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Original research article

Effects of bamboo vinegar powder on growth performance and mRNA expression levels of interleukin-10, interleukin-22, and interleukin-25 in immune organs of weaned piglets

Yongjiu Huo [a, 1], Zhengxu Liu [a, 1], Han Xuan [a], Chunbin Lu [b], Lihuai Yu [a], Wenbin Bao [a], Guoqi Zhao [a, *]

[a] College of Animal Science and Technology, Yangzhou University, Yangzhou 225009, China
[b] Animal Disease Control and Prevention Centre of Jiangyin City, Jiangyin 214400, China

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ABSTRACT

The aim of this study was to explore the effects of bamboo vinegar powder on growth performance, diarrhea situation and mRNA expression levels of cytokines i.e., interleukin-10 (*IL-10*), interleukin-22 (*IL-22*), and interleukin-25 (*IL-25*) in immune organs of weaned piglets, and to accumulate theoretical data for the application of bamboo vinegar powder in weaned piglet production. Forty-five crossbred (Duroc × Landrace × Yorkshire, all male) weaned piglets with similar body weight (6.74 ± 0.17 kg) at 31 days of age were randomly assigned to 5 treatments with 3 replicates per treatment and 3 piglets in each replicate. The five treatments were as follows: CON (a basal diet), ANT (the basal diet + 0.12% antibiotics), BV1 (the basal diet + 0.1% bamboo vinegar powder), BV5 (the basal diet + 0.5% bamboo vinegar powder), BV10 (the basal diet + 1.0% bamboo vinegar powder). This experiment lasted 35 days. The growth performance and diarrhea situation were recorded. The relative mRNA expression levels of *IL-10*, *IL-22* and *IL-25* in liver, spleen, duodenum and mesenteric lymph nodes were detected by real-time PCR. Feed: gain of BV5 was significantly lower than that of CON ($P < 0.05$). In comparison with CON, diarrhea rate and diarrhea index of BV1 and BV5 all tended to decrease ($P < 0.1$). Compared with CON, mRNA expression level of *IL-10* in liver of ANT tended to be lower ($P < 0.1$) and these of BV1, BV5 and BV10 were significantly reduced ($P < 0.05$). The mRNA expression levels of *IL-10* in duodenum of ANT, BV1, BV5 and BV10 were all lower than those of CON, of which BV10 had significantly decreased *IL-10* mRNA expression in duodenum ($P < 0.05$). The mRNA expression levels of *IL-22* in duodenum of ANT, BV1, BV5 and BV10 all tended to be inhibited compared with CON ($P < 0.1$). With the increase of bamboo vinegar powder dosage, mRNA expression levels of *IL-25* in spleen and mesenteric lymph nodes of BV1, BV5 and BV10 tended to be up-regulated. Overall, bamboo vinegar powder could improve growth performance, and regulate mRNA expression levels of *IL-10*, *IL-22* and *IL-25* in immune organs of weaned piglets. The dosage at 0.5% showed optimum effects.

1. Introduction

Antibiotic is one of the major measures to prevent and treat diseases of digestive tract of piglets. However, antibiotics might cause bacterial resistance and suppression on pigs' immune function (Liu et al., 2013). Therefore, European Union passed the law to prohibit the usage of antibiotics in feed from 2006 and this restriction on antibiotics has also become a trend in China. Thus, it is valuable to search for eco-friendly material, which could regulate on immune system of pigs, to replace antibiotics for pig production

* Corresponding author.
E-mail address: guoqizhao2015@126.com (G. Zhao).
[1] These authors contributed equally to this work.
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(Dong et al., 2005). Bamboo vinegar product is one of the eco-friendly products focused by researchers.

Bamboo forest is known as “Second Forest” in China and the country also has the richest bamboo resource around the world. Nevertheless, the actual utilization efficiency of bamboo wood in China is only about 30% (Zhu et al., 2014). Bamboo vinegar is by product of bamboo charcoal industry, which is brown transparent liquid collected from condensed vapor during pyrolysis process of bamboo charcoal production with unique smoky aroma. It contains rich chemical components e.g., organic acids, phenolic compounds, ketones, aldehydes, alcohols and heterocyclic compounds, with pH at range of 2.5 to 3.0. Bamboo vinegar has been used as chemical fertilizers synergist, disinfection, deodorizer, antioxidant and antibacterial in agriculture and daily life (Cui and Wu, 2010). Yan et al. (2012) found that adding bamboo vinegar in diet could improve daily gain and feed conversion of fattening pigs. Also, blood lymphocytes percentage were significantly increased and intestinal *Escherichia coli* was drastically reduced in bamboo vinegar group. Ruttanavut et al. (2009) revealed that dietary bamboo charcoal powder including vinegar liquid tended to improve growth performance and intestinal mucosa morphology of Aigamo ducks. Jiang et al. (2013) reported that 1% bamboo vinegar in feed could relieve the small intestinal mucosa damage and decline of oxidation resistance of piglets caused by deoxynivalenol through improving gut barrier function and anti-oxidation ability.

