

Full Length Research Paper

Potential of bamboo vinegar with liquid probiotics on growth performance, fecal microbiology and fecal odorous gas emissions from finishing pigs

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Received 26 January, 2016; Accepted 24 February, 2016

In this study, the effects of dietary supplementation with bamboo vinegar liquid probiotics (BVLP) on finishing pigs were investigated. A total of 105 crossbred (Landrace × Yorkshire) finishing pigs were allotted to five treatments of three replicate pens (7 pigs/pen) following a completely randomized design. Results elucidated that, there were no significant differences observed in weight gain, feed intake and feed conversion efficiency among the dietary supplementation. Carcass weight and carcass yield grade did not differ significantly; however, the back fat thickness tended to be decreased in the 0.2% BVLP group compared to control ($P < 0.10$). In addition to that, fecal *Escherichia coli* content was significantly suppressed in response to the antibiotic and 0.2, 0.4, and 0.8% BVLP supplementation relative to the control ($P > 0.05$); where no significant differences were found between antibiotics and BVLP supplemented groups. Furthermore, among malodorous gases, ammonia and hydrogen sulfide gas was significantly down trended in the antibiotic and 0.2, 0.4, and 0.8% BVLP supplemented groups and no significant differences were observed between antibiotic and BVLP supplemented groups. Significant diminution of the sulfur dioxide was found after antibiotic and 0.2, 0.4, and 0.8% BVLP supplementation compared to control ($P < 0.05$); where antibiotic and 0.4% BVLP also significantly differed. Moreover, the mercaptan gas was substantially decreased in the 0.4% BVLP supplemented group relative to control ($P < 0.05$), while no significant differences were observed between the antibiotic and BVLP groups. Taken together, supplementation of BVLP could be potentially utilized in the diet of finishing pigs for the suppression of the fecal pathogenic *Escherichia coli* and fecal odorous gas emissions, without negative impact on the growth performance.

Key words: Bamboo vinegar liquid probiotics, gaseous emissions, microbiology, growth performance, finishing pigs.

INTRODUCTION

Animal production is increasing rapidly in Asia (Huynh et al., 2007). While developing countries produced 31% of the world's meat in 1980, they are expected to produce 60% by 2020 (Delgado et al., 1999). Among the projected 60%, around 13.2% will be produced in Southeast Asia

alone. In such countries, pigs serve as an important source of family income (Steinfeld, 1998). Feed additives are compounds added to diets to enhance animal performance, either directly or indirectly. Antibiotics, which are the most controversial additive, were used at

the therapeutic level for disease treatment (high dose-short duration) or at the sub-therapeutic level for growth promotion (low dose-long duration) until last decades. A great deal of debate regarding the continued use of sub-therapeutic antibiotics in livestock feeds has taken place due to concern surrounding the development of antibiotic resistant bacteria. In fact, the European Union has banned the use of growth promoters in the feed of food animals (European Commission, 1998), and similar efforts are being made in Denmark, Korea, the United States and elsewhere throughout the world. Therefore, pork producers are looking for replacements for growth-promoting antibiotics.

Among various antibiotic alternatives, use of probiotics is a common approach that has shown potential. Probiotics are a class of feed additives composed of living bacteria and/or yeast cultures that are provided to improve desirable microflora balance within the small and large intestine (McKean, 2004). Most common mixtures contain one or more of the *Lactobacillus* species, *Bacillus subtilis*, *Streptococcus faecium*, *Saccharomyces cerevisiae* and other commensal species. These mixtures are thought to work either directly, by excluding harmful bacteria or reducing intestinal pH, or indirectly, by favoring the development of other desirable health promoting microorganisms that compete with harmful bacteria to reduce their presence in the gut. The desired effect is improved weight gain and feed efficiency via improved gut digestion and reduced pathogenic organism loads. Ko and Yang (2008) found that finishing pigs receiving probiotics in their feed showed equal or superior daily gain, intake, and feed efficiency as pigs fed antibiotics.

