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Effects of Dietary Biochar Including Vinegar Liquid on Growth Performance, Nutrient Digestibility, Blood Characteristics and Fecal Noxious Gas Emission in Weaned Piglets

[1]J. Han, [1]F. Zhang, [1]L.B. Du, [1]X.H. Han, [2]W.F. Chen and [2]J. Meng
[1]College of Animal Science and Veterinary Science,
[2]Liaoning Biochar Engineering and Technology Research Center,
Shenyang Agricultural University, 110866 Shenyang, P.R. China

Abstract: Increasing pressure to sustain and improve environment has accelerated searching for feed additives with environmental and economic benefits in animal production. This experiment investigated the effect of dietary Biochar Including Vinegar Liquid (BIOV) supplements on weaned pigs growth performance, nutrient digestibility, blood characteristics and fecal noxious gas emission in a 28 days feeding trial. A total of 60 crossbred piglets with an initial BW of 8.07±0.29 kg (28 days of age) were randomly assigned to five dietary treatments with 4 replications per treatment and 3 pigs per pen in a completely randomized design. Dietary treatments included NC (Negative Control; basal diet), PC (Positive Control; basal diet+0.05% oxytetracycline), BV1 (basal diet+1% BIOV), BV2 (basal diet+2% BIOV) and BV3 (basal diet+3% BIOV). Although, PC treatment showed highest Average Daily Gain (ADG) and gain: feed these indices were not found differ with BV1 and BV2 treatments through the 28 days trial ($p<0.05$). The pigs in BV treatments presented better nitrogen digestibility than PC and NC group ($p<0.05$) while Dry Matter (DM) and Digestibility Energy (DE) were not affected by BV treatment ($p>0.05$). Also, the concentration of leucocytes, lymphocyte and IgG were optimal in PC treatment whereas there were no significant differ between antibiotic and BIOV additive ($p>0.05$). Meanwhile, all the BIOV added treatments decreased BUN and cortisol compared to NC treatment ($p<0.05$). Moreover, the fecal ammonia, hydrogen sulfide, amine, R.SH and acetic acid gas emissions were decreased, fecal pH was boosted in BV2 and BV3 treatments compared to PC and NC group ($p<0.05$). Therefore, the results of the present study suggest that BIOV is an animal feed additive with environmental and economic benefits which can be a potential alternative to antibiotics.

Key words: Biochar, fecal noxious gas, growth performance, blood characteristics, weaned pigs

INTRODUCTION

A great deal of public concerns have been focused on the large quantities of manure released by intensive animal production which lead to malodor through emission of noxious gas consisting primarily of volatilization of ammonia (NH_3), Hydrogen Sulfide (H_2S) as well as volatile organic compounds due to nutrients losses (Bustamante *et al*., 2012). Exposure to high level of noxious gas not only exerts adversely impact on well-being of animals and workers but creates considerable environmental issues (Ushida *et al*., 2003; Wang *et al*., 2008). As a result, several efforts have been made to search for application of natural substances as feed additive with environmental and economic benefits.

Carbonaceous adsorbents are the very natural substances which are made from agroforestry residue and characterized with adsorption capacity due to complex network of pores of various shape and size (Chen *et al*., 2008; Uchimiya *et al*., 2011) can prevent the noxious substances forming and absorbing in gastrointestinal tract as non-digestible carrier (Van *et al*., 2006). Many previous findings primarily focused on bamboo charcoal, (activated) charcoal have converged to suggest their abilities to enhance nutrient digestibility (Van *et al*., 2006; Leng *et al*., 2012) reduce toxicity of aflatoxin (Edrington *et al*., 1997; Teleb *et al*., 2004; Kana *et al*., 2010) lead to better growth performance (Hsu, 2011; Kana *et al*., 2011) modulate fecal microflora populations (Watarai and Tana, 2005; Chu *et al*., 2013a) decrease slurry noxious gas emission (Chu *et al*., 2013b) and improve immune responses (Chu *et al*., 2013a). On the other hand, vinegar liquid is byproduct during the preparation of carbonaceous adsorbents which is composed principally of >200 chemical components with acetic acid and phenolic compound being the main