Preservability of bamboo vinegar liquid is not great enough, which would limit the large-scale application of bamboo vinegar in feed industry. Hence, the bio-company cooperating with our research team converted bamboo vinegar liquid into solid powder with dextrin to enhance the application of bamboo vinegar in feed industry. Weaning stress would weaken immune response ability of piglets, resulting in an increase of diarrhea rate and a decrease of growth rate (Xun et al., 2015). Interleukin-10 (IL-10), interleukin-22 (IL-22) and interleukin-25 (IL-25) are the cytokines deeply connected with immune system of piglets (Yue et al., 2012; Craig et al., 2012). Moreover, there is no paper on direct effects of bamboo vinegar powder on post-weaning diarrhea (PWD) of weaned piglets until now. Therefore, the objective of this study was to evaluate the effects of bamboo vinegar powder on growth performance and diarrhea situation of weaned piglets. The effects of bamboo vinegar powder on *IL-10*, *IL-22* and *IL-25* mRNAs' expression of weaned piglets were also explored by real-time PCR for the first time. We hope our study could provide a theoretical basis for the application of bamboo vinegar powder in pig production.

2. Materials and methods

The experimental protocols describing the management and care of animals were carried out in accordance with the guidelines approved by the Animal Care and Use Committee of Yangzhou University.

2.1. Experimental design

Forty-five crossbred (Duroc × Landrace × Yorkshire) weaned piglets (all male, body weight 6.74 ± 0.17 kg) at 31 days of age were randomly assigned to 5 treatments according to live weight with 3 replicates (pens) per treatment and 3 piglets in each pen. The 5 treatments were as follows: CON (a basal diet), ANT (the basal diet + 0.12% compounding antibiotics i.e., 10% bacitracin zinc at 0.008%, 10% colistin at 0.004%, 10% roxarsone at 0.01% and carrier), BV1 (the basal diet + 0.1% bamboo vinegar powder), BV5 (the basal diet + 0.5% bamboo vinegar powder), BV10 (the basal diet + 1.0% bamboo vinegar powder). Antibiotics and bamboo vinegar powder were added to the basal diet by substituting carrier of the premix. The pigs in each pen received normal vaccine and disinfection procedures according to the pig farm's regulation, with free access to feed and drinking. The trial lasted for 35 days. The basal diet was a powder compound feed formulated referring to NRC (2012), composition and nutrient levels of which is shown in Table 1.

Table 1
Composition and nutrient levels of basal diet (air dry basis).

Item	Content
Ingredients, %	
Corn	62
Soybean meal	25
Wheat bran	8
Pre-mix[1]	5
Total	100
Nutrient level,[2] **%**	
Digestible energy, MJ/kg	13.05
Crude protein	17.41
Ash	4.51
Ca	0.61
Total phosphorus	0.68
Available phosphorus	0.35
Lysine	0.99
Methionine	0.39

[1] Per 1 kg premix provided VA 1,125,000 IU, VD_3 250,000 IU, VE 2000 mg, VK_3 204 mg, VB_1 207 mg, VB_2 600 mg, VB_6 246 mg, VB_{12} 2.5 mg, nicotinic acid 2475 mg, calcium pantothenate 1350 mg, folic acid 120 mg, biotin 5 mg and copper sulfate 19,500 mg, ferrous sulfate 22,500 mg, zinc sulfate 14,145 mg, manganese sulfate 4800 mg, calcium iodate (5%) 100 mg, sodium selenite (1%) 33 mg, cobalt chloride (1%) 5 mg.
[2] Nutrient levels were calculated values.

