Asian-Aust. J. Anim. Sci.
Vol. 22, No. 2 : 267 - 274
February 2009

www.ajas.info

Effect of Wood Vinegar on the Performance, Nutrient Digestibility and Intestinal Microflora in Weanling Pigs

J. Y. Choi[1], P. L. Shinde[1], I. K. Kwon[2], Y. H. Song[1] and B. J. Chae[1,]*

College of Animal Resources Science, Kangwon National University, Chuncheon 200-701, Korea

ABSTRACT : Two experiments were conducted to investigate the feeding value of wood vinegar in weanling pigs. In Experiment 1, weanling pigs (n = 224; Landrace×Yorkshire×Duroc, 21±3 d-old, initial BW 6.12±0.10 kg) were assigned to four dietary treatments. Different levels of wood vinegar were added to the diets as dietary treatments (0, 0.1, 0.2 and 0.3%). Each treatment comprised 4 replicates with 14 piglets in each. Experimental feeding was conducted for 28 d in two phases (phase I, d 0 to 14 and phase II, d 15 to 28). Feeding of wood vinegar linearly ($p<0.05$) improved the phase I, phase II and overall ADG and increased (linear, $p<0.05$) the overall and phase II ADFI. Linear improvements in the apparent fecal digestibility of dry matter ($p = 0.013$), gross energy ($p = 0.019$) and crude protein ($p = 0.033$) were observed as the level of wood vinegar was increased in the diet of pigs. Experiment 2 was conducted to compare dietary wood vinegar with commonly used growth promoters, organic acid (mixture of 21% phosphoric acid, 3.25% propionic acid, 2.8% formic acid, 10% calcium formate and 5% calcium propionate) and antibiotic (aparamycin). A total of 288 weanling piglets (Landrace×Yorkshire×Duroc, 22±2 d-old, initial BW 6.62±0.31 kg) were assigned to four treatments with four replicates (18 piglets/pen) for 28 days and fed in 2 phases: phase I, d 0 to 14 and phase II, d 15 to 28. The dietary treatments were control (corn-soybean meal basal diet without antibiotics) and diets containing 0.2% antibiotic, 0.2% organic acid and 0.2% wood vinegar. Pigs fed antibiotic showed higher ($p<0.001$) ADG and better feed efficiency followed by pigs fed wood vinegar and organic acid diets while those fed the control diet had lowest ADG and poorest feed efficiency. The overall and phase I ADFI was highest ($p<0.001$) in pigs fed wood vinegar and lowest in pigs fed the control diet. Apparent fecal digestibility of dry matter, gross energy and crude protein was significantly higher ($p<0.05$) in pigs fed the antibiotic diet when compared with pigs fed the control but comparable among pigs fed antibiotic, organic acid and wood vinegar diets. Higher populations of *Lactobacillus* ($p = 0.004$) were noted in the ileum of pigs fed the wood vinegar diet, while the population of coliforms in the ileum and cecum was higher ($p<0.001$) in pigs fed the control diet when compared with pigs fed antibiotic, organic acid or wood vinegar diets. These results indicated that wood vinegar could improve the performance of weanling pigs by improving the nutrient digestibility and reducing harmful intestinal coliforms; moreover performance of pigs fed wood vinegar was superior to those fed organic acid. (**Key Words :** Wood Vinegar, Performance, Nutrient Digestibility, Intestinal Microflora, Weanling Pigs)

INTRODUCTION

Since their discovery, antibiotics have been used as therapeutic and growth-promoting agents and this has lead to improvements in the performance of animals (Doyle, 2001). However, the development of bacterial resistance (Ogawara, 1981; Russell, 1991) and the problem of antibiotic residues in animal products have lead to regulatory pressure and public perception of the need to ban antibiotics from animal feeds (Han, 2007). Thus it is necessary to identify alternative to antibiotics to maintain growth performance (Bae et al., 1999). Organic acids, probiotics, prebiotics, and phytogenic substances have been tested as possible alternatives to replace antibiotics (Kamel, 2001; An et al., 2008). The addition of organic acids like citric, fumaric, formic and propionic acid to the diets of pigs is one of the most widely used alternative for antibiotics and has been reported to improve their performance (Kirchgessner et al., 1997; Partanen and Mroz, 1999). Their effects have been related to reduction in the growth of coliform bacteria (Partanen, 2001), known to be involved in

* Corresponding Author: Byung-Jo Chae. Tel: +82-33-250-8616, Fax: +82-33-244-4946, E-mail: bjchae@kangwon.ac.kr

[1] Department of Animal Resources Science, College of Animal Resources Science, Kangwon National University, Chuncheon 200-701, Korea.

[2] Department of Animal Product and Food Science, College of Animal Resources Science, Kangwon National University, Chuncheon 200-701, Korea.