Corresponding Author: J. Han, College of Animal Science and Veterinary Science, Shenyang Agricultural University, 110866 Shenyang, P.R. China

ingredients (Mu *et al*., 2004) and used to function as insecticide, bactericide and deodorant (Akakabe *et al*., 2006; Baimark and Niamsa, 2009). Recent research in animal production evidenced its efficiency in performance improvement (Kook *et al*., 2002, 2005; Wang *et al*., 2012; Yan *et al*., 2012; Chu *et al*., 2013a), noxious gas emission reduction (Yan *et al*., 2012) and apparent digestibility promotion (Yan *et al*., 2012) and fecal microflora regulation (Wang *et al*., 2012; Yan *et al*., 2012; Chu *et al*., 2013a). Remarkably, beneficial effects achieved when carbonaceous adsorbent and vinegar liquid were incorporated to diets in livestock production have gained extensive attention (Samanya and Yamauchi, 2002; Mekbungwan *et al*., 2004; Ruttanavut *et al*., 2009). All the aforementioned researches offer resoluble avenues for manure noxious gases without hindering performance.

Biochar as carbonaceous adsorbents produced by pyrolysis of various biomass under oxygen-limited conditions (Lehmann *et al*., 2006) has been closely involved in the environment-related issues caused by agricultural production and has gained considerable attention in decreasing or manipulating some odours related to swine manure (Doydora *et al*., 2011). Besides the advantage of territorial unlimited source of raw material, popular price and environmentally friendly production process, biochar also has specific characteristic of lower carbon content, more richer chemical composition including, better functional group, bigger specific surface as well as porosity which provided increased oxidation resistance and applicable habitat for microbial besides adsorptive power comparing to bamboo charcoal and activated charcoal. The earlier application attempting to demonstrate the beneficial effects of dietary Biochar Including Vinegar (BIOV®) produced by Jinhefu Agricultural Development Ltd. (Liaoning, China) on growth performance are not cited in the scientific literature but are used locally in China. In addition they act as antibiotic substitute and its effects on the emission of fecal noxious gas have not been determined. Therefore, it is interesting to evaluate how the environmental and economical benefits would be achieved to some extent after feeding the mixture.

In the herein study, we assessed whether BIOV inclusion to diet could substitute for antibiotics by examining effects on growth performance and nutrition digestibility in weanling pigs. Moreover, the fecal noxious gas emission was also carried out to evaluate the environmental benefit of the mixture in meat production.

MATERIALS AND METHODS

Preparation of biochar including vinegar: Biochar made from rice straw (BIO-R®) was prepared by Jinhefu Agricultural Development Ltd. (Liaoning, China). Briefly, rice straw was first air dried to lower moisture content below 16% and crushed to pass through 2 mm sieve and then was added onto the burner of semi-closed granule carbonization furnace for 10~25 cm high and ignited to carbonize at 150~450°C. Subsequently, remained straw material was supplemented for 6~12 cm every time when 450°C obtained to remove oxygen. Finally, a layer of made-up biochar was covered topside for 10~25 cm high and cool water was sprayed to put out the fire when the red-hot made-up biochar was observed visually. The procedures used to produce BIO-R are patented, China. The surface scanning image of rice straw and BIO-R demonstrating retained the basic structural characteristics of raw materials which has changed more smoothly and clearly was presented in Fig. 1 (TM-1000, Hitachi, Japan) and the primary physical and chemical characteristics were summarized in Table 1.

In the process of carbonization up to 700°C, the vapor was cooled to collect and kept for >1 year. Then,

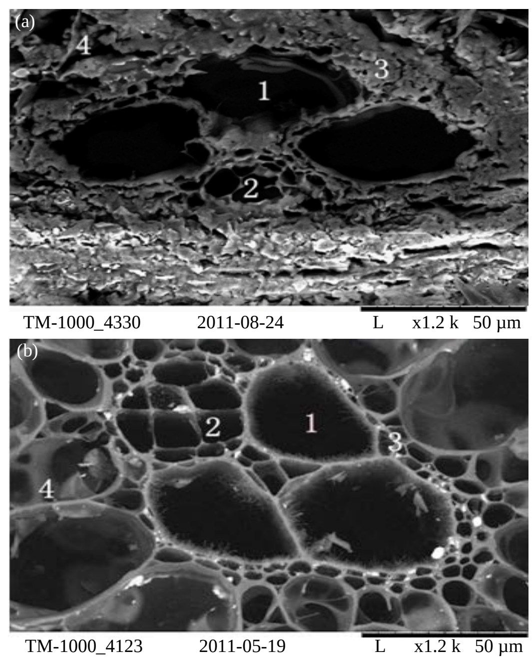

Fig. 1: The surface scanning image of a) rice straw and b) rice straw biochar; 1: xylem; 2: phloem; 3: bundle; 4: parenchyma cell

