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Effect of bamboo vinegar as an antibiotic alternative on growth performance and fecal bacterial communities of weaned piglets

H.F. Wang [a,b,*], J.L. Wang [a], C. Wang [a], W.M. Zhang [b], J.X. Liu [b], B. Dai [c]

[a] Department of Animal Science, College of Forestry and Biotechnology, Zhejiang A & F University, Hangzhou 311300, PR China
[b] College of Animal Sciences, MOE Key Laboratory of Molecular Animal Nutrition, Zhejiang University, Hangzhou 310029, PR China
[c] Zhejiang Guomao Feed Limited Corporation, Hangzhou 310012, PR China

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ABSTRACT

The aim of the study was to investigate the effects of bamboo vinegar as an antibiotic alternative in the diet of weaned piglets on their growth performance and fecal bacterial communities. The compound composition of bamboo vinegar was analyzed by gas chromatography–mass spectrometry (GC–MS). One hundred and twenty weaned piglets (Duroc×Landrace×Yorkshire), with an average weight of 8.4 kg, were randomly assigned to five treatments, with three pens per treatment. The diets included bamboo vinegar at levels of 0, 0.2, 0.4 or 0.8%, or antibiotics, and designated as control, BV2, BV4, BV8 and antibiotic, respectively. Feed intake and weight gain of pigs were recorded at the start and at the end of the feeding trial. At the end of the experiment, fecal samples of four pigs from each treatment were taken to analyze the fecal bacterial communities analyzed by using 16S rDNA-based techniques. Amplicons of the V6–V8 variable regions of bacterial 16S rDNA were analyzed by denaturing gradient gel electrophoresis. Thirty four peaks (compounds) were identified or characterized in acetic ether extract from bamboo vinegar. The main group from bamboo vinegar was phenolic compounds, ketone and furfural. Daily weight gain of the pigs in BV4 and antibiotic was significantly higher than pigs in the control group. No significant difference was observed in daily weight gain among pigs fed diet containing bamboo vinegar and antibiotics. There was no significant difference in feed intake and feed to gain ratio among different treatment. The serum glutathione peroxidase activity of pigs in BV2 or BV4 was significantly higher than that of pigs in antibiotics treatment ($P<0.05$). The pigs in BV2 had significantly higher serum glutamic–oxaloacetic transaminase activity than those in control ($P<0.05$). No significant differences were found in serum superoxide dismutase, hydrogen peroxide, hydrogen peroxidase, oxidation resistance, malondialdehyde and glutamicpyruvic transaminase activities among different treatments ($P>0.05$). The richness and Shannon index of diversity were significantly lower for the pigs on the diet containing antibiotics than that of control or diets containing 0.2 or 0.4% bamboo vinegar, and tended to decrease with the increase of bamboo vinegar inclusion in the diets. The results demonstrate that bamboo vinegar in feed exerts an impact on the fecal bacterial community of piglets. The reasonable inclusion of bamboo vinegar, like antibiotics in piglet diet benefited for a better performance of piglets in this experiment. The result suggested that bamboo vinegar could be used as a potential additive in animal production as antibiotic alternative.

1. Introduction

Serious infections caused by bacteria that have become resistant to commonly used antibiotics have become a major global healthcare problem in the 21st century. They

* Corresponding author at: Zhejiang A & F University, College of Forestry and Biotechnology, Department of Animal Science, Lin'an Hangzhou 311300, PR China. Tel.: +86 571 63743313; fax: +86 571 63740898.
E-mail address: wanghaifengzj@yahoo.cn (H.F. Wang).

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not only are more severe and require longer and more complex treatments, but they are also significantly more expensive to diagnose and to treat (Alfonso, 2005). In recent years, there has been increasing concern that the use of antibiotics in food-producing animals, particularly their long term use for growth promotion, contributes to the emergence of antibiotic-resistant bacteria in animals. In the recent years, incidence of multidrug resistance in pathogenic and opportunistic bacteria has been increasingly documented (Jones et al., 2004). Great attention has been paid to food-producing animals as one of several potential sources of antibiotic-resistant bacteria in humans (Janet et al., 2002).

Due to increasing resistance to antibiotics by many bacteria, plant extracts and plant compounds are of new interest as antiseptics and antimicrobial agents in dermatology (Augustin and Hoch, 2004; Blaschek et al., 2004; Norton, 2000). Although the active constituents may occur in lower concentrations, plant extracts may be a better source of antimicrobial compounds than synthetic drugs (Cox and Balick, 1994).