2.2. Experimental material

Bamboo vinegar powder was a product from Jiangyin Zhong Li Bio-tech Co., Ltd, and has undergone the following technological process: bamboo vinegar refined liquid + dextrin → low temperature spray drying → bamboo vinegar powder. Our research team tested its major components by gas chromatography–mass spectrography (GC–MS) in former experiment and the results showed that bamboo vinegar powder was composed of phenolic compounds (such as phenol, 2,6-dimethoxy, 2.77%), aldehydes [such as 2-furaldehyde, 5-(hydroxymethyl), 9.78%], ketones [such as 4-hydroxydihydro-2(3H)-furanone, 0.81%], organic acids (such as acetic acid, 2.26%), heterocycle compounds (such as 2-butyltetrahydrothiophene, 3.55%) and alcohol (such as furfuryl alcohol, 0.37%), having the potential to regulate growth performance of pigs (Liu et al., 2014).

2.3. Growth performance

The body weight of each piglet was recorded at 31 and 66 days of age under limosis, respectively. Feed consumption per replicate was recorded every day. The average daily feed intake (ADFI), average daily gain (ADG) and ratio of feed to gain (F:G) were calculated.

2.4. Diarrhea rate and diarrhea score

Diarrhea situation of piglets were observed one after another every 1400 h during the experiment. Diarrhea rate per replicate was calculated in accordance with the following formula:

$$\text{Diarrhea rate}\,(\%) = [(\text{number of piglets with diarrhea} \times \text{diarrhea days})/(\text{number of experimental piglets} \times \text{experimental days})] \times 100.$$

Diarrhea score per replicate was evaluated by feces in the pen, referring to rectum and mental status of piglets. A score of 0 represents normal and firm feces; 1 represents slight diarrhea; 2 represents definitely unformed and moderately fluid feces; and 3 represents very watery and frothy diarrhea (Zhang et al., 2013). Diarrhea score per replicate was calculated in accordance with the following formula:

$$\text{Diarrhea score} = \text{The sum of fecal score}/\text{number of experimental piglets}.$$

2.5. Immunity-related mRNAs' expression

At the end of the experiment (66 days of age of piglets), two piglets of each replicate (30 piglets in total) were randomly selected to be sacrificed. Afterwards, liver, spleen, duodenum and mesenteric lymph nodes were removed rapidly, and flushed with a 0.9% sodium chloride solution. Then tissue samples were snap-frozen in liquid nitrogen and stored at −80 °C pending analysis. The tissue samples were homogenized with tissue-tearor homogenizer (BioSpec Products Inc., USA), after which total RNAs were isolated by TRIzol reagent kit [TIANGEN Biotech (Beijing) Co., Ltd., China]. Reverse transcription PCR was operated according to instructions of PrimeScript RT Master Mix kit (Takara Bio Inc., Japan), applied for PCR programs as follows: amplification at 37 °C for 15 min, denaturation at 85 °C for 5 s, termination at 4 °C for saving. The cDNA was stored at −40 °C for later analysis.

Gene-specific primers were designed using Primer3 and BLAST with annealing temperatures of about 60 °C and synthesized by Invitrogen (Thermo Fisher Scientific Inc., USA). Parameters of primer pairs of *IL-10*, *IL-22*, *IL-25* and glyceraldehyde-3-phosphate dehydrogenase (*GAPDH*, reference gene) were shown in Table 2. Real-time PCR reaction was carried out using Roche FastStart Essential DNA Green Master kit, which was applied on Light Cycler 96 fluorescent quantitate PCR instrument (F. Hoffmann-La Roche Ltd, Switzerland) following such reaction conditions: pre-denaturation at 95 °C for 600 s; denaturation at 95 °C for 5 s; anneal at 60 °C for 10 s, extension at 72 °C for 10 s; 45 cycles for one reaction.

2.6. Calculation and statistics

For gene expression, all samples were assayed in triplicate and data were normalized to the reference gene (*GAPDH*). Relative gene expression was calculated and analyzed by the $2^{-\triangle\triangle Ct}$ (Kenneth and Thomas, 2001).

Data were analyzed using one-way ANOVA of SPSS 19.0 (SPSS, 2010. User's tutorial. Version 19.0. IBM Corporation, Armonk, NY), and differences among the treatment means were tested using LSD method, with a $P < 0.1$ indicating difference trend, a $P < 0.05$ indicating significance and $P < 0.01$ indicating high significance. All the data were expressed as mean ± standard error (SE).