Various active substances are present in medicinal plants or extracts, including natural antibiotics. Accordingly, functional medicinal plants have been applied as replacements for antibiotics (Harris and Webb, 1990; Martin and Nisbet, 1992; Berg, 1998; Lyons and Jacques, 2000; Kwon et al., 2005; Sarker et al., 2010d). Among medicinal plants, bamboo and bamboo vinegar are very effective due to their natural active substances. Bamboo vinegar is a by-product of bamboo carbonization derived from the smoke of carbonized bamboo that has a unique tart flavor and smoky smell. Bamboo vinegar contains over 200 ingredients that are all from natural sources and known to be safe for use on humans and animal. The acetic acid contained in bamboo vinegar softens the skin cuticle and relieves dermatitis, itch, and athlete's foot. The functional and medicinal properties of bamboo vinegar, such as its antioxidant, antimicrobial, and antimutagenic activities have been studied for decades (Lin et al., 2008), and it has recently drawn the attention of medical researchers. Suga et al. (2003) isolated an

antioxidative phyllostadiemier from *Phyllostachys edulis*, a common bamboo species. The combination of medicinal plants with probiotics has shown the potential to improve many aspects of broilers (Sarker et al., 2010a, 2010b, 2010c), pigs (Ko and Yang, 2008) and calves (Sarker et al., 2010e). To the best of our knowledge, there were limited researches has been conducted on the combination of bamboo vinegar and probiotics on the growth performance, fecal microbiology and fecal odorous gas emissions in finishing pigs. Odorous gas emissions from the swine industry are considered as a major problem and continuous research has been conducted to minimize the emissions through different dietary manipulations (Van der Peet-Schwering et al., 1999). This study was conducted to investigate the effects of bamboo vinegar liquid probiotics (BVLP) on fecal microbiology and fecal malodorous gas emissions from finishing pigs and to ascertain whether it has a positive or negative impact on the growth performance of the finishing pigs.

MATERIALS AND METHODS

Animal and experimental design

A total of 105 crossbreed (Landrace × Yorkshire) finishing pigs were housed in concrete floor pens for a period of six weeks. The pigs were assigned to five dietary treatments in a completely randomized design. Each treatment consisted of three replicates with seven pigs per replication. The five dietary treatments were a control (no additives), antibiotics (control diet + 30 ppm chlortetracycline), and diets supplemented with 0.2, 0.4 or 0.8% BVLP. The nutrient composition of the control diet (Table 1) was in accordance with the suggested nutrient requirements for finishing pigs (NRC, 1994). BVLP used in this experiment were composed of 17.73% crude protein, 2.90% crude fat, 9.71% crude fiber and 10.37% crude ash. In addition, 4.2×10^7 cfu/g *Lactobacillus acidophilus*, 5.8×10^6 cfu/g *Lactobacillus plantarum*, 2.6×10^7 cfu/g *B. subtilis* and 6.2×10^9 cfu/g *S. cerevisiae* were included in the BVLP (Table 2). The chemical compositions of the five experimental feeds were analyzed as described by the AOAC (1990) (Table 3). The experimental protocol was applied based on the guidelines of the Animal Care and Management Committee of the Suncheon National University, Republic of Korea.

Measurements and analyses

Body weight, feed intake, and feed conversion ratios were measured every two weeks. Analyses of carcass grade, fecal microbiology, and malodorous gas emissions were conducted at the end of the experiment as described subsequently.

Measurement of body weight, feed intake and feed conversion ratio

The body weight of pigs was measured every two weeks from the

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Table 1. Formula and chemical composition of basal diet for finishing pigs.

Ingredient	Amount (%)
Yellow Corn	45.15
Wheat	25.00
Wheat bran	4.00
Soybean meal	16.00
Limestone	0.78
Calcium phosphate	1.10
Salt	0.25
Vit-min. premix ¹⁾	0.55
Animal fat	2.50
Molasses	4.50
L-Lysine	0.17
Chemical composition²⁾	
ME (kcal/kg)	3,265.00
Crude Protein (%)	16.00
Ca (%)	0.50
Available P (%)	0.45
Lysine (%)	0.80
Methionine (%)	0.27