Bamboo vinegar (BV) is a brown-red transparent liquid produced during pyrolysis of bamboo charcoal and contains more than 200 types of chemical components, in which acetic acid is the main component (Mu et al., 2004). Bamboo charcoal is manufactured by introducing dried bamboo into a kiln with a flame tunnel, heating the bamboo slowly to 600–900 °C, holding for 4 days, sealing the tunnel, holding for 4 days, cooling to room temperature, and withdrawing the charcoal (Zhang et al., 2003). In this process of carbonization, the vapor is cooled and the resulting solution is recovered as bamboo vinegar. Hence, bamboo vinegar, is a by-product of the bamboo charcoal manufacturing industry, including more than 200 kinds of organic compounds, such as acetic acid, phenolic compounds, alkone compounds, alcohol compounds, aldehyde compounds, and others (Ikimoto and Ikeshima, 2000; Nomura, 2004; Uchimura et al., 2000). Previous studies revealed that these organic compounds in bamboo vinegar may have practical applications even when they present in only trace quantities (Lin, 1995; Uchimura et al., 2000). As a result, the commercial production of bamboo vinegar is being increased in China and Japan, where it is highly valued for its various effective uses for example as a natural insecticide. Acetic acid and phenolic compounds contained in wood vinegar have been reported as anti-germination agents (Yatagai, et al., 2002) and termiticides (Mu et al., 2004). Bamboo vinegar and biomass slurry exhibit similar fungicidal and termiticidal properties of wood vinegar, due to their acetic acid and phenolic compound contents (Kartal et al., 2004; Nakayama et al., 2001). However, little is known about the effect of feeding bamboo vinegar alternative antibiotics on bacterial communities and performance of pig.

Therefore, the object of this study was to evaluate the effect of bamboo vinegar as an antibiotic alternative on serum antioxidant status, growth performance and bacterial communities of weaned piglets.

2. Material and methods

2.1. Analysis of components in bamboo vinegar

The bamboo vinegar was extracted with an equivalent volume of acetic ether at room temperature for 24 h and the part of acetic ether with compounds was separated for later analysis. The compounds were isolated with an Agilent 7890A gas chromatograph with the mass detector 5975C. A DB-5 fused silica capillary column (30 m × 250 μm × 0.25 μm) was used to separate the compounds in bamboo vinegar. Column temperature was held at 50 °C for 2 min and then programmed at 2 °C/min up to 100 °C and for 1 min, at 8 °C/min up to 180 °C and for 1 min, finally at 10 °C/min up to 250 °C for 2 min. The flow rate of He was held at 1 ml/min, the split ratio was adjusted to 50. Mass detector was set in EI mode at 70 eV, and the interface temperature was maintained at 230 °C. Tentative identifications of volatile components were from peak retention times and mass spectral data. Identification was confirmed by using standard compounds when available. Composition measurements were calculated from the peak areas of the components.

2.2. Animals and diets

One hundred and twenty weaned piglets (Duroc × Landrace × Yorkshire), with an average weight of 8.4 ± 1.5 kg, were randomly assigned to five treatments based on live weight, with three replicates per treatment. Eight animals were kept in a pen. Bamboo vinegar was a product from Zhejiang Agriculture & Forestry University, located in Zhejiang Province, China. The diet was mixed with bamboo vinegar at levels of 0, 0.2, 0.4 or 0.8%, or antibiotics (antibiotic mixture included aureomycin, colistin sulfate, kitamycin at levels 75, 20 and 50 mg/kg, respectively) and supplemented with water as balance at levels of 0.8, 0.6, 0.4 and 0%, and designated as control, BV2, BV4, BV8 and antibiotics, respectively. The pigs in each pen were kept in a concrete-floored pen with an underground heating device, free access to feed and drinking water. The trial lasted for 25 days. Four pigs from each treatment were selected to sample feces. All samples were stored at −20 °C for later analysis.

Dry matter (DM) and ash contents of feeds were determined according to method 942.05 (AOAC, 1999). The sample was analyzed for Kjeldahl N (method 954.01), ether extract (method 920.39) and crude fiber (method 978.10, AOAC, 1999). Neutral detergent fiber was determined according to methods by Van Soest et al. (1991) without sulfite and heat stable amylase, excluded of residual ash and expressed as NDFom. The ingredients and compositions of diet for pigs are presented in Table 1.

2.3. Growth performance

The initial and final body weights of pigs were recorded, respectively, and the corresponding feed consumptions were recorded per group in 25 days feeding periods. The daily feed intake, average daily gain and feed conversion ratio were calculated.