3. Results

3.1. Effects of bamboo vinegar powder on growth performance of weaned piglets

Final weight of ANT tended to increase compared with CON ($P < 0.1$); final weight of bamboo vinegar powder experimental groups all increased compared with CON and had no significant difference from ANT. In comparison with CON, ADG of ANT and BV1 tended to increase ($P < 0.1$). The ADG of bamboo vinegar powder experimental groups had no significant difference from ANT. In comparison with CON, F:G of BV5 was significantly decreased ($P < 0.05$) and F:G of BV1 tended to decrease ($P < 0.1$) (Table 3).

3.2. Effects of bamboo vinegar powder on diarrhea situation of weaned piglets

In comparison with CON, diarrhea rate of ANT, BV1 and BV5 all tended to decline ($P < 0.1$); diarrhea score of BV1 and BV5 also tended to decrease ($P < 0.1$) (Table 4).

3.3. mRNA expression levels of immunity-related genes

3.3.1. Effects of bamboo vinegar powder on relative mRNA expression levels of immunity-related genes in liver

Known from Fig. 1, mRNA expression level of *IL-10* in liver of ANT tended to be lower than that of CON ($P < 0.1$). In comparison with CON, mRNA expression levels of *IL-10* in liver of BV1 and BV5 were significantly decreased ($P < 0.05$); mRNA expression level of *IL-10* in liver of BV10 was highly significantly decreased ($P < 0.01$). The mRNA expression levels of *IL-22* and *IL-25* in liver were under the limit of detection and thus not detected.

3.3.2. Effects of bamboo vinegar powder on relative mRNA expression levels of immunity-related genes in spleen

Known from Fig. 2, mRNA expression level of *IL-10* in spleen of BV10 tended to be lower than that of ANT ($P < 0.1$); mRNA expression level of *IL-10* in spleen of BV10 was significantly decreased compared with BV1 and BV5 ($P < 0.05$). Compared with CON, mRNA expression level of *IL-25* in spleen of ANT tended to increase ($P < 0.1$). The mRNA expression level of *IL-25* in spleen of

Table 2
Parameters of primer pairs for *IL-10*, *IL-22*, *IL-25*, *GAPDH* genes of pigs.

Gene	Genbank accession No.	Primer sequence (5′ to 3′)	Product, bp
IL-10	NM_214041.1	F: CTGACTGCCTCCCACTTTCT R: TTTGGGGAATGAGGTCAGGG	150
IL-22	XM_001926156.1	F: AACTTCCAGCAGCCCTACAT R: TGGGGAACAGCACTTCTTCA	184
IL-25	XM_005666258.1	F: GCCCCTTGGAGATACGAGTT R: CGGTAGAAGACGGTCTGGTT	164
GAPDH	NM_001206359.1	F: ACATCATCCCTGCTTCTACTGG; R: CTCGGACGCCTGCTTCAC;	187

IL-10 = interleukin-10; *IL-22* = interleukin-22; *IL-25* = interleukin-25; *GAPDH* = glyceraldehydes phosphate dehydrogenase.

Table 3
Effects of bamboo vinegar powder on growth performance of weaned piglets[1].

Item	CON	ANT	BV1	BV5	BV10
Initial weight, kg	6.51 ± 0.38	7.04 ± 0.34	6.79 ± 0.46	6.71 ± 0.28	6.57 ± 0.53
Final weight, kg	16.53 ± 1.07	19.66 ± 1.20	19.09 ± 0.97	18.66 ± 0.94	18.94 ± 1.55
ADG, g/d	286.48 ± 32.16	360.49 ± 30.06	351.36 ± 19.72	341.47 ± 20.88	353.60 ± 34.11
ADFI, g/d	605.65 ± 79.02	704.98 ± 12.34	650.86 ± 14.79	625.20 ± 54.42	678.53 ± 13.28
F:G	2.20 ± 0.19[a]	1.94 ± 0.04[ab]	1.85 ± 0.07[ab]	1.81 ± 0.13[b]	1.94 ± 0.05[ab]

[a,b] In the same row, values with no letter or the same letter superscripts mean no significant difference ($P > 0.05$), while with different small letter superscripts mean significant difference ($P < 0.05$).
[1] CON: a basal diet; ANT: the basal diet + 0.12% compounding antibiotics i.e., 10% bacitracin zinc at 0.008%, 10% colistin at 0.004%, 10% roxarsone at 0.01% and carrier; BV1: the basal diet + 0.1% bamboo vinegar powder; BV5: the basal diet + 0.5% bamboo vinegar powder; BV10: the basal diet + 1.0% bamboo vinegar powder.