¹⁾Vit-min. mix provided the following nutrients per kg of premix: vitamin A, 6,000 IU; vitamin D₃, 800 IU; vitamin E, 20 IU; vitamin K₃, 2 mg; thiamin, 2 mg; riboflavin, 4 mg; vitamin B₆, 2 mg; vitamin B₁₂, 1 mg; pantothenic acid, 11 mg; niacin, 10 mg; biotin, 0.02 mg; Cu (copper sulfate), 21 mg; Fe (ferrous sulfate), 100 mg; Zn (zinc sulfate), 60 mg; Mn (manganese sulfate), 90 mg; I (calcium iodate), 1.0 mg; Co (cobalt nitrate), 0.3 mg; Se (sodium selenite), 0.3 mg² Calculated value.

initial day to the final day of the experiment to calculate body weight gain. The feed intake of pigs was recorded every two weeks by offering a weighed quantity of feed and weighing the residue. The feed conversion ratio was calculated by dividing feed intake by the body weight gain of pigs.

Measurement of meat carcass grade

Carcass quality traits in terms of slaughter weight, back fat thickness, and carcass grade were determined according to the Korean carcass grading system (Animal Products Grading Service, 1998).

Microbial analysis

For *Escherichia coli* and *Salmonella* enumeration, fecal sample of two pigs from each treatments were collected at the end of the experiment. Fresh fecal samples were collected from the rectum of the pigs in sterile polythelene bags. During fecal collection stimulation of internal and external sphincters were applied to avoid contamination of the samples. In brief, 1 g aliquots of feces from each treatment group were diluted in 10 ml of saline solution, after which 1 ml was serially diluted to make dilutions of 10¹ to 10¹¹. Microbial plates were then inoculated with three dilutions of each. Samples were plated on MacConkey Sorbitol Agar and Salmonella Shigella Agar for *E. coli* and *Salmonella*, respectively. Triplicate agar plates were then incubated at 37°C for 24 h. The total number of colonies for each *E. coli* and *Salmonella* were enumerated. Finally, the bacterial counts were expressed as log₁₀ cfu/ml.

Table 2. The name, strain and number of microflora used for fermentation of bamboo vinegar liquid with chemical composition.

Item	Contents
Number of microflora in BVLP ¹⁾	
<i>Lactobacillus acidophilus</i> KCTC 3111	4.2 × 10 ⁷ cfu/g
<i>Lactobacillus plantarum</i> KCTC 3104	5.8 × 10 ⁶ cfu/g
<i>Bacillus subtilis</i> , KCTC 3239	2.6 × 10 ⁷ cfu/g
<i>Saccharomyces cerevisiae</i> KCTC 7915	6.2 × 10 ⁹ cfu/g
Chemical composition (g/100g) ²⁾	
Moisture	8.98
Crude protein	17.73
Crude fat	2.90
Crude fiber	9.71
Crude ash	10.37

¹⁾BVLP=bamboo vinegar liquid probiotics. ²⁾Calculated chemical composition.

Gas measurements

At the end of the experiment, emissions of malodorous gases from feces were measured using a handheld GASTEC-GV 100 cylinder pump (Gastec Corporation, Japan). Specifically, ammonia (NH₃), hydrogen sulfide (H₂S), sulfur dioxide (SO₂), and total mercaptans (CH₃SH) were measured using individual gas detector tubes. Gastec detector tube (3 LA, 3M for NH₃; 4 LB, 4LK for H₂S; 5 LA for SO₂; 70L for Mercaptan). During measurement, the tube was open and 100 ml of headspace air was sampled approximately 2.0 cm above the sample surface. The concentration of lethal gases was expressed as ppm/100 ml.

Statistical analysis

The data obtained were analyzed using the SAS Package Program (1995) to estimate variance components for a completely randomized design. Duncan's multiple range tests (1955) were used to identify significant differences between treatment means. A P<0.05 was considered to indicate significance; while a tendency was considered at P<0.10.

RESULTS AND DISCUSSION

Growth performance

The effects of BVLP on growth performance are shown in Table 4. There were no significant differences in final body weight, weight gain, feed intake, or feed conversion ratio in response to the 0.2, 0.4 and 0.8% BVLP and antibiotic treatments (P>0.05). In a previous study, Sarker et al. (2010a) found that the same levels of BVLP had no effect on weight gain in Ross broilers. Pollmann et al. (1980) and Kim et al. (1993) reported that microbial additives had little effect on the feed provided to finishing pigs. In general, in the animal industry, the benefits of herbs and other plant extracts can help improve the amount of feed intake, digestive enzymes, and immunity (Wenk, 2003). Tsinas et al. (1998) found that the addition of oreganum essential oil led to increased feed intake and feed conversion ratio relative to that of a control group.