2.4. Blood sampling and analyses

Blood samples were collected from 12 h fasted animals at the end of the experiment. Blood (10 ml) was drawn from the jugular vein and centrifuged at 3000 *g* for 15 min. Serum was frozen at −10 °C and later thawed for analysis of superoxide dismutase (SOD), lactate dehydrogenase (LDH), glutathion (GSH), glutathione peroxidase (GSH-Px), hydrogen peroxide

Table 1
Ingredients and composition of diets.

	Control	BV2[a]	BV4[a]	BV8[a]	Antibiotic[b]
Ingredients (%)					
Corn	55.5	55.5	55.5	55.5	55.5
Soybean meal	20.0	20.0	20.0	20.0	20.0
Wheat grain	6.0	6.0	6.0	6.0	6.0
Expanded soybean	5.0	5.0	5.0	5.0	5.0
Soybean oil	2.2	2.2	2.2	2.2	2.2
Bamboo vinegar	0.0	0.2	0.4	0.8	0.0
Water	0.8	0.6	0.4	0.0	0.8
Whey power	2.5	2.5	2.5	2.5	2.5
Fish meal	5.5	5.5	5.5	5.5	5.5
L-lysine	0.2	0.2	0.2	0.2	0.2
Dicalciumphosphate	0.7	0.7	0.7	0.7	0.7
Limestone	0.5	0.5	0.5	0.5	0.5
Salt	0.1	0.1	0.1	0.1	0.1
Vitamin–mineral premix[c]	1.0	1.0	1.0	1.0	1.0
Chemical compositions (%)					
Dry matter[d]	88.3	87.8	87.7	88.1	88.0
Crude protein[d]	19.6	19.8	19.5	19.3	19.5
Crude fiber[d]	3.1	3.3	3.0	3.2	3.3
Ash[d]	4.9	4.6	5.0	5.2	4.9
Lysine[e]	1.45	1.45	1.45	1.45	1.45
DE/(MJ/kg)[e]	14.8	14.8	14.8	14.8	14.8

[a] BV2, BV4, BV8: bamboo vinegar was added to the diets at levels of 0.2, 0.4 or 0.8%, respectively.
[b] Antibiotic mixture included: aureomycin, colistin sulfate, kitamycin at levels 75, 20 and 50 mg/kg, respectively.
[c] Vitamin–mineral premix supplied for piglets (per kg of diet): vitamin A 22,500 IU, vitamin D_3 4950 IU, vitamin E 89 IU, vitamin K_3 2.7 mg, vitamin B_1 5.2 mg, vitamin B_2 15 mg, vitamin B_6 8.1 mg, vitamin B_{12} 4.5 mg, pantothenic acid 42 mg, niacin 52 mg, folic acid 3.3 mg, biotin 0.3 mg, choline chloride 1500 mg, Zn 120 mg, Fe 175 mg, Cu 130 mg, Mn 48 mg, I 0.5 mg, and Se 0.6 mg.
[d] Actually determined values.
[e] Calculated values.

(H_2O_2), hydrogen peroxidase (CAT), oxidation resistance (T-AOC), hydroxy radical resistance (T-HRR), malondialdehyde (MDA), glutamic–pyruvic transaminase (GPT) and glutamic–oxaloacetic transaminasetotal (GOT) by automatic biochemistry analyzer (HITACHI 7020). Test kits were purchased from Diasys diagnostic systems (Shanghai) Co. Ltd.

2.5. PCR-DGGE analysis

Genomic DNA was obtained using an extraction method according to Wang et al. (2007). Primer U968-GC (50-CGC CCG GGG CGC GCC CCG GGC GGG GCG GGG GCA CGG GGG GAA CGC GAA GAA CCT TAC-30) and L140lr (50-GCG TGT GTA CAA GAC CC-30) (Nübel et al., 1996) were used to amplify V6–V8 regions of 16S rDNA using TC-512 PCR System (TECHNE, UK). This primer pair is specific for bacterial 16S rDNA and yields amplicons of 470-bp length. The GC clamp in primer U968-GC creates PCR products suitable for separation by denaturing gradient gel electrophoresis (DGGE). PCR was performed with the Taq DNA polymerase kit from TaKaRa (TaKaRa Biotechnology (Dalian) Co., Ltd., DaLian, China). Based on the manufacturer's instruction, the PCR reaction (50 μl) used 0.25 μl of Taq polymerase (1.25 U), 1 μl of primers U968-GC and L1401 (5 pmol), 1 μl of ten-fold diluted DNA template (approximately 1 ng), 5 μl of ten-fold PCR buffer, 3 μl of $MgCl_2$ (50 μM) and lastly UV sterile water. The samples were amplified in an Eppendorf PCR system, with 30 cycles of 94 °C for 30 s, 56.5 °C for 1 min, and 72 °C for 1 min. Aliquots of 5 μl were analyzed by electrophoresis on an agarose gel (1.0%) to check the sizes and amounts of the amplicons. Amplicons of V6–V8 of 16S rDNA were used for sequence-specific separation by DGGE. DGGE was performed on 8% polyacrylamide gels containing acrylamide, bisacrylamide, formamide and a gradient of 30–50% of urea in 0.5×TAE buffer using BioRad DGGE system according to the method of Wang et al. (2007).