Table 1: Physical and chemical characteristics of biochar used in this study (% as is basis)

Items	Chemical composition (%)	Items	Physical parameters
Fixed carbon	66.00	pH	7.8
Volatile matter	23.00	Specific surface area	4.3 m^2/g
Ash	7.20	Porosity	0.018 mL g^{-1}
moisture	3.80	Average pore diameter	15 nm
Total humic acid	5.10	Total pore volume	0.021 mL g^{-1}
Nitrogen	0.12	Iodine adsorption value	2.1 mg g^{-1}

Table 2: Effects of dietary Biochar Including Vinegar Liquid (BIOV) on growth performance in weaned pigs

Items	Treatments					SEM	p-values
	NC	PC	BV1	BV2	BV3		
ADG (g)							
Days 0-14	294.050^{a}	330.480^{c}	321.190^{bc}	314.760^{abc}	303.100^{ab}	4.510	0.049
Days 14-28	531.900^{a}	615.710^{b}	580.470^{ab}	596.420^{b}	560.950^{ab}	9.950	0.040
Days 0-28	412.140^{a}	474.280^{c}	450.830^{bc}	458.810^{c}	429.880^{ab}	6.530	<0.010
ADFI (g)							
Phase 1 (days 0-14)	417.670	452.970	441.950	434.460	426.480	4.940	0.180
Phase 2 (days 14-28)	959.000	980.640	973.280	973.460	965.480	6.540	0.910
Overall (days 0-28)	688.330	716.800	707.610	703.960	695.980	4.550	0.360
Gain:Feed							
Phase 1 (days 0-14)	0.704	0.730	0.726	0.724	0.711	0.004	0.250
Phase 2 (days 14-28)	0.554^{a}	0.628^{b}	0.596^{ab}	0.613^{b}	0.581^{ab}	0.009	0.048
Overall (days 0-28)	0.614^{a}	0.637^{c}	0.629^{bc}	0.632^{c}	0.621^{ab}	0.007	<0.010

$^{a-c}$Means in a row with no common superscripts significantly differ ($p<0.05$)

the solution was distilled to remove the harmful substances such as tar. The vinegar mainly composed of acetic acid, propionic acid, butyric acid and phenol compounds as determined by gas chromatography assay. Vinegar for 2 kg was adsorbed into 8 kg of BIO-R to prepare the BIOV.

Animals, management, designing: All the procedures relating to the use of live animals were performed in accordance with the guidelines of Pig Production Association of Liaoning Province and were approved by Animal Care Committee of Shenyang Agricultural University (China).

A total of 60 piglets [(Landrance x Yorkshire) x Duroc] weaned at 28 days old with average initial body weight of 8.07±0.29 kg were given feeding trial lasting for 28 days. Pigs were allotted to 1 of 5 treatment groups using a completely randomized block design including NC (Negative Control; basal diet), PC (Positive Control; basal diet+0.05% oxytetracycline), BV1 (basal diet+1% BIOV), BV2 (basal diet+2% BIOV) and BV3 (basal diet+3% BIOV). There were 4 replicate pens per treatment and with 3 pigs per pen Table 2. The pigs were housed in an environmentally controlled confinement house (temperature = 26±2°C and relative humidity = 63±5%) with concrete-slotted floor. Each pen (2.0×2.1 m) was equipped with a one-sided self-feeder and a nipple drinker to allow the pig *ad libitum* access to feed and water throughout the experiment. The basal diet was based on maize and soybean meal presented in Table 3. Treatment additive was included in the basal diet at the expense of the same amount of wheat bran. The diets were formulated to meet or exceed the NRC recommendations for all nutrients.

Growth performance and blood characteristic analyses: The BW of pig was weighed individually and feed consumption was measured per pen on day 0, 14 and 28 of trial. These values were utilized in the determination of Average Daily Gain (ADG), Average Daily Feed Intake (ADFI) and gain:feed.