Received June 24, 2008; Accepted September 22, 2008

Table 1. Ingredient and chemical composition of the basal diets used for feeding trial (Exp. 1)[1]

Ingredients (%)	Phase I (d 0 to 14)	Phase II (d 15 to 28)
Corn	42.73	51.39
Soybean meal (48%)	8.24	14.53
Whey powder	12.30	15.38
Lactose	12.00	-
Bakery byproduct	5.00	6.00
Spray dried porcine plasma	6.00	4.00
Soy protein concentrate	4.00	-
Fish meal	5.00	4.00
Soy oil	2.00	2.00
L-lysine HCl (78%)	0.43	0.37
DL-methionine (100%)	0.12	0.08
MCP	0.59	0.61
Limestone	0.84	0.89
Salt	0.20	0.20
Choline chloride (25%)	0.05	0.05
Vitamin premix[2]	0.30	0.30
Mineral premix[3]	0.20	0.20
Calculated chemical composition (%)		
ME (kcal/kg)	3,380	3,360
Crude protein	21.00	20.00
Calcium	0.82	0.82
Available phosphorus	0.4	0.4
Lysine	1.60	1.44
Methionine+cystine	0.82	0.76

[1] Wood vinegar was added to the basal diets at 0.0, 0.1, 0.2 and 0.3% as dietary treatments.
[2] Supplied per kg diet: 9,600 IU vitamin A, 1,800 IU vitamin D_3, 24 mg vitamin E, 1.5 mg vitamin B_1, 12 mg vitamin B_2, 2.4 mg vitamin B_6, 0.045 mg vitamin B_{12}, 1.5 mg vitamin K_3, 24 mg pantothenic acid, 45 mg niacin, 0.09 mg biotin, 0.75 mg folic acid.
[3] Supplied per kg diet: 162 mg Fe, 96 mg Cu, 72 mg Zn, 46.49 mg Mn, 0.9 mg I, 0.9 mg Co, 0.3 mg Se.

digestive disorders.

Wood vinegar is the product obtained by distilling the smoke arising from burning wood and it is a complex mixture of 80-90% water, and 10-20% organic compounds. In addition wood vinegar contains several phenolic compounds such as guaiacol and cresol, and organic acids like acetic, formic and propionic acids. It can be refined by fractional distillation to produce a food-grade product (Sakaguchi et al., 2007). Wood vinegar is being used to remove the odor of landfill site leachate (Huh et al., 1999) and ammonia in animal farms (Park et al., 2003). Wood vinegar has been shown to induce a significant increase in egg production and improvements in the feed efficiency of laying hens (Sakaida et al., 1987; Li and Ryu, 2001) and to enhance intestinal calcium absorption in rats (Kishi et al., 1999). Nonetheless, there are fewer reports on the effect of wood vinegar in pigs and thus further scientific investigations are needed.

Therefore, we conducted this study to determine the optimal inclusion level of wood vinegar (Exp. 1) and to comparatively evaluate the use of wood vinegar as an alternative to antibiotic in the diet of weanling pigs (Exp. 2).

MATERIALS AND METHODS

Experimental design, animals and their diets

In Exp. 1, 224 crossbred weanling pigs (Landrace×Yorkshire×Duroc; average BW of 6.12±0.10 kg; 21±3 d of age) were used to investigate the effect of adding different levels of wood vinegar in the diet on performance and nutrient digestibility. Pigs were randomly allotted to four treatments based on body weight and gender (male: female, 1:1). Each treatment had 4 replications with 14 pigs per replicate. Experimental feeding of mash diets was done for 28 days in two phases: phase I (d 0 to 14) and phase II (d 15 to 28). The ingredient and chemical composition of basal diets for both phases is presented in Table 1. Wood vinegar was added to the basal diets at 0.0, 0.1, 0.2 and 0.3% as dietary treatments. All nutrients met or exceeded NRC (1998) requirements. The diets of 0.0, 0.1, 0.2 and 0.3% wood vinegar treatments had 21.25, 21.18, 21.23 and 21.22% crude protein and 1.66, 1.62, 1.65 and 1.67% lysine in phase I diets, and 19.2, 19.5, 19.3 and 19.2% crude protein and 1.35, 1.35, 1.34 and 1.36% lysine in phase II diets, respectively.

In Exp. 2 comparisons were made among different growth promoters (antibiotic, organic acid and wood vinegar) added to the diets of piglets by studying their growth performance, apparent fecal nutrient and ileal amino acid digestibility and intestinal microflora. Weanling pigs (n = 224; Landrace×Yorkshire×Duroc; average body weight of 6.62±0.31 kg; 21±3 d of age) were randomly allotted to four treatments based on body weight and gender (male: female, 1:1). Each treatment had 4 replications with 14 pigs per replicate. The dietary treatments were control (corn-soybean meal basal diet without antibiotics) and basal diets added with 0.2% antibiotic (aparamycin), 0.2% organic acid and 0.2% wood vinegar. The ingredient and chemical composition of experimental diets is presented in Table 2. All nutrients met or exceeded NRC (1998) requirements. The experimental diets in mash form were fed for 28 days in 2 phases: phase I (d 0 to 14) and phase II (d 15 to 28). The analyzed composition of control, antibiotic, organic acid and wood vinegar diets used during phase I showed 20.83, 20.78, 20.82 and 20.80% crude protein and 1.53, 1.50, 1.54 and 1.51% lysine, respectively; and phase II diets had 19.98, 20.00, 19.96 and 19.91 % crude protein and 1.30, 1.31, 1.29 and 1.28% lysine, respectively.

In both experiments, pigs were housed in partially slotted and concrete floor pens of 1.90×2.54 m size with a self feeder and nipple drinker to allow *ad libitum* access to the feed and water. The experiments underwent proper ethical standards and were approved by the Animal Care and Use Committee of Kangwon National University.