DGGE analysis of all samples was repeated twice to confirm pattern consistency. All gels were scanned at 400 dpi. The richness (number of DGGE bands) and similarity indices were calculated from the densitometric curves of the scanned DGGE profiles with Molecular Analyst 1.12 (Biorad) software, using the Pearson product–moment correlation coefficient. Similarity indices were calculated for pairs of DGGE profiles for feces samples, and dendograms were produced to cluster pigs by the similarity. As a parameter for the structural diversity of the microbial community, the Shannon index of general diversity was calculated according to Konstantinov et al. (2003).

2.6. Statistics

For feed intake and feed conversion ratio, pen was considered as the experimental unit. Data were analyzed as a completely randomized design using the General Linear Models (GLM) procedure of SAS (1996). Differences among means for the four treatments were tested using Duncan's new multiple range test. For weight gain, richness and Shannon index of general diversity, the pig was considered as the experimental unit.

3. Results

3.1. Components of bamboo vinegar

Fig. 1 shows a typical chromatogram of bamboo vinegar. About 34 peaks were identified or characterized in acetic ether extract from bamboo vinegar. Composition of the components, calculated from the peak areas, appears in Table 2. The main group from bamboo vinegar was phenolic compounds (such as phenol 19.0%, 2,6-dimethoxy-phenol 13.2%, 2-methoxy-phenol 8.6%, 1,2-benzenediol 7.3%, 4-methyl-phenol 5.9%), ketone (1-hydroxy-2-butanone 3.8%, 2-hydroxy-3-methyl-2-cyclopenten-1-one 3.3%, 2-methyl-2-cyclopenten-1-one 1.2%,) and furfural 5.7%.

3.2. Performance

The pigs in BV4 and antibiotic were significantly higher in final weight and daily weight gain than pig in control ($P<0.05$, Table 3). No significant differences were observed in the final weight and daily weight gain among pigs fed diets containing bamboo vinegar or antibiotics ($P>0.05$, Table 3). There was no significant difference in feed intake and feed to gain ratio among different treatments ($P>0.05$, Table 3).

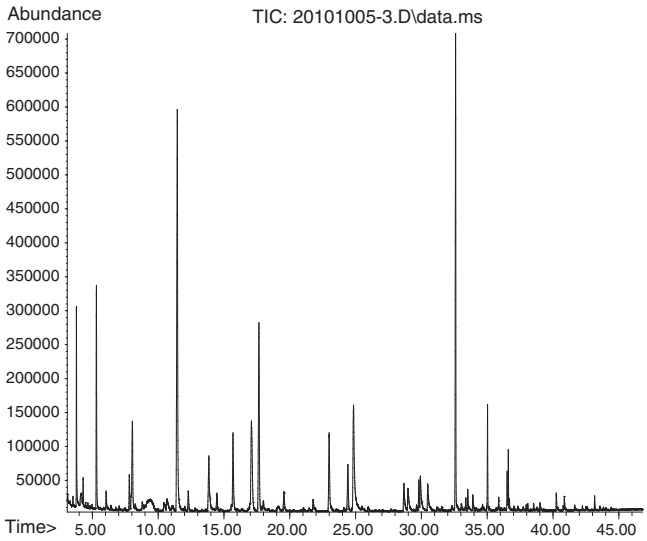

Fig. 1. Typical chromatograms obtained by GC–MS of the volatile fraction from bamboo vinegar.

3.3. Serum parameter

The pigs in BV8 were significantly lower in T-HRR and GSH than those in the antibiotics group ($P<0.05$, Table 4). The GSH-Px of pigs in BV2 or BV4 was significantly higher than that of pigs in antibiotic treatment ($P<0.05$, Table 4). The pigs in BV2 had significantly higher GOT than those in control ($P<0.05$, Table 4). No significant differences were found in SOD, H2O2, CAT, T-AOC, MDA and GPT among different treatments ($P>0.05$, Table 4).