Table 3: Ingredient composition and nutrient contents of the basal diet

Items	Ingredient composition (%)
Corn	52.20
Soybean meal	21.60
Fish meal	4.50
Soybean protein concentrate	5.00
Wheat bran	4.00
Whey power	6.20
Soybean oil	3.00
Limestone	0.80
Dicalcium phosphate	1.00
Sodium chloride	0.20
L-Lysine·HCl (98%)	0.15
DL-Methionine (98%)	0.25
Choline chloride	0.10
Vitamin-mineral premix[1]	1.00
Nutrient content[2]	
Metabolizable energy (MJ kg^{-1})	13.59
Crude protein	21.15
Calcium	0.79
Total phosphorus	0.72
Lysine	1.36
Methionine	0.54

[1]Vitamin premix containing (per kg): vitamin A 12,000 IU; vitamin D3 3,000 IU; vitamin E 60 IU; vitamin K3 3 mg; thiamine 3 mg; riboflavin 7.5 mg; niacin 45 mg; pantothenic acid 30 mg; pyridoxine 4.8 mg; vitamin B12 0.04 mg; folic acid 1.2 mg; biotin 0.22 mg; Cu (copper sulfate) 15 mg; Zn (zinc sulfate) 120 mg; Fe (ferrous sulfate) 120 mg; Mn (manganese sulfate) 50 mg; I (calcium iodate) 0.6 mg; Se (sodium selenite) 0.3 mg; [2]Crude protein, calcium, phosphorus were analyzed values. The metabolizable energy and amino acid contents were calculated

On day 28 of trial, two shares of peripheral blood were sampled from the jugular veins of 8 pigs (2 pigs per pen) randomly selected from per treatment at 20 min after last feed. Approximately, 5.0 mL of blood samples were collected into non-heparinised vacuum tubes and centrifuged at 1,960×g for 15 min at room temperature and the serum was harvested. Thereafter, the samples were frozen and stored at at -20°C until further analysis. The serum concentrations of Immunoglobulin G (IgG) and cortisol were assayed using pig ELISA kits (R and D Inc., Los Angeles, CA, USA). The value of Total Protein (TP) and Blood Urea Nitrogen (BUN) were obtained with commercial assay kits (Nanjing Jianchen Biological Co., Ltd. Nanjing, China).

Another share was sampled with vacuum tubes with EDTA K_2 anti-coagulant for evaluation of whole blood.

The concentration of Red Blood Cell (RBC), White Blood Cell (WBC) lymphocyte and granuloctye in the whole blood were measured using an automatic blood analyzer (Uritest-3000, Youlite Co., Ltd. Guangxi, China).

Nutrition digestibility: The experimental diets were provided with 0.2% Chromic Oxide (Cr_2O_3) as an indigestible marker for 7 days prior to collection period to calculate the Coefficients of Total Tract Apparent Digestibility (CTTAD) of DM, Nitrogen (N) and GE. At the end of experiment, fresh fecal samples were collected from 3 pigs in each pen for 3 consecutive days. All the fecal and the feed samples were stored in a freezer at -20°C until analyses were performed. Before chemical analysis, the samples were thawed and dried at 70°C in an air-forced oven for 72 h and then were ground finely to pass through a 1 mm screen. The determination of DM, N and DE followed the procedures outlined by AOAC (2000). Chromium was analyzed via UV absorption spectrophotometry (Shimadzu, UV-1201, Kyoto, Japan). Nitrogen levels were assessed using Semi Micro Kjeldahl Method. GE was determined by measuring the combustion heat of samples using a calorimeter (IKA, C2000 basic, Staufen, Germany). The CATTD of DM, N and DE were calculated using indirect methods according to equation:

$$\text{Apparent digestibility (\%)} = 100\text{-}100\left[\frac{(E_1 \times F_2)}{(E_2 \times F_1)}\right]$$

Where:

E_1 and E_2 = The concentration of Cr in diet and fecal, respectively

F_1 and F_2 = The content of nutrient in diet and fecal, respectively

Fecal noxious gas emission: Two pigs were randomly selected from each pen to sample urine and feces, respectively which were stored immediately at -20°C until analysis. When used, the sampled urine and feces from each pig were thawed, homogenized and then mixed well in a 1:1 wt./wt. ratio according to the method described by Cho *et al.* (2008). The stock slurries in triplicate were stored in 2.6 L plastic boxes with a small hole in the middle of one side wall that was sealed with adhesive plaster. Two of the boxes being used to measure gas emission and one being for determining pH. The slurry was fermented for 24 h at room temperature (25°C) and then the gas was sampled using gas sampling pump (Gastec, GV-100S, Kanagawa-Ken, Japan) and Gastec detector tube No. 3 La for NH_3, No. 4 LL for H_2S, No. 70 for R.SH (Gastec, Kanagawa-Ken, Japan). When gas sampled, plastic box was shaken manually for ~30 sec in order to disrupt any crust formation on the surface of the slurry sample and to homogenize the samples. The adhesive plaster was then pulled down and 100 mL of headspace air ~2.0 cm above the slurry surface was sampled. The pH of this slurry was measured directly using a pH meter (Sartorius, PB-10, Gottingen, Germany).