Table 2. Ingredient and chemical composition of the basal diets used for feeding trial (Exp. 2)[1]

Ingredients (%)	Phase I (d 0 to 14)	Phase II (d 15 to 28)
Corn	51.42	49.38
Soybean meal (48%)	26.39	34.72
Whey powder	13.00	-
Bakery byproduct	-	6.00
Fish meal	3.61	-
Rice bran	-	4.00
Soy oil	2.88	2.80
L-lysine HCl (78%)	0.37	0.25
DL-methionine (100%)	0.08	0.17
MCP	0.61	-
TCP	-	1.75
Limestone	0.89	0.31
Salt	0.20	0.25
Choline chloride (25%)	0.05	0.05
Vitamin premix[2]	0.30	0.12
Mineral premix[3]	0.20	0.20
Calculated chemical composition (%)		
ME (kcal/kg)	3,360	3,380
Crude protein	20.00	20.50
Calcium	0.82	0.80
Available phosphorus	0.39	0.42
Lysine	1.43	1.35
Methionine+cystine	0.77	0.75

[1] Dietary treatments were: Control (basal diet without antibiotic), Antibiotic (basal diet added with 0.2% aparamycin), organic acid (basal diet added with 0.2% organic acid), Wood vinegar (basal diet added with 0.2% wood vinegar). Aparamycin, organic acid and wood vinegar were added at 0.2% of diet at the expense of corn.

[2] Supplied per kg diet: 9,600 IU vitamin A, 1,800 IU vitamin D_3, 24 mg vitamin E, 1.5 mg vitamin B_1, 12 mg vitamin B_2, 2.4 mg vitamin B_6, 0.045 mg vitamin B_{12}, 1.5 mg vitamin K_3, 24 mg pantothenic acid, 45 mg niacin, 0.09 mg biotin, 0.75 mg folic acid.

[3] Supplied per kg diet: 162 mg Fe, 96 mg Cu, 72 mg Zn, 46.49 mg Mn, 0.9 mg I, 0.9 mg Co, 0.3 mg Se.

Wood vinegar (Vital Force L®) used in the present study was obtained from Kangwon Mogcho Industrial Co. Ltd., Yeongwol-gun, Gangwon-do, Korea. Oak (*Quercus acutissima*) chips of Yeongwol-gun were burned at 500 to 700°C then the smoke was cooled by a water cooling system and distilled. The crude vinegar was stored for more than 6 months and then the supernatant was collected, purified and used. The chemical composition of wood vinegar as analyzed by the Korean Forest Research Institute (KFRI, 2002) showed 3.99% acetic acid and 10.89% propionic acid. The organic acid was obtained from Korean Milk Product Inc. (Pyeongtaek, Gyeonggi-do, Korea), and was composed of 21% phosphoric acid, 3.25% propionic acid, 2.8% formic acid, 10% calcium formate and 5% calcium propionate.

Measurements and sampling

In both experiments the individual pigs were weighed at the end of each phase and the feed offered and refusals from each pen were noted to calculate average daily gain (ADG), average daily feed intake (ADFI) and feed efficiency (F/G).

In order to study the apparent fecal nutrient digestibility, all pigs were fed their originally assigned diets mixed with chromic oxide (0.25%) from d 21 to 28 of phase II in Exp. 1 and d 7 to 14 of phase I in Exp. 2. The fecal samples were collected from d 25 to 28 and d 11 to 14 in Exp. 1 and 2, respectively. The fecal samples were freshly collected from each pen, then dried in a forced-air drying oven at 60°C for 72 h and ground with a 1 mm mesh Wiley mill for chemical analysis. Moreover, in Exp. 2, at the end of phase I, 2 representative pigs from each replicate (8 pigs per treatment) reflecting average body weights were sacrificed by electrocution. Immediately after slaughter, their digestive tract was excised. The ileal (about 20 cm from the ileo-ceacal junction) chyme was collected in a sterilized plastic bottle, kept on ice and then brought to the laboratory and freeze-dried until analyzed for apparent ileal amino acid digestibility. Also chyme from the ileum (for total anaerobic bacteria, *Lactobacillus spp*. and Coliforms) and cecum (for total anaerobic bacteria, *Bifidobacterium spp*. and Coliforms) was aseptically collected and a weighed amount was suspended in sterile phosphate buffer solution containing cysteine (0.05% wt/vol), and used for enumeration of bacteria populations.

Chemical analysis

Analysis of the experimental diets and excreta was done according to the methods of AOAC (1990). Gross energy was measured by a bomb calorimeter (Model 1216, Parr Instrument Co., Molin. IL), and chromium was determined with an automated spectrophotometer (Shimadzu, Japan), according to the procedure of Fenton and Fenton (1979). Following acid hydrolysis in 6 N HCl at 105°C for 24 h, amino acid concentrations were analyzed by HPLC (Waters 486, USA). Sulfur-containing amino acids were analyzed after cold performic acid oxidation (Moore, 1963) overnight with subsequent hydrolysis.