Table 2
Volatile composition of the acetic ether extract of bamboo vinegar.

Compound	Composition (%)
1-Hydroxy-2-butanone	3.8
Hexanoic acid	0.4
Cyclopentanone	0.6
Furfural	5.7
Tetrahydro-, acetate 2-furanmethanol	0.6
2-methyl-2-cyclopenten-1-one	1.2
Butyrolactone	3.6
2,5-Hexanedione	0.4
Phenol	19.0
2,5-Dihydro-3,5-dimethyl-2-furanone	0.8
2-Hydroxy-3-methyl-2-cyclopenten-1-one	3.3
2,3-Dimethyl-2-cyclopenten-1-one	0.7
2-Methyl-phenol	3.3
4-Methyl-phenol	5.9
2-Methoxy-phenol	8.6
3-Ethyl-2-hydroxy-2-cyclopenten-1-one	0.9
2,3-dimethyl-phenol	0.6
4-Ethyl-phenol	3.8
2-Methoxy-4-methyl-phenol	2.1
1,2-Benzenediol	7.3
3-Methoxy-1,2-benzenediol	1.6
3-Methyl-1,2-benzenediol	1.3
4-Ethyl-2-methoxy-phenol	1.2
Hydroquinone	1.4
4-Methyl-1,2-benzenediol	1.4
2,6-Dimethoxy-phenol	13.2
2,2-Dimethylpropyl 2-ethylhexanoate	0.4
4-Ethylcatechol	0.7
Vanillin	0.7
3-Hydroxy-4-methoxybenzoic acid	2.4
6,7-Dihydro-3,6-dimethyl-, (R)-4(5H)-benzofuranone	0.9
1,2,3-Trimethoxy-5-methyl benzene	1.2
1-(4-Hydroxy-3,5-dimethoxyphenyl)-ethanone	0.5
4-Hydroxy-3,5-dimethoxy-, hydrazide benzoic acid	0.4

3.4. Bacterial communities in feces of pig fed on different levels of bamboo vinegar

A representative analysis of the PCR fragments generated with primers 968-GC and 1401r and analyzed by DGGE. In order to compare the diversity of the predominant bacterial populations in pigs fed diet containing different levels of bamboo vinegar, the numbers of predominant fragments in the DGGE profiles were calculated for feces samples (Fig. 2). The richness varied from 26 to 41 for feces samples. The richness was significantly lower for the pigs on diet containing antibiotics (26.7) than that of control (40.0) or diets containing 0.2 (41.0) or 0.4% (39.5) bamboo vinegar ($P<0.05$, Fig. 2). The richness tended to decrease with the increase of bamboo vinegar inclusion in the diet. The Shannon index of diversity, H′, was lower in fecal samples for pigs on the antibiotic diet (1.42) compared with the control (1.59) or diets containing 0.2 (1.60) or 0.4% (1.58) bamboo vinegar ($P<0.05$, Fig. 2). Similar to the richness, the Shannon index of diversity tended to decrease with the increase of bamboo vinegar inclusion in the diet (Fig. 2).

Dendograms showing clustering of the DGGE profiles of feces based on pairwise correlation coefficients are presented in Fig. 3. The similarity indices in profiles of the individual pigs ranged from 0.43 to 0.73. In general, with some exception, the fecal communities in piglets formed different small clusters according to the type and abundance of additive included in the diet. For example, BV2 and the control diet tended to cluster together and separate from antibiotics, BV4 and BV8 which also cluster together (Fig. 3).

Table 3
Performance of piglets fed different levels of bamboo vinegar.

	Control	BV2	BV4	BV8	Antibiotic	SEM
Initial weight (kg)	8.34	8.39	8.38	8.39	8.38	0.131
Final weight (kg)	18.68^{b}	19.62^{ab}	19.89^{a}	19.51^{ab}	20.23^{a}	0.392
Daily weight gain(g)	414^{b}	449^{ab}	460^{a}	445^{ab}	474^{a}	13.0
Feed intake (g)	682	718	746	718	757	10.8
Feed to gain ratio	1.648	1.594	1.622	1.611	1.593	0.1264

^{a,b}Means in the same row bearing different superscript are significantly different ($P<0.05$).