Statistical analysis: The pen was used as the experimental unit for the analysis of growth performance whereas individual piglet was used as experimental unit for analysis of blood parameters and noxious gas emission. All data were statistically analyzed by one-way analysis of variance using the procedure of 16.0 Software. Differences among all treatments were separated by Duncan's multiple test. Results were expressed as means and Standard Error of the Means (SEM). Difference significance was taken at $p<0.05$ and there was tendency toward statistical significance if $p<0.10$.

RESULTS

Growth performance: Piglets in PC treatment gained the highest ADG and gain:feed which were not found differ with BV1 and BV2 treatments throughout the experimental period ($p>0.05$). Considering the effect following BIOV additive, better performance were achieved in 1% BIOV supplement during the first 14 days and 2% BIOV additive during the last 14 days, respectively. In addition, no significant difference was observed on ADFI among piglets fed experimental diet through the entire experimental period ($p>0.05$).

Nutrient digestibility: The pigs in BV treatments presented better nitrogen digestibility (Table 4) than PC and NC group ($p<0.05$) while Dry Matter (DM) and Digestibility Energy (DE) were not affected by BV treatment ($p>0.05$). DM digestibility were increased slightly by dietary BIOV treatment compared to CON treatment, however, there was no significant difference ($p>0.05$).

Blood characteristics: As presented in Table 5, although, no significant differences were noted on RBC, TP and granulocyte among the dietary treatments ($p>0.05$) the concentration of WBC, BUN as well as IgG were significantly affected ($p<0.05$) by experimental diets. The concentration of leucocytes, lymphocyte and IgG were optimal in PC treatment whereas these indicators were not found significant differ between antibiotic and BIOV additive ($p>0.05$). Meanwhile, all the BIOV added treatments decreased BUN and cortisol compared to NC treatment ($p<0.05$).

Table 4: Effects of dietary biochar including vinegar liquid on nutrient digestibility of weaned pigs

	Treatments						
Items (%)	NC	PC	BV1	BV2	BV3	SEM	p-values
DM	69.81	70.55	70.69	72.59	71.55	0.65	0.76
Nitrogen	68.34[a]	69.06[ab]	71.55[c]	72.25[c]	70.68[bc]	0.46	<0.01
DE	70.94	69.64	70.03	70.73	71.77	0.60	0.87

[a, b]Means in a row with no common superscripts significantly differ ($p<0.05$)

Table 5: Effects of dietary Biochar Including Vinegar liquid (BIOV) on blood characteristics of weaned pigs

	Treatments						
Items	NC	PC	BV1	BV2	BV3	SEM	p-values
Erythrocytes ($\times10^6$/mm^3)	5.43	5.27	5.66	5.18	5.36	0.07	0.210
Leucocytes ($\times10^3$/mm^3)	19.73[a]	22.05[b]	21.86[b]	21.45[b]	21.06[b]	0.27	0.020
Lymphocyte (%)	58.68[a]	64.73[b]	61.68[ab]	62.37[ab]	60.63[ab]	0.72	0.070
Granulocyte (%)	33.25	27.64	30.04	29.46	31.83	0.83	0.260
TP (g dL^{-1})	6.87	6.84	6.43	6.75	6.89	0.13	0.820
BUN (mg dL^{-1})	15.39[a]	12.60[b]	11.21[b]	10.95[b]	10.25[b]	0.55	<0.010
IgG (mg mL^{-1})	9.12[a]	13.23[c]	11.20[abc]	12.72[bc]	10.39[ab]	0.50	0.024
Cortisol (ng mL^{-1})	40.29[a]	37.45[b]	37.07[b]	36.31[b]	37.23[b]	0.50	0.080

[a-c]Means in a row with no common superscripts significantly differ ($p<0.05$)