Analysis of microbial populations

The microbial assay was carried out by the procedure suggested by Torrallardona et al. (2003). One gram of mixed intestinal contents was diluted with 9 ml of Buffer-fields phosphate buffer dilution solution, followed by further serial dilutions in Buffer-fields phosphate buffer dilution solution. The microbial groups analyzed were total anaerobic bacteria (Tryptic soy agar), *Bifidobacterium spp.* (MRS agar), *Lactobacillus spp*. (MRS agar) and Coliform bacteria (Voilet red bile agar). Duplicate plates were inoculated with 0.1 ml sample and incubated. The anaerobic conditions were generated using an anaerobic jar with a gas generator envelope (GasPak Plus, disposable H_2 and CO_2 generating system with palladium catalyst). The final

Table 3. Effect of dietary wood vinegar levels on growth performance of weanling pigs (Exp. 1)

Item	Wood vinegar (%)				SEM[1]	p-value[2]	
	0.0	0.1	0.2	0.3		Linear	Quadratic
Phase I (d 0 to 14)							
ADG (g)	282	286	293	299	3.17	0.044	0.885
ADFI (g)	420	419	422	424	1.25	0.214	0.507
F/G	1.50	1.46	1.44	1.42	0.02	0.102	0.856
Phase II (d 15 to 28)							
ADG (g)	417	422	434	441	4.31	0.032	0.864
ADFI (g)	657	659	663	667	1.63	0.022	0.734
F/G	1.58	1.56	1.53	1.51	0.02	0.140	1.000
Overall (d 0 to 28)							
ADG (g)	349	354	363	370	3.75	0.035	0.873
ADFI (g)	529	541	542	543	2.21	0.016	0.117
F/G	1.52	1.53	1.49	1.47	0.02	0.201	0.566

[1] Standard error of the means.
[2] Linear and quadratic effect of increasing concentration of wood vinegar (0.0 to 0.3%) in the diet.

Table 4. Effect of dietary wood vinegar levels on the apparent fecal nutrient digestibility of weanling pigs (Exp. 1, d 28)

Item	Wood vinegar (%)				SEM[1]	p-value[2]	
	0.0	0.1	0.2	0.3		Linear	Quadratic
Dry matter	83.38	83.67	84.78	85.24	0.30	0.013	0.871
Gross energy	83.19	83.31	84.15	84.83	0.27	0.019	0.568
Crude protein	81.64	81.82	82.38	83.40	0.30	0.033	0.460

[1] Standard error of the means.
[2] Linear and quadratic effect of increasing concentration of wood vinegar (0.0 to 0.3%) in the diet.

anaerobic atmosphere consisted of 6.5 to 7.5% CO_2, 25 to 35% H_2, with the balance being N_2. The bacterial populations were transformed (log_{10}) before statistical analysis and expressed as log_{10} cfu/g of intestinal contents.

Statistical analysis

Data generated was subjected to statistical analysis using the SAS statistical software package. In Exp. 1, linear and quadratic contrasts were used to compare effects of increasing dietary wood vinegar levels. In Exp. 2, data was analyzed by ANOVA and when significant differences were noted, the means were separated by using LSD's multiple range test. The replicate was the experimental unit of analysis in both experiments. The level of significance was accepted at p<0.05.

RESULTS

Experiment 1

During phase I, linear (p = 0.044) improvements in the ADG with increasing amount of wood vinegar in the diet were observed, but there were no differences (p>0.05) in ADFI and F/G (Table 3). In phase II, ADG (p = 0.032) and ADFI (p = 0.022) increased linearly as dietary wood vinegar level was increased, although the F/G (p>0.05) remained unaffected. Consequently, for the overall period, ADG (linear, p = 0.035) and ADFI (linear, p = 0.016) were higher in pigs fed with increasing levels of wood vinegar. Linear improvements in the apparent fecal digestibility of dry matter (p = 0.013), gross energy (p = 0.019) and crude protein (p = 0.033) with an increase in dietary wood vinegar level were recorded (Table 4).

Experiment 2

Pigs fed with the antibiotic diet showed the highest (p<0.001) ADG during phase I, II and the overall period, followed by pigs fed wood vinegar and organic acid diets, while pigs fed the control diet had the lowest ADG throughout the study (Table 5). The ADFI during phase I and the overall period was highest (p<0.001) in pigs fed the wood vinegar diet and lowest in pigs fed the control diet. Pigs fed the antibiotic diet showed better feed efficiency (p<0.001) throughout the feeding period, while pigs fed the wood vinegar diet had better feed efficiency than pigs fed organic acid and control diets. The apparent fecal digestibility of dry matter was higher (p = 0.049) in pigs fed the antibiotic diet when compared with pigs fed the control diet, while the digestibility of gross energy was significantly higher (p = 0.045) in pigs fed antibiotic, organic acid and wood vinegar diets than pigs fed the control diet (Table 6). The digestibility of crude protein was higher (p = 0.014) in pigs fed antibiotic and wood vinegar diets than those fed the control diet. But no differences in the apparent nutrient digestibility were observed when compared among pigs fed with antibiotic, organic acid and wood vinegar diets.

In general pigs fed the control diet had the lowest apparent ileal digestibility of all individual amino acids

Table 5. Effect of dietary treatments on growth performance of weanling pigs (Exp. 2)

Item	Treatments[1]				SEM[2]	p-value
	Control	Antibiotic	Organic acid	Wood vinegar		
Phase I (d 0 to 14)						
ADG (g)	298[d]	349[a]	315[c]	339[b]	5.27	<0.001
ADFI (g)	459[d]	466[c]	472[b]	489[a]	2.85	<0.001
F/G	1.54[a]	1.34[d]	1.50[b]	1.44[c]	0.02	<0.001
Phase II (d 15 to 28)						
ADG (g)	397[d]	423[a]	406[c]	413[b]	2.55	<0.001
ADFI (g)	672	678	680	677	1.32	0.240
F/G	1.69[a]	1.60[c]	1.68[a]	1.64[b]	0.01	<0.001
Overall (d 0 to 28)						
ADG (g)	348[d]	388[a]	361[c]	377[b]	3.96	<0.001
ADFI (g)	557[c]	559[bc]	563[b]	570[a]	1.43	<0.001
F/G	1.60[a]	1.44[d]	1.56[b]	1.51[c]	0.02	<0.001

[a, b, c, d] Values with different superscripts in the same row significantly differ (p<0.05).
[1] Control (basal diet without antibiotic), antibiotic (basal diet added with 0.2% aparamycin), organic acid (basal diet added with 0.2% organic acid), wood vinegar (basal diet added with 0.2% wood vinegar).
[2] Standard error of the means.