Table 4
Effects of bamboo vinegar powder on diarrhea situation of weaned piglets[1].

Item	CON	ANT	BV1	BV5	BV10
Diarrhea rate, %	52.70 ± 10.81	29.21 ± 5.01	27.94 ± 10.17	26.51 ± 8.31	46.19 ± 3.33
Diarrhea score	23.86 ± 8.75	11.39 ± 0.48	9.94 ± 3.75	10.03 ± 5.25	15.83 ± 2.83

[1] CON: a basal diet; ANT: the basal diet + 0.12% compounding antibiotics i.e., 10% bacitracin zinc at 0.008%, 10% colistin at 0.004%, 10% roxarsone at 0.01% and carrier; BV1: the basal diet + 0.1% bamboo vinegar powder; BV5: the basal diet + 0.5% bamboo vinegar powder; BV10: the basal diet + 1.0% bamboo vinegar powder.

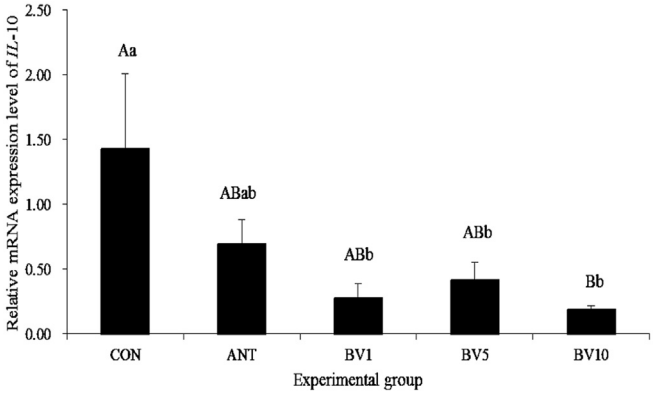

Fig. 1. Effects of bamboo vinegar powder on relative mRNA expression level of interleukin-10 (*IL-10*) gene in liver. Bars with no letter or the same letters mean no significant difference ($P > 0.05$), while with different small letters mean significant difference ($P < 0.05$), and with different capital letters mean highly significant difference ($P < 0.01$). CON: a basal diet; ANT: the basal diet + 0.12% compounding antibiotics i.e., 10% bacitracin zinc at 0.008%, 10% colistin at 0.004%, 10% roxarsone at 0.01% and carrier; BV1: the basal diet + 0.1% bamboo vinegar powder; BV5: the basal diet + 0.5% bamboo vinegar powder; BV10: the basal diet + 1.0% bamboo vinegar powder.

ANT was significantly higher than that of BV1 ($P < 0.05$). The mRNA expression level of *IL-22* in spleen was under the limit of detection and thus not detected.

3.3.3. Effects of bamboo vinegar powder on relative mRNA expression levels of immunity-related genes in duodenum

As shown in Fig. 3, mRNA expression levels of *IL-10* in duodenum of ANT and bamboo vinegar experimental groups were all lower than those of CON, of which BV10 showed a significant decrease ($P < 0.05$). Compared with CON, mRNA expression levels of *IL-22* in duodenum of ANT, BV1, BV5 and BV10 all tended to decline ($P < 0.1$), of which ANT showed a significant decline ($P < 0.05$). The mRNA expression level of *IL-25* in duodenum was under the limit of detection and thus not detected.

3.3.4. Effects of bamboo vinegar powder on relative mRNA expression levels of immunity-related genes in mesenteric lymph nodes

As illustrated in Fig. 4, there were no significant differences in the mRNA expression levels of *IL-10*, *IL-22* and *IL-25* in mesenteric