Table 6: Effects of dietary Biochar Including Vinegar liquid (BIOV) on fecal noxious gas emission of weaned pigs

	Treatments						
Items	NC	PC	BV1	BV2	BV3	SEM	p-values
Fecal pH	6.421[a]	6.438[ab]	6.441[ab]	6.474[bc]	6.499[c]	0.009	0.017
Fecal gas emission (ppm)							
Ammonia	7.587[a]	7.463[a]	6170.000[b]	5.327[b]	5.240[b]	0.299	<0.010
Hydrogen sulfide	8.726[a]	8.494[a]	7.137[ab]	6.304[b]	5.746[b]	0.379	0.014
Amine	26.646[a]	27.026[a]	18.836[b]	15.913[b]	13.850[b]	1.599	<0.010
R·SH	15.066[a]	14.203[a]	12.678[ab]	10.789[b]	11.087[b]	0.562	0.026
Fecal votatile fatty concentration (μmol N^{-1})							
Acetic acid	88.217[a]	82.087[ab]	74.387[ab]	71.753[b]	69.229[b]	2.613	0.096
Propionic acid	57.870	53.513	54.740	50.070	47.883	3.949	0.959
Butyric acid	16.239	15.764	16.068	14.129	16.203	0.718	0.910
Valeric acid	9.229	9.612	9.067	8.854	8.541	0.492	0.980

[a-c]Means in a row with no common superscripts significantly differ ($p<0.05$)

Fecal noxious gas emission: Pigs fed with BIOV at 2 and 3% level evidenced significantly higher fecal pH ($p<0.05$) than NC and PC group (Table 6). The emission of fecal noxious gas such as ammonia (decreased by 29.79 and 30.34%, respectively) hydrogen sulfide (27.76 and 34.15%), amine (40.28 and 48.02%) as well as R·SH (28.39 and 26.41%) were significantly lower in BV2 and BV3 compared to the NC ($p<0.05$). Also, noxious gas emission in BV2 and BV3 appeared significant differ with PC treatment. The concentration of fecal votatile fatty including propinonic acid, butyric acid and valeric acid were not affected by BIOV at 2 and 3% additives besides acetic acid.

DISCUSSION

Along with the concerns related to the limited use of antibiotics demanding that alternative feed additives should be explored in animal production, carbonaceous adsorbents have been increasingly exploited as antibiotic alternatives and reported to have beneficial effects on livestock in recent years. Earlier report showed that super activated charcoal inclusion to the diet with Aflatoxins (AF) resulted in BW gains compared to the growing broilers fed AF (Edrington *et al*., 1997). Recent research regarding bamboo charcoal at 0.3 and 0.6% level in diet conducted by Chu *et al.* (2013b) suggested ADG acceleration and gain: feed improvement in fattening pigs which was as effectively as antibiotics treatment in Chu *et al*. (2013a) another research. A few of researchers demonstrated increased growth performance in livestock due to bamboo vinegar administration (Kook *et al*., 2002, 2005; Yan *et al*., 2012). Although, the foregoing findings have converged to suggest the role of promoted performance of adsorbents or vinegar, the relevant study of incorporating these two materials touches the experiment closely.

The relevant study in piglet conducted by Mekbungwan *et al.* (2004) assessed the tendency of improved feed efficiency and indicated Charcoal powder and Wood Vinegar (CCWV, 4:1) incorporated up to 3% level. The other reports in chick by Samanya and

Yamauchi (2002) reviewed that the CCWV incorporated at 1 and 3% level in diet was marginally effective in boosting ADG but was of little benefit on ADFI and feed efficiency. Ruttanavut *et al*. (2009) in ducks reported that the growth performance tended to be improved with 1% mixture of bamboo charcoal powder including vinegar liquid. It follows that previous reports concerning this mixture supplementation in livestock demonstrated a variety of results. In the present study, the optimal effect on weight gain of 1% BIOV inclusion to the diet of weaned pigs in the first 14 days and 2% BIOV on G:F besides ADG in the last 14 days were observed. Whereas, 3% BIOV maybe over-dosage and detrimental to the piglet. The tar smell of BIOV in slightly maybe result in unaffected ADFI due to sensitive taste and smell of piglets. In addition, BIOV performed a positive effect which was comparable with antibiotics treatment. Collectively, the differentiation results in dosage and effect of this mixture between above reports and the present report may be associated with the species of experimental animal and the mixture. These results also suggest that BIOV are potential substitutes of antibiotics.