Table 6. Effect of dietary treatments on the apparent nutrient digestibility in weanling pigs (Exp. 2, d 14)

Item	Treatments[1]				SEM[2]	p-value
	Control	Antibiotic	Organic acid	Wood vinegar		
At 14 day						
Dry matter	81.67[b]	84.63[a]	82.06[ab]	83.63[ab]	0.49	0.049
Gross energy	82.46[b]	84.31[a]	84.13[a]	84.14[a]	0.28	0.045
Crude protein	81.11[b]	83.22[a]	82.28[ab]	82.62[a]	0.26	0.014

[a, b] Values with different superscripts in the same row significantly differ (p<0.05).
[1] Control (basal diet without antibiotic), antibiotic (basal diet added with 0.2% aparamycin), organic acid (basal diet added with 0.2% organic acid), wood vinegar (basal diet added with 0.2% wood vinegar.
[2] Standard error of the means.

Table 7. Effect of dietary treatments on the apparent ileal digestibility of amino acids in weanling pigs (Exp. 2, d 14)

Item	Treatments[1]				SEM[2]	p-value
	Control	Antibiotic	Organic acid	Wood vinegar		
Essential amino acids						
Arginine	77.26[c]	80.71[a]	79.78[ab]	77.67[bc]	0.51	0.023
Histidine	69.96[b]	71.97[ab]	74.89[a]	74.38[a]	0.73	0.037
Isoleucine	69.96[c]	76.90[a]	76.92[a]	73.65[b]	0.80	<0.001
Leucine	72.67[c]	76.70[b]	76.66[b]	79.45[a]	0.67	<0.001
Lysine	71.87[b]	79.25[a]	76.45[b]	78.40[ab]	0.80	<0.001
Methionine	74.07[c]	77.10[b]	78.63[b]	81.66[a]	0.79	<0.001
Phenylalanine	73.41[c]	76.79[b]	80.02[a]	79.71[a]	0.75	<0.001
Threonine	68.35[c]	72.6[b]	73.46[b]	75.60[a]	0.74	<0.001
Valine	69.12[b]	73.28[a]	74.74[a]	74.67[a]	0.67	<0.001
Non-essential amino acids						
Alanine	73.06[d]	75.82[c]	77.00[b]	79.53[a]	0.62	<0.001
Aspartic acid	75.44[b]	77.09[b]	79.98[a]	81.00[a]	0.63	<0.001
Cystine	61.92[c]	63.44[bc]	65.65[ab]	67.41[a]	0.65	<0.001
Glutamic acid	76.12[c]	78.74[b]	80.32[ab]	81.87[a]	0.62	<0.001
Glycine	63.07[b]	68.95[a]	68.54[a]	71.15[a]	0.88	<0.001
Proline	72.03[b]	77.56[a]	75.30[a]	75.58[a]	0.62	<0.001
Serine	73.39[b]	75.16[b]	78.64[a]	78.00[a]	0.62	0.019
Tyrosine	69.72[b]	72.38[a]	71.67[ab]	73.67[a]	0.50	<0.001

[a, b, c, d] Values with different superscripts in the same row significantly differ (p<0.05).
[1] Control (basal diet without antibiotic), antibiotic (basal diet added with 0.2% aparamycin), organic acid (basal diet added with 0.2% organic acid), Wood vinegar (basal diet added with 0.2% wood vinegar.
[2] Standard error of the means.

Table 8. Effect of dietary treatments on intestinal microbial populations (Exp. 2, d 14)

Microbial population (log_{10} cfu/g)	Treatments[1]				SEM[2]	p-value
	Control	Antibiotic	Organic acid	Wood vinegar		
Ileum						
Total anaerobic bacteria	8.55	7.76	7.81	7.90	0.21	0.566
Lactobacillus spp.	7.27[c]	7.43[b]	7.53[ab]	7.86[a]	0.09	0.004
Coliform bacteria	5.85[a]	3.85[b]	3.90[b]	3.82[b]	0.23	<0.001
Cecum						
Total anaerobic bacteria	8.77	8.70	8.78	8.74	0.06	0.969
Bifidobacterium spp.	8.69	8.66	8.64	8.68	0.14	0.999
Coliform bacteria	7.73[a]	5.89[b]	5.86[b]	5.95[b]	0.21	<0.001

[a, b, c] Values with different superscripts in the same row significantly differ (p<0.05).

[1] Control (basal diet without antibiotic), antibiotic (basal diet added with 0.2% aparamycin), organic acid (basal diet added with 0.2% organic acid), Wood vinegar (basal diet added with 0.2% wood vinegar.

[2] Standard error of the means.

(Table 7). Ileal digestibility of arginine was lower (p = 0.023) in pigs fed wood vinegar when compared with pigs fed the antibiotic diet while isoleucine digestibility of pigs fed the wood vinegar diet was lower than pigs fed antibiotic and organic acid diets. The apparent ileal digestibility of leucine, methionine, threonine and alanine was higher (p<0.001) in pigs fed wood vinegar when compared with pigs fed antibiotic and organic acid diets.