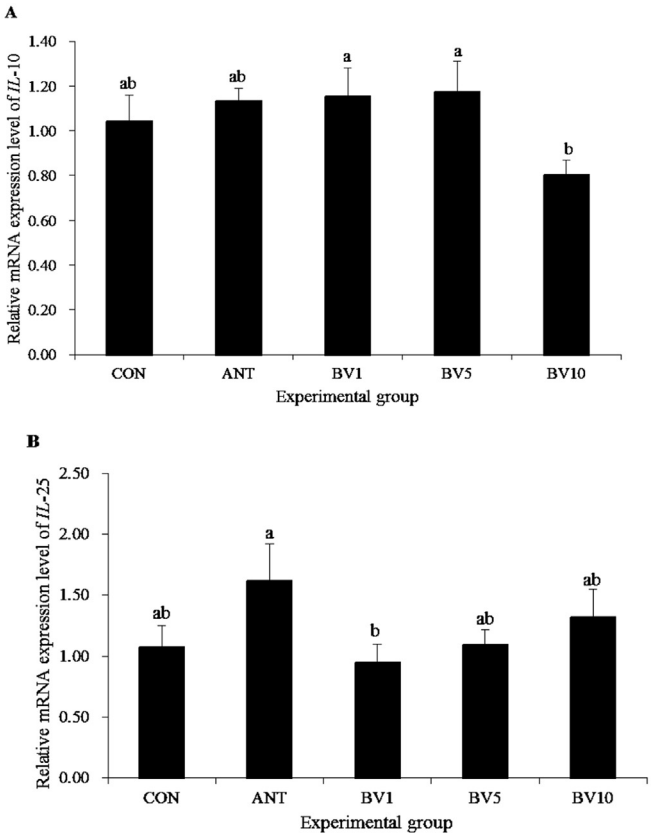

Fig. 2. Effects of bamboo vinegar powder on relative mRNA expression levels of (A) interleukin-10 (*IL-10*) and (B) interleukin-25 (*IL-25*) genes in spleen. Bars with no letter or the same letters mean no significant difference ($P > 0.05$), while with different small letters mean significant difference ($P < 0.05$), and with different capital letters mean highly significant difference ($P < 0.01$). CON: a basal diet; ANT: the basal diet + 0.12% compounding antibiotics i.e., 10% bacitracin zinc at 0.008%, 10% colistin at 0.004%, 10% roxarsone at 0.01% and carrier; BV1: the basal diet + 0.1% bamboo vinegar powder; BV5: the basal diet + 0.5% bamboo vinegar powder; BV10: the basal diet + 1.0% bamboo vinegar powder.

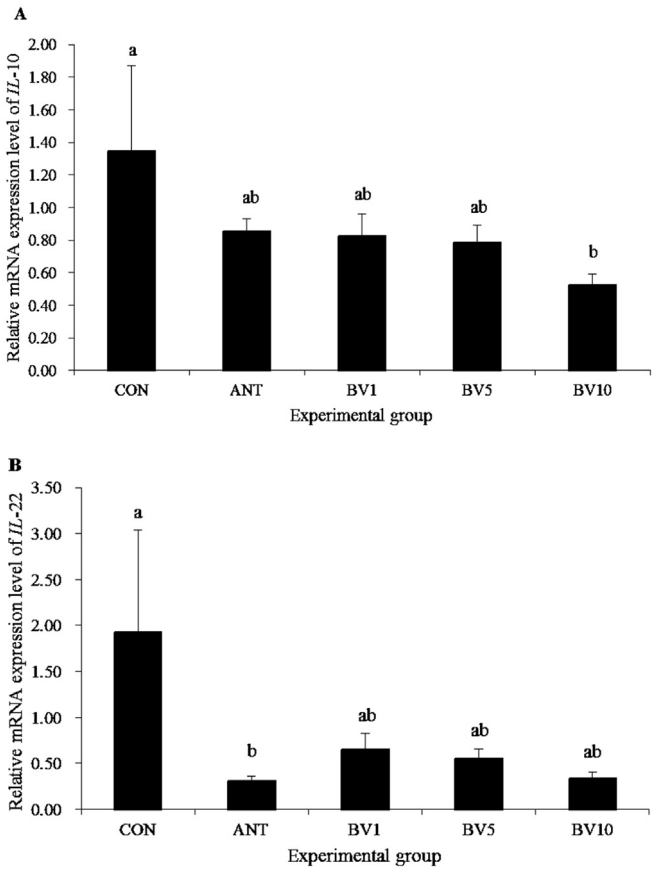

Fig. 3. Effects of bamboo vinegar powder on relative mRNA expression levels of (A) interleukin-10 (*IL-10*) and (B) interleukin-22 (*IL-22*) genes in duodenum. Bars with no letter or the same letters mean no significant difference ($P > 0.05$), while with different small letters mean significant difference ($P < 0.05$), and with different capital letters mean highly significant difference ($P < 0.01$). CON: a basal diet; ANT: the basal diet + 0.12% compounding antibiotics i.e., 10% bacitracin zinc at 0.008%, 10% colistin at 0.004%, 10% roxarsone at 0.01% and carrier; BV1: the basal diet + 0.1% bamboo vinegar powder; BV5: the basal diet + 0.5% bamboo vinegar powder; BV10: the basal diet + 1.0% bamboo vinegar powder.