4. Discussion

Previous studies about bamboo vinegar utilization were focused on pesticides in agriculture or forestry, or disinfectant in medicine. Recently, bamboo vinegar products have been developed that are beneficial for the human skin, it is good against allergies, can be used in health drinks, it is virus/fungi resistant (Hageta, 2004; Kobahasi, 2004; Mizuki, 2004; Yoshie, 2004). Watarai et al. (2008) found that the use of bamboo vinegar combined with bamboo charcoal was an efficient treatment for cryptosporidiosis in calves. However, there are no reports about bamboo vinegar used as feed additive in animal production. The evaluation of toxicological safety by Sprague Dawley (SD) rats and mice has proven that the bamboo vinegar was safe to animals as oral medicine (Chen et al., 2007). Therefore, bamboo vinegar can be regarded as a secure feed additive as an alternative to antibiotics in food animal production. The inclusion of 0.5 and 1.0% SB (bamboo charcoal powder including vinegar liquid) induced better production performance, and the results confirmed that dietary SB can be used as a natural substance to supplement chicken diets as an alternative to antibiotics (Yamauchi et al., 2010). The duck growth performance tended to be improved with increasing SB from 0 to 1% supplemented in basal commercial diet (Ruttanavut et al., 2009). Pigs fed antibiotic showed higher ($P<0.001$) ADG and better feed efficiency followed by pigs fed 0.2% wood vinegar and 0.2% organic acid diets while those fed the control diet had lowest ADG and poorest feed efficiency. The overall ADFI was highest ($P<0.001$) in pigs fed wood vinegar and lowest in pigs fed the control diet (Choi et al., 2009). In this study, adding 0.4% bamboo vinegar in feed was effective in improving the performance of piglets, and comparable with the pigs fed diets containing antibiotics. Due to the higher feed intake of pigs in BV4 and antibiotics, the final weight and daily weight gain for pigs in both treatments were significantly higher than those in control, therefore feed to gain ratio was not significantly different among treatments. The physical condition was good for animals on bamboo vinegar, similar to animals treated with antibiotics during the whole experiment. The results manifested that the bamboo vinegar has the potential to replace antibiotics.

As well known, antibiotics such as aureomycin, colistin sulfate and kitamycin use in this experiment have been widely employed in food-producing animals to favor growth promotion and inhibit the pathogenic bacteria to reduce the outbreak of fecal disease. *Escherichia coli*, *Staphylococcus aureus*, and *Candida albicans* were found to be killed by bamboo vinegar diluted at 1:8 (Jiang, 2005). Luo et al. reported that the bamboo vinegar had antimicrobial activity on *E. coli* even at dilution of 1:100 (Luo et al., 2004). Previous works (Lin and Shiah, 2006; Lin et al., 2006; Shiah et al., 2006) had established a clear positive relationship between bamboo vinegar and fungi resistance. The wood vinegars prepared from different plant resources can be used as antifungal agents to replace formic and acetic acids. Stronger antifungal activity of wood vinegars was shown than that of both formic and acetic acid. The antifungal property of wood vinegars was in order of coconut shell wood vinegar > bamboo wood vinegar > Eucalyptus wood vinegar according to their phenolic compound contents (Yodthong and Niamsa, 2009).

It was not surprising to find that the use of antibiotics significantly decreased the richness (Collier et al., 2003) compared with the control or BV2 and BV4 treatment. Based on

Table 4
Serum parameter of piglets fed different levels of bamboo vinegar.

	Control	BV2	BV4	BV8	Antibiotic	SEM
SOD (U/ml)	201.2	206.6	205.5	203.9	206.3	6.05
LDH (U/ml)	13.61^{ab}	17.11^{a}	11.93^{ab}	10.17^{b}	15.00^{ab}	1.891
GSH (mg/l)	4.22^{ab}	4.51^{a}	3.72^{ab}	3.21^{b}	4.28^{a}	0.329
GSH-Px (U/ml)	603.3^{ab}	697.4^{a}	695.6^{a}	628.8^{ab}	543.5^{b}	38.52
H_2O_2 (mmol/l)	6.54	7.08	3.62	3.46	5.43	0.701
CAT (U/ml)	3.29	4.31	3.43	2.96	3.45	0.733
T-AOC (U/ml)	3.43	4.19	4.22	4.07	3.50	0.637
T-HRR (U/ml)	1318.0^{ab}	1326.5^{ab}	1094.2^{ab}	902.1^{b}	1520.4^{a}	140.76
MDA (nmol/ml)	2.25	2.94	2.72	2.47	3.25	0.531
GPT (U/ml)	15.68	17.68	18.75	18.14	21.03	2.853
GOT (U/ml)	13.67^{b}	24.91^{a}	14.78^{b}	10.90^{b}	16.98^{ab}	3.177

SOD, superoxide dismutase; LDH, lactate dehydrogenase; GSH, glutathion; GSH-Px, glutathione peroxidase; H_2O_2, hydrogen peroxide; CAT, hydrogen peroxidase; T-AOC, oxidation resistance; T-HRR hydroxy radical resistance; MDA malondialdehyde; GPT, glutamicpyruvic transaminase; GOT, glutamic–oxaloacetic transaminase.
^{a,b}Means in the same row bearing different superscript are significantly different ($P<0.05$).