The population of total anaerobic bacteria both in the ileum and cecum were comparable among the dietary treatments (Table 8). Pigs fed the wood vinegar diet had higher (p = 0.004) *Lactobacillus* population in the ileal contents when compared with pigs fed antibiotic and control diets, while the population of *Lactobacillus* was comparable among pigs fed wood vinegar and organic acid diets. The population of coliform bacteria in the ileal and cecal contents was higher (p<0.001) in pigs fed the control diet than pigs fed antibiotic, organic acid and wood vinegar diets, while the *Bifidobacterium* were not affected by the dietary treatments.

DISCUSSION

There has been growing concern about the negative effect of the use of antibiotics in pig diets (Monroe and Polk, 2000). Therefore, numerous studies are being conducted to find alternatives to replace antibiotics in diets for young pigs. Organic acids are currently considered as one of the attractive additives for weanling pigs (Jensen, 1998; Partanen and Mroz, 1999) to replace antibiotics in feed. Organic acids added to diets can maintain a low pH of gastric contents and subsequently modify or decrease the intestinal microflora (Thomlison and Lawrence, 1981; Kirchegessner and Roth, 1982; Burnell et al., 1988). It has been reported that wood vinegar also shows strong acid activity at pH 3 and contains 280 different components, the major ones being acetic and propionic acid (Kim, 1996) and antioxidant substances like phenolic compounds (Loo et al., 2008); thus wood vinegar is also termed as natural organic acids (Sasaki et al., 1999).

The pigs fed 0.1, 0.2 and 0.3% wood vinegar diets had 1.4, 4.0 and 6.0% higher overall daily gain, respectively, than pigs fed diets without added wood vinegar. At the same time their daily feed intake was 2.3, 2.5 and 2.7% higher while there were no differences in the feed efficiency. These higher gains may be attributed to the higher feed intake as well as higher apparent nutrient digestibility in pigs fed increasing wood vinegar levels. In line with our findings, improvements in body weight gain and feed intake have been reported in Hanwoo cattle (Kook and Kim, 2003) and meat-type duck (Kook et al., 2002) when bamboo vinegar was added to their diets.

In Exp. 2, the pigs fed diets with added growth promoters in the form of antibiotics, organic acid and wood vinegar had better growth performance and higher apparent and ileal amino acid digestibility than pigs fed diets devoid of growth promoters. Nevertheless, feeding of antibiotics has resulted in the best growth performance of pigs. Improvements in the performance of pigs fed with antibiotics have been well documented (Yen and Pond, 1990; Doyle, 2001; Li et al., 2008). The organic acids added to diets influence the performance of pigs by lowering the gastric pH (Oh, 2004), activating endogenous enzyme secretion (Thaela et al., 1998), improving mineral absorption (Kirchgessner and Roth, 1982), stimulating intermediary metabolism (Grassmann et al., 1992), acting as an energy source for the gastrointestinal tract (Bosi et al., 1999), and reducing the number of pathogenic bacteria (Kirchgessner et al., 1997). In our study, pigs fed wood vinegar had better performance than those fed organic acids. It can be speculated that wood vinegar might have similar effects in influencing the performance of pigs due to the presence of different organic acids (Sasaki et al., 1999); in addition wood vinegar has anti-oxidative effects (Pszczola, 1995).

The different additives used in Exp. 2 were effective in reducing the population of coliforms in the ileum and cecum. However, pigs fed wood vinegar diets had higher

Lactobacilli in their ileum than pigs fed control and antibiotic diets. These results suggest that antibiotics not only reduce the harmful coliforms but they also inhibit the beneficial bacteria. Similar findings have been reported by Cromwell (1991) and Dibner et al. (2007). Watarai and Tana (2005) noticed that wood vinegar added to the diet of birds inhibited the growth of harmful intestinal *Salmonella enteritidis* but promoted the beneficial microbes such as *Enterococcus faecium* and *Bifidobacterium thermophilum*. In line with the latter findings, wood vinegar added to the diets of pigs selectively reduced the harmful coliforms and was favorable towards beneficial bacteria. The organic acids supplemented in the diet of pigs are also effective in reducing pathogenic bacteria (Bolduan et al., 1988; Kluge et al., 2006) and help beneficial microorganisms to dominate the gastrointestinal tract (Mathew et al., 1991).

The reduction of pathogenic bacteria in the intestinal tract can be expected to improve nutrient digestibility and growth performance, alleviating weaning stress and lowering the inflammatory responses to sub-clinical infections (Anderson et al., 1999; Blank et al., 2001). Previous results have demonstrated improved amino acid and energy digestibility due to the reduction of pathogenic bacteria in the gastrointestinal tract (Dibner and Buttin, 2002). Also, the greater population of *Lactobacilli* in the ileum of pigs fed wood vinegar diets might inhibit harmful coliforms in the intestinal tract by blocking possible intestinal receptors of these pathogens or by secreting toxic metabolites against gram negative bacteria (Cranwell et al., 1976; Danielson et al., 1989).

Thus the findings of our study suggest that wood vinegar added to the diets of pigs could improve the performance, apparent nutrient digestibility and selectively inhibit the harmful coliforms. Moreover, even though the feeding of antibiotics resulted in better performances in pigs, the performance of pigs fed wood vinegar was better than pigs fed organic acids.

ACKNOWLEDGMENT

The authors sincerely acknowledge the partial financial support provided by the Institute of Animal Resources at Kangwon National University, Korea.