Table 3. Chemical composition of experimental feed for finishing pigs (%).

Parameter	Control	Antibiotics	BVLP ¹⁾		
			0.2%	0.4%	0.8%
Moisture	11.36 ± 0.05	11.72 ± 0.05	11.54 ± 0.04	11.43 ± 0.07	11.60 ± 0.04
Crude protein	17.91 ± 0.33	16.15 ± 0.65	16.77 ± 0.26	17.20 ± 0.64	15.52 ± 0.14
Crude fat	5.24 ± 0.08	5.88 ± 0.16	4.65 ± 0.24	5.58 ± 0.18	5.34 ± 0.28
Crude fiber	4.37 ± 0.27	4.54 ± 0.26	4.62 ± 0.27	4.39 ± 0.03	4.83 ± 0.27
Crude Ash	5.09 ± 0.23	4.00 ± 0.13	4.88 ± 0.19	4.95 ± 0.17	4.71 ± 0.35
NFE ²⁾	56.03 ± 0.43	57.70 ± 0.36	57.54 ± 0.11	56.45 ± 0.87	58.00 ± 0.05

Values are mean ± standard error and within the same row are not significantly different (P>0.05). ¹⁾BVLP: Bamboo vinegar liquid probiotics;

²⁾ NFE: nitrogen free extract

Table 4. Effects of dietary BVLP on the growth performance of finishing pigs.

Parameter	Control	Antibiotics	BVLP ¹⁾		
			0.2%	0.4%	0.8%
Initial weight (kg)	59.68 ± 1.16	62.02 ± 1.16	59.36 ± 1.51	58.71 ± 2.93	57.05 ± 1.60
Final weight (kg)	105.85 ± 1.39	106.36 ± 1.39	102.69 ± 1.87	104.67 ± 1.30	104.21 ± 2.33
Feed intake (kg)	146.53 ± 2.91	135.10 ± 2.91	149.00 ± 10.56	140.72 ± 3.57	133.48 ± 5.87
Weight gain (kg)	46.17 ± 2.28	44.34 ± 2.28	43.33 ± 1.10	45.95 ± 1.84	47.16 ± 1.06
FCR (Feed : Gain) ²⁾	3.20 ± 0.23	3.07 ± 0.19	3.45 ± 0.33	3.08 ± 0.20	2.83 ± 0.08

Values are mean ± standard error and within the same row are not significantly different (P>0.05). ²⁾FCR: Feed conversion ratio; ¹⁾BVLP: bamboo vinegar liquid probiotics.

Table 5. Effects of dietary BVLP on carcass grades of finishing pigs.

Parameter	Control	Antibiotics	BVLP ¹⁾		
			0.2%	0.4%	0.8%
Carcass weight (kg)	82.33± 1.46	79.20± 1.35	77.92± 1.61	80.10± 2.01	80.07± 1.16
Backfat thickness (mm)	23.58± 1.18	23.27± 1.08	21.00± 1.13	22.60± 0.76	21.43± 0.97
Carcass yield grade ²⁾	4.42± 0.23	3.93± 0.25	3.75± 0.25	4.10± 0.35	4.21± 0.26

Values are mean ± standard error and within the same row are not significantly different (P>0.05). Carcass Yield Grade: A-5, B-4, C-3, D-2; ¹⁾ BVLP: bamboo vinegar liquid probiotics.

Carcass grade

The effects of BVLP on the carcass grade of finishing pigs are shown in Table 5. The values of carcass weight, back fat thickness and carcass yield grade did not differ significantly, although a decreasing trend in backfat thickness was observed in 0.2% BVLP treated groups (P<0.10). There is a negative association between carcass quality grade and carcass yield grade to some extent (Moon et al., 2003). However, higher backfat thickness indicated better carcass grade according to Korean grading system (Moon et al., 2006); while for lean

meat demand, lower backfat thickness is the desired criteria for the consumers based on the US grading system (Boleman et al., 1998).