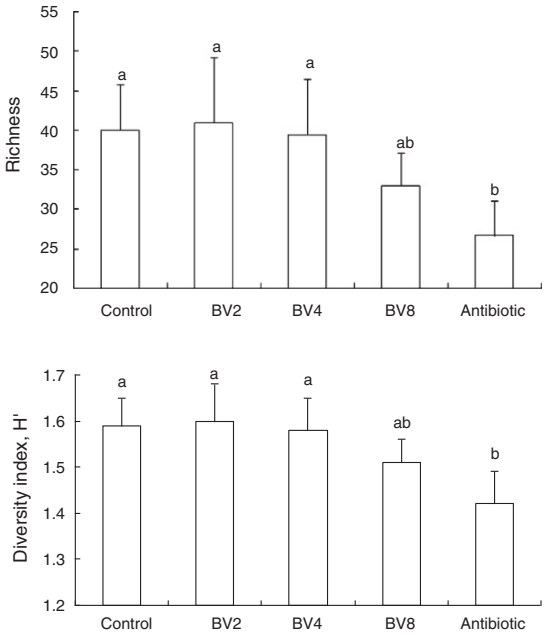

Fig. 2. The richness and Shannon diversity index, H′, of feces samples from pigs fed control, BV2, BV4, BV8 or antibiotic diets.[a,b]Means in the bar bearing different superscript are significantly different ($P<0.05$).

the fecal bacterial communities from piglets no.18–20 fed the diet containing antibiotics, a distinct cluster was formed in DGGE profiles. It is necessary to note that the bamboo vinegar exerted an impact on fecal bacterial communities of pigs according to the reduction of richness and diversity index at 0.4 and 0.8% bamboo vinegar in the diet. The variation of bacterial communities observed in the high concentration treatment of bamboo vinegar may be attributed to the inhibitory effect of the bamboo vinegar on the fecal bacteria. The acetic acid concentration in vinegar was 2% in the vinegar, this computes to a concentration of 0.004–0.016% acetic acid in the diets containing bamboo vinegar in the trial. Although, the acetic acid in the diets might influence pH of feed, the action on pH in the intestine was insignificant due to the buffering capacity of the body (our unpublished data), thus acetic acid from bamboo vinegar in diets may not affect the fecal bacterial communities of piglets. It is inferred that the effect of bamboo vinegar on bacterial communities attributes to the active components such as phenolic compounds in bamboo vinegar. The bamboo vinegar could exert impact on animal fecal microbial communities like antibiotics do, but may not increase antibacterial resistance as antibiotics do. The richness (band numbers) and total bacteria were decreased ($P<0.05$) in ileal luminal contents of pig treated with tylosin and an antibiotic rotation (chlorotetracycline sulfathiazole penicillin; bacitracin and roxarsone; lincomycin; carbadox; virginiamycin, with each for a week) for 5 weeks (Collier et al., 2003). The inclusion of antibiotic amoxicillin at a daily dose of 100 mg/kg body weight significantly decreased the richness and Shannon index of intestinal bacterial communities of mice in comparison with saline control (Yuan et al., 2010). The similar result was found in this study that the antibiotic significantly decreased the richness and Shannon index of intestinal bacterial communities of pig in comparison with the control. Higher populations of lactobacillus 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, 0.2% organic acid or 0.2% wood vinegar diets (Choi et al., 2009). These results indicated that wood vinegar could reduce harmful intestinal coliforms, but increase the probiotics (Choi et al., 2009). It is inferred that the action mode of bamboo vinegar like wood vinegar most