REFERENCES

An, B. K., B. L. Cho, S. J. You, H. D. Paik, H. I. Chang, S. W. Kim, C. W. Yun and C. W. Kang. 2008. Growth performance and antibody response of broiler chicks fed yeast derived β-glucan and single-strain probiotics. Asian-Aust. J. Anim. Sci. 21:1027-1032.

Anderson, D. B., R. T. McCracken, J. M. Aminov, R. Simpson, M. Mackie, M. W. A. Verstegen and H. R. Gaskins. 1999. Gut microbiology and growth-promoting antibiotics in swine. Pigs New Inf. 20:115-122.

AOAC. 1990. Official method of analysis (15th ed). Association of Official Analytical Chemists. Arlington. VA.

Bae, K. H., T. G. Ko, J. H. Kim, W. T. Cho, Y. K. Han and In K. Han. 1999. Use of metabolically active substances to substitute for antibiotics in finishing pigs. Kor. J. Anim. Sci. 41:23-30.

Blank, R., W. C. Sauer, R. Mosenthin, J. Zentek, S. Huang and S. Roth. 2001. Effect of fumaric acid supplementation and dietary buffering capacity on the concentration of microbial metabolites in ileal digesta of young pigs. Can. J. Anim. Sci. 81:345-353.

Bolduan, G., H. Jung, R. Schneider, J. Block and B. Klenke. 1988. Influence of propionic- and formic acid on piglets. J. Anim. Physiol. Anim. Nutr. 59:72-78.

Bosi, P., H. J. Jung, In K. Han, S. Perini, J. A. Cacciavillani, L. Casini, D. Creston, C. Gremokolini and S. Mattuzzi. 1999. Effects of dietary buffering characteristic and protected or unprotected acids on piglet growth, digestibility and characteristics of gut content. Asian-Aust. J. Anim. Sci. 12:1104-1110.

Burnell, T. W., G. L. Cromwell and T. S. Staly. 1988. Effects of dried whey and copper sulfate on the growth responses to organic acid in diets for weanling pigs. J. Anim. Sci. 66:1100-1108.

Cranwell, P. D., D. E. Noakes and K. J. Hill. 1976. Gastric secretion and fermentation in the suckling pig. Br. J. Nutr. 36:71-86.

Cromwell, G. L. 1991. Antimicrobial agents. In: Swine Nutrition (Ed. E. R. Miller, D. E. Ullrey and A. J. Lewis). Butterworth-Heinemann, Stoneham, Massachusetts, USA. pp. 297-314.

Danielson, A. P., E. R. Peo, K. M. Sahan, P. J. Whaler and M. A. Amer. 1989. Anticholesteremic property of *Lactobacillus acidophilus* yogurt fed to mature boars. J. Anim. Sci. 67:966-974.

Dibner, J. J. and P. Buttin. 2002. Use of organic acids as a model to study the impact of gut microflora on nutrition and metabolism. J. Appl. Poult. Res. 11:453-463.

Dibner, J. J., C. Knight, G. F. Yi and J. D. Richards. 2007. Gut development and health in the absence of antibiotic growth promoters. Asian-Aust. J. Anim. Sci. 20:1007-1014.

Doyle, M. E. 2001. Alternatives to antibiotic use for growth promotion in animal husbandary. Briefings Food Research Institute, University of Wisconsin, Madison.

Fenton, T. W. and M. Fenton. 1979. An improved method for chromic oxide determination in feed and feces. Can. J. Anim. Sci. 59:631-634.

Grassmann, E., F. X. Roth and M. Kirchgessner. 1992. Metabolic effects of formic acid in daily use: 6. Nutritive value of organic acids in piglet rearing. J. Anim. Physiol. Nutr. 67:250-257.

Han, K. N., I. K. Kwon, J. D. Lohakare, S. Heo and B. J. Chae. 2007. Chito-oligosaccharides as an alternative to antimicrobials in improving performance, digestibility and microbial ecology of the gut in weanling pigs. Asian-Aust. J. Anim. Sci. 20:556-562.

Huh, K. S., E. D. Jeong and U. H. Paek. 1999. A study on odor removal of landfill site leachate by pyroligenous liquid. J. Kor. Environ. Sci. Soc. 8:607-610.

Jensen, B. B. 1998. The impact of feed additives on the microbial ecology of the gut in young pigs. J. Anim. Feed Sci. 7:45-64.

Kamel, C. 2001. Tracing modes of action and the roles of plant extracts in non-ruminants. In: Recent Advances in Animal Nutrition (Ed. P. C. Garnsworthy and J. Wiseman). Nottingham Univ. Press, Nottingham, UK. pp. 135-150.

KFRI. 2002. Standard procedures for forestry practice. Korea Forest Research Institute, Seoul, Korea.

Kim, P. G. 1996. Subacute toxicity study of refined wood vinegar. Bulletin of Natural Science Youngin University 1:35-49.

Kirchegessner, M. and F. X. Roth. 1982. Fumaric acid as a feed additive in pig nutrition. Pig News Inf. 3:259.

Kirchgessner, M., B. R. Paulicks and F. X. Roth. 1997. Effects of supplementations of diformate complexes (FormiLHS) on growth and carcass performance of piglets and fattening pigs in response to application time. Agribiol. Res. 50:1-10.

Kishi, M., M. Fudaya, Y. Tsukamoto, T. Nagasawa, K. Takehana and N. Nishizawa. 1999. Enhancing effect of dietary vinegar on the intestinal absorption of calcium in ovariectomized rats. Biosci. Biotech. Biochem. 63:905-910.