Fecal microbiology

E. coli is a rod shaped, Gram-negative, facultative anaerobe, lactose-fermenting, and non-endospore-forming microorganism. *E. coli* is the most common intestinal bacterium of the Enterobacteriaceae family and its presence outside the intestine is often used as an

Table 6. Effect of dietary BVLP on fecal microbiology in finishing pigs (log₁₀cfu/g).

Microbiology	Control	Antibiotics	BVLP ¹⁾		
			0.2%	0.4%	0.8%
<i>E. coli</i>	6.67 ^a ±0.36	5.18 ^b ±0.31	5.27 ^b ±0.38	5.09 ^b ±0.35	5.4 ^b ±0.25
<i>Salmonella</i>	7.46±0.81	6.20±1.05	6.03±0.99	5.79±1.01	6.26±0.95

^{a,b}Means with different superscripts within the same row are significantly different (P<0.05). BVLP¹⁾: Bamboo vinegar liquid probiotics.

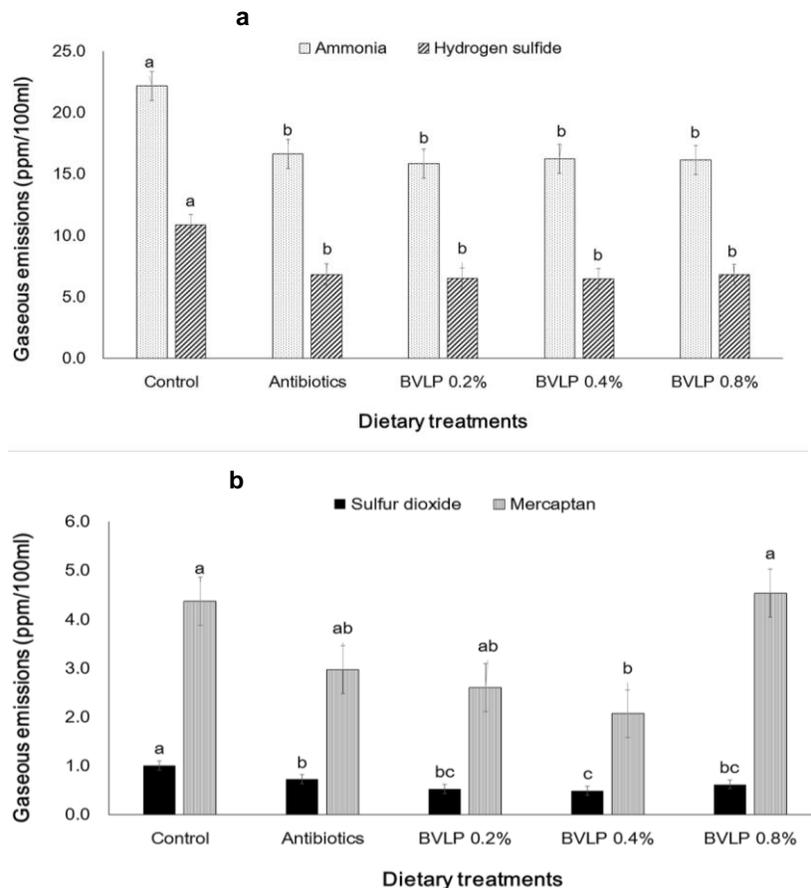


Figure 1. Effect of BVLP¹⁾ on fecal malodorous gas emissions (ppm/100 ml) from finishing pigs (a) ammonia and hydrogen sulfide gas; and (b) sulfur dioxide and mercaptan gas. ^{a,b,c}Means with different superscripts within the same bar are significantly different (P<0.05). Error bar indicated standard error. BVLP: Bamboo vinegar liquid probiotics.

indicator of fecal pollution and in surveillance programs of antimicrobial resistance (Wu et al., 2008). Screening and enumeration of antimicrobial resistant *E. coli* directly from samples is needed to identify emerging resistance and obtain quantitative data for epidemiological investigations (Aarestrup, 2004).