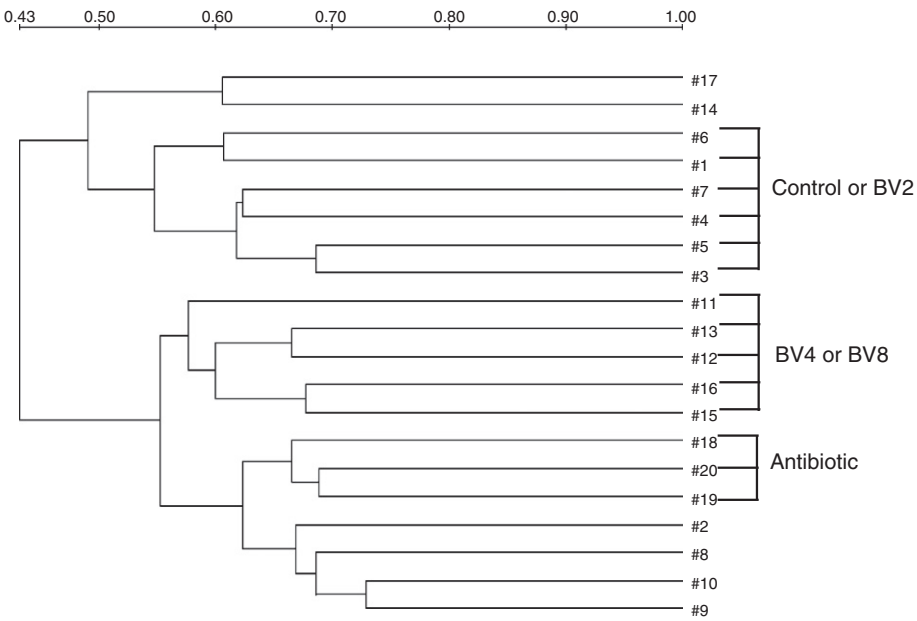

Fig. 3. Similarity index of DGGE profiles obtained from feces of twenty pigs. Symbol: control (1–4), piglets on control diet; BV2 (5–8), BV4 (9–12) or BV8 (13–16), piglets on diets containing 0.2%, 0.4% or 0.8% bamboo vinegar, respectively; antibiotic (17–20), piglets on diets containing antibiotics.

probably differs from antibiotic in action mode on intestinal bacterial communities, although the bamboo vinegar produces a fecal microbial community profile more similar to antibiotics.

Besides the action on bacteria, bamboo vinegar had antioxidant ability due to more than 200 organic compounds present, such as phenolic compounds, alkone compounds, alcohol compounds, aldehyde compounds, and others (Ikimoto and Ikeshima, 2000; Nomura, 2004; Uchimura et al., 2000). Main kinds of phenolic compounds were also found in bamboo vinegar extracted with ethyl acetate by GC–MS analysis in this study. Chang et al. (2004) also reported that the best dosage is confirmed to be 5 g bamboo vinegar per kg of lard for bamboo vinegar as antioxidant in lard. Bamboo vinegar showed similar antioxidant properties to tea polyphenol when used in equivalent weight ratio 4 g/kg in lard, which indicated that bamboo vinegar is a favorable antioxidant (Chang et al., 2004). The inclusion of ascorbic acid a well known antioxidant in pig diet increased the superoxide dismutase and catalase activities in liver, kidney, brain and heart, and glutathione peroxidase and glutathione reductase activities in serum and liver (Suresh et al., 1999). SOD, GSH-Px and CAT are the main antioxidant enzymes in the body (Krajcovicova et al., 2003). These enzymes may scavenge unwanted O_2^- and H_2O_2, and ROOH produced by free radicals. For example, SOD catalyzes superoxide radical dismutation: $O_2^- + O_2^- + 2H^+ \rightarrow H_2O_2 + O_2$. The resulting hydrogen peroxide in turn is decomposed by the enzymes GSH-Px and CAT (Rzeuski et al., 1998). As shown above, the increased activities of SOD, GSH-Px and CAT would increase the ability for elimination of free radicals. T-AOC, used to reflect the total capacity of antioxidant systems in the body in recent years, is an integrative index (Tao et al., 2006). The GSH-Px and oxidation resistance activity in serum of pigs fed diet with bamboo vinegar were higher than those in control and antibiotic treatment in this study, which indicated that the bamboo vinegar had a good antioxidant activity for pig. The antioxidant activity of bamboo vinegar could be responsible for the contribution to the health of pigs by scavenging free radicals and improving the immune defense function in the animal.

The results demonstrate that the bamboo vinegar in feed could exert an impact on fecal bacterial communities of piglets as well as positive effect on growth performance and serum parameters. The reasonable inclusion of bamboo vinegar in the diet favors a better performance and produces fecal bacteria community profile similar to antibiotic in this study. Taken together, our results suggest that bamboo vinegar could be used as a potential additive in animal production as antibiotics alternative.

Conflict of interest

We declare that the authors do not have any possible conflicts of interest in the article “Effect of bamboo vinegar as an antibiotic alternative on growth performance and fecal bacterial communities of weaned piglets”.

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