Kluge, H., J. Broz and K. Eder. 2006. Effect of benzoic acid on growth performance, nutrient digestibility, nitrogen balance, gastrointestinal microflora and parameters of microbial metabolism in piglets. J. Anim. Physiol. Anim. Nutr. 90:316-324.

Kook, K. and K. H. Kim. 2003. The effects of supplemental levels of bamboo vinegar on growth performance, serum profile and meat quality in fattening Hanwoo cow. Korean J. Anim. Sci. Technol. 45:57-68.

Kook, K., J. E. Kim, K. H. Jung, J. P. Kim, H. B. Koh, J. I. Lee, C. R. Kim and K. H. Kim. 2002. Effect of supplemental bamboo vinegar on production and meat quality of meat-type ducks. Kor. J. Poult. Sci. 29:293-300.

Li, H. L. and K. S. Ryu. 2001. Effect of feeding various vinegar on performance and egg quality of laying hens. Kor. J. Anim. Sci. Technol. 43:655-662.

Li, Z., G. Yi, J. Yin, P. Sun, D. Li and C. Knight. 2008. Effects of organic acids on growth performance, gastrointestinal pH, intestinal microbial populations and immune responses of weaned pigs. Asian-Aust. J. Anim. Sci. 21:252-261.

Loo, A. Y., K. Jain and I. Darah. 2008. Antioxidant activity of compounds isolated from the pyroligneous acid, Rhizophora apiculata. Food Chem. 107:1151-1160.

Mathew, A. G., A. L. Sutton, A. B. Scheidt, D. M. Forsyth, J. A. Patterson and D. T. Kelly. 1991. Effects of a propionic acid containing feed additive on performance and intestinal microbial fermentation of the weanling pigs. In: Proc Sixth Int. Symposium on the Digestive Physiology in Pigs. PUDOC. Wageningen, The Netherlands. pp. 464-469.

Monroe, S. and R. Polk. 2000. Antimicrobial use and bacterial resistance. Curr. Opin. Microbiol. 3:496-501.

Moore, S. 1963. On the determination of cystine as cysteric acid. J. Biol. Sci. 38:235-237.

NRC. 1998. Nutrient requirements of swine (10th Ed). National Academy Press, Washington. DC.

Ogawara, H. 1981. Antibiotic resistance in pathogenic and producing bacteria, with special reference to beta lactam antibiotics. Microbiol. Rev. 45:591-619.

Oh, H. K. 2004. Effects of dietary supplements on growth, nutrient digestion and intestinal morphology in monogastric animals. Ph.D. Thesis, Seoul National University, Korea.

Park, J. H., G. I. Jun and C. H. Jeong. 2003. Ammonia removal characteristics by pyroligneous liquid at livestock farmhouse. J. Kor. Environ. Sci. Soc. 12:1309-1313.

Partanen, K. H. and Z. Mroz. 1999. Organic acids for performance enhancement in pig diets. Nutr. Res. Rev. 12:117-145.

Partanen, K. 2001. Organic acids: their efficacy and modes of action in pigs. In: Gut Environment of Pigs (Ed. A. Piva, K. E. Bach Knudsen and J. E. Lindberg). Nottingham Univ. Press, Nottingham, UK. pp. 201-230.

Pszczola, D. E. 1995. Tour highlights production and uses of smoke-based flavor. Food Tech. 49:70-74.

Russell, A. D. 1991. Mechanism of bacterial resistance to non-antibiotic food additives and food and pharmaceutical preservatives. J. Appl. Bacteriol. 71:191-201.

Sakaguchi, H., N. Uyama and H. Uyama. 2007. Preserving boiled eggs with a sterilization system employing microbial laccase and wood vinegar. Anim. Sci. J. 78:668-671.

Sakaida, T., K. Enya and T. Tanaka. 1987. Effect of wood vinegar compound on egg production and egg quality of white leghorn hens. Jap. Poult. Sci. 24:44-49.

Sasaki, K., M. Tsunekawa, S. Tanaka, M, Fudushima and H. Konno. 1999. Effect of natural organic acids on microbially mediated dissolution of pyrite in acidic environments. J. MMIJ. 115(4):233-239.

Thaela, M. J., M. S. Jensen, S. G. Pierzynowski, S. Jacob and B. B. Jensen. 1998. Effect of lactic acid supplementation on pancreatic secretion in pigs after weaning. J. Anim. Feed Sci. 7 (Suppl. 1):181-183.

Thomlison, J. R. and T. L. J. Lawrence. 1981. Dietary manipulation of gastric pH in the prophylaxis of enteric disease in weaned pigs: Some field observations. Vet. Record. 109:120-122.

Torrallardona, K., M. R. Conde, I. Badiola, J. Polo and J. Brufau. 2003. Effect of fishmeal replacement with spray-dried animal plasma and colistin on intestinal structure, intestinal microbiology, and performance of weanling pigs challenged with *Escherichia coli* K99. J. Anim. Sci. 81:1220-1226.

Watarai, S. and Tana. 2005. Eliminating the carriage of *Samonella enterica* serovar Enteritidis in domestic fowls by feeding activated charcoal from bark containing wood vinegar liquid (Nekka-Rich). Poult. Sci. 84:515-521.

Yen, J. T. and W. W. G. Pond. 1990. Effect of carbadox on net absorption of ammonia and glucose into hepatic portal vein of growing pigs. J. Anim. Sci. 68:4236-4242.