The effects of bamboo vinegar liquid on the fecal *E. coli* contents of finishing pigs are shown in Table 6. The *E. coli* and *Salmonella* content in feces decreased in response to all levels of BVLP and the antibiotic relative to the control. Although, the antimicrobial mechanisms are not clear, medicinal plant extracts are known to have wide antimicrobial activities. Chu et al. (2013) reported

the reduction of *Salmonella* after 0.3% bamboo vinegar supplementation in fattening pigs which support our current result of all level of BVLP supplementation. Guo et al. (2004) and Lucy (2002) reported that herb polysaccharides extract decreases *E. coli* to balance the number of intestinal microbes, which is similar to the results of the present study.

Fecal malodorous gas emissions

The effects of BVLP on fecal malodorous gases emissions were measured at 21 days of the experiment are as shown Figure 1a and b. The fecal ammonia (NH₃)

and hydrogen sulfide (H₂S) were significantly lower in the antibiotic, 0.2, 0.4 and 0.8% BVLP groups than the control (Figure 1a). Where sulfur dioxide (SO₂) and mercaptan (CH₃SH) gas was significantly (P<0.05) lower in the 0.4% BVLP relative to control and 0.8% BVLP group and no other significant differences were observed among other groups (Figure 1b). Owing to the concern of odor from swine operations, products that potentially reduce odor emissions are being marketed. Dietary manipulation can be effective nutritional approach to reduce gaseous emissions where many factors should be considered, such as manure composition, manure pH, temperature, organic material, and antibiotics in the diets are responsible for the emissions of malodorous gas by the feces of pigs (Jongbloed et al., 1999; Sutton et al., 1999). One of the best options to reduce odor emissions are dietary manipulations that decrease nitrogen, sulfur, and phosphorus excretion is low N, Cu and Zn supplementation in the diet (Van der Peet-Schwering et al., 1999). Changing of the diet through supplementation of BVLP might be responsible for diminishing the gaseous emissions through alteration of urea concentration and pH of slurry (Van der Peet-Schwering et al., 1999). In addition, fermentable carbohydrate and crude protein content after BVLP supplementation in the diet may have played an important role in reducing the gaseous emission from the slurry in the present experiment (Le et al., 2005). Since bamboo vinegar composed of acetic acid and phenolic compounds, therefore, the higher level of BVLP might trigger to increase the volatile fatty acids rather than decrease, by altering the pH and enzymatic actions (Lin et al., 2008; Chu et al., 2013); which was opined to be the reason of difference of SO₂ and CH₃SH between higher and lower level of BVLP in the current study. Higher level of BVLP increases the chance of availability of benzoic acid, which can interact with other dietary nutrients (methionine and sulfur) and increase the SO₂ and CH₃SH in comparison with the lower level of BVLP (Eriksen et al., 2010).

Conclusion

There were no significant differences in weight gain, feed intake and feed conversion efficiency of finishing pigs observed in response to the dietary supplementation of antibiotic and BVLP. In addition to that, the carcass weight and carcass yield grade did not differ significantly among the dietary treatments. However, backfat thickness tended to be decreased in the 0.2% BVLP supplemented group in comparison with control. Furthermore, fecal microbial analysis indicated that, fecal *E. coli* content was significantly suppressed after antibiotic and 0.2, 0.4 and 0.8% BVLP supplementation relative to the control. Furthermore, among malodorous gases, ammonia and hydrogen sulfide gas was significantly down trended in the antibiotic and 0.2, 0.4 and 0.8% BVLP supplemented groups, whereas no

significant differences were observed between antibiotic and BVLP supplemented groups. Significant diminution of the sulfur dioxide was found after antibiotic and 0.2, 0.4 and 0.8% BVLP supplementation as compared to the control, and the antibiotic and 0.4% BVLP group differed significantly. Moreover, the mercaptan gas was substantially decreased in the 0.4% BVLP supplemented group relative to the control and no significant differences were observed between the antibiotic and BVLP groups. In general, the current results indicated that BVLP as a dietary supplement has the potential to reduce the pathogenic fecal *E. coli* and fecal odorous gas emissions of finishing pigs without a significant negative impact on the performance. Further detailed research is required to observe the impact of BVLP on meat quality parameters.

Conflict of Interests

The authors have not declared any conflict of interests.

ACKNOWLEDGEMENT

This work (Grants No. C0193546) was supported by Business for Academic-Industrial Cooperative Establishments funded Korea Small and Medium Business Administration in 2014.

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