insignificant decrease in gastric pH (Scipioni et al., 1978; Burnell et al., 1988; Risley et al., 1992; Gabert and Sauer, 1995). Partanen and Mroz (1999) had suggested that these differences in results could be due to either a difficulty in sampling from the stomach of the weanling pigs or due to variations in the sampling time. Results from the current study agree with Risley et al. (1992) and Clark and Batterham (1989) where no significant effect of supplemental organic acids on the occurrence of scours were observed in the weanling pigs.

Collectively, this study indicates that the supplementation of phytobiotics in the nursery pig diets

with or without organic acids was not beneficial when it was measured in research facility without sufficient disease challenges to the pigs. Nursery pigs fed diets supplemented with phytobiotics or a combination of phytobiotics and organic acids, however, performed similar to the nursery pigs fed diets supplemented with antibiotic supplementation as well. More studies are needed to find if supplementation of phytobiotics and organic acids in the nursery pig diets can successfully replace the use of antibiotics in stressed and disease challenged conditions.

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