The previous trials in ruminants conducted by Van *et al*. (2006) and Leng *et al*. (2012) have clearly confirmed a more important effect of single bamboo charcoal or biochar on nutrients digestibility. In the present study, the findings regarding increased N digestibility but unaffected CCTAD for DE and DM by dietary BIOV are in accordance with the recent findings by Yan *et al*. (2012) who evaluated the effects of bamboo vinegar in the diets of finishing pigs and found the positive effect of linearly increased N digestibility. This propose that vinegar liquid maybe research on thanks to acetic acid and phenolic compound as its main ingredients which have been verified to influence nutrient digestibility by modulating the balance of intestinal microflora by Kishi *et al*. (1999) and Shiomi *et al*. experiments. At the same time, abundant porosity and specific surface area that characterize biochar supply appropriate conditions for intestinal microflora (Sun *et al*., 2013). Improvement in N digestibility might be due to the coadjutant ingredients in the BIOV. However, what and how the underlying mechanism of regulating intestinal microbiota in pigs by dietary BIOV need further research. The result of this study also indicates that better performance partially due to a significant improvement in N digestibility.

The determination of blood cell and antibody aimed at assessing the ability of BIOV to resist pathogens in present results. Leukocytes and lymphocytes serving as prevalent indicators of immune response can recognize non-specifically different pathogens and can predict underlying infection. Likewise, IgG is the high levels of antibody switched from white blood cells when encountering continued antigen exposure. In the current study, considering the possible reason for an increase in circulating leukocytes number, lymphocyte ratio as well as the IgG concentration by BIOV maybe ascribe to the acetic acid and phenolic compound in the BIOV. These ingredients evidenced immunity enhancement and anti-disease properties via controlling the balance of intestinal microflora in rodents (Kishi *et al*., 1999) and human (Knekt *et al*., 2002). BUN as a waste during protein metabolism elevates when diseases and damaged kidneys occur due to the failure of clearing urea from the bloodstream (Johnson *et al*., 1972). Cortisol is released by hypothalamus-pituitary-adrenal in response to stress and lead to suppressed immune system (Johnson, 1997). Reduced BUN and cortisol concentration in piglets as a result of BIOV inclusion to diet might demonstrate its efficacy in protecting pigs from diseased. As a matter of fact, these results accord with the research conducted by Chu *et al*. (2013a, b) in which improved immune response in fattening pigs was obtained following bamboo charcoal or bamboo vinegar additive and propose a potential resistance to disease in piglets by dietary BIOV due to obviously increased of immune-related criterion.

The effect of carbonaceous adsorbents on harmful gases emission reduction has widely reported. Asada reported that bamboo charcoal decreased ammonia and benzene due to its pore volume and micro-pore range. Chu *et al*. (2013a, b) demonstrated the suppressed noxious gas emission from feces of fattening pig just because of the micro-pores in bamboo charcoal. Yan *et al*. (2012) reported that bamboo vinegar may be attributable to the fact that it exerts some positive effects on fecal noxious gas in finishing pigs by modulating intestine microbial population and increasing nutrient digestibility. As much as 2 and 3% dietary BIOV markedly decreased noxious gas emission including ammonia, hydrogen sulfide, amine and R·SH from piglets litter in current experiment which is consistent with foregoing reports. The improved N digestibility in the experiment may have significant environmental benefits through reduced amounts of feces ammonia Nitrogen (NH_3-N). The current observation response to BIOV may be associated with direct adsorption capacity and improving nutrient digestibility owing to physical function in the gastrointestinal tract by biochar and chemical function derived from vinegar.

CONCLUSION

The present study demonstrates that dietary BIOV could increase growth performance and nutrient digestibility while decrease feces noxious gas emission. BIOV maybe enhance the ability of immune response and anti-stress due to improved blood characteristics of weaned piglets. BIOV in 2% level is the appropriate additive with environmental and economic benefits which can be a potential alternative to antibiotics. However, there is side effect to piglets in 3% level. These results also suggest that optimal growth performance may be attributable in part to improved nutrient digestibility and feeding environment resulted by microflora modulation following BIOV additive. Further, research involving thorough understanding of how and why BIOV function on intestinal microflora will be the foundation for improving animal well-being and profitability by BIOV.

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