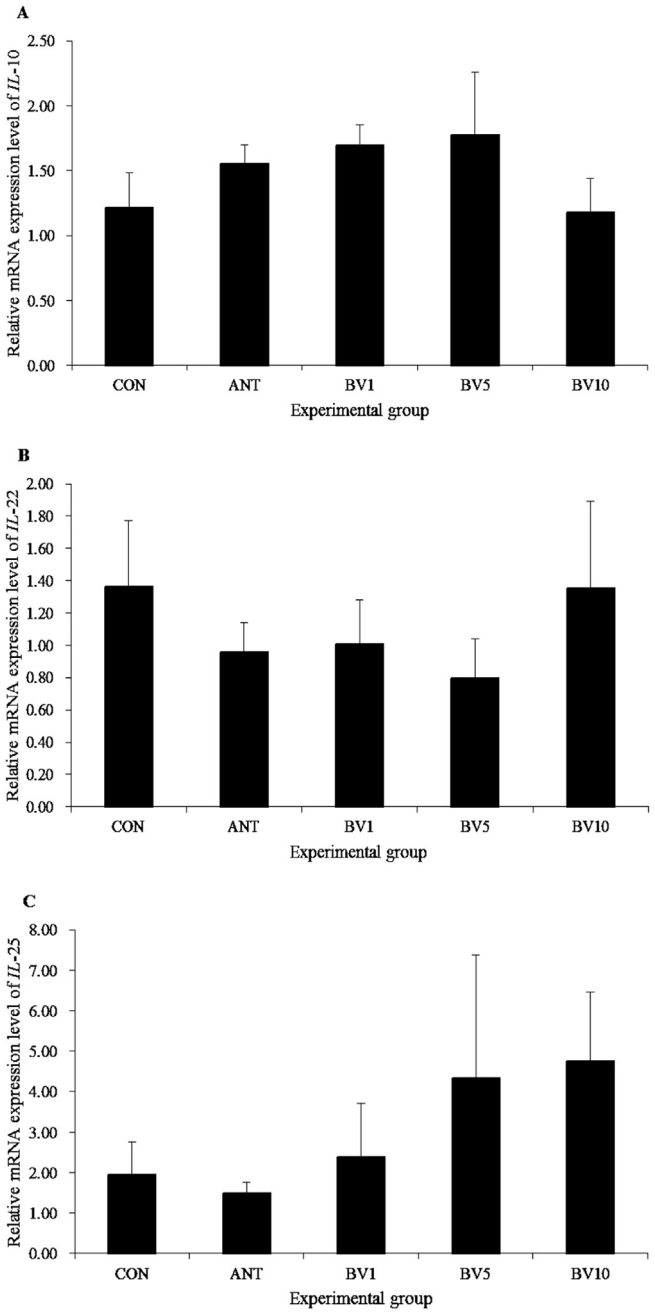

Fig. 4. Effects of bamboo vinegar powder on relative mRNA expression levels of (A) interleukin-10 (*IL-10*), (B) interleukin-22 (*IL-22*) and (C) interleukin-25 (*IL-25*) genes in mesenteric lymph nodes. Bars with no letter or the same letters mean no significant difference ($P > 0.05$), while with different small letters mean significant difference ($P < 0.05$), and with different capital letters mean highly significant difference ($P < 0.01$). CON: a basal diet; ANT: the basal diet + 0.12% compounding antibiotics i.e., 10% bacitracin zinc at 0.008%, 10% colistin at 0.004%, 10% roxarsone at 0.01% and carrier; BV1: the basal diet + 0.1% bamboo vinegar powder; BV5: the basal diet + 0.5% bamboo vinegar powder; BV10: the basal diet + 1.0% bamboo vinegar powder.

lymph nodes among all these groups. The mRNA expression levels of *IL-10* in mesenteric lymph nodes of ANT, BV1 and BV5 were all higher than those of CON, while that of BV10 was lower than that of CON. The mRNA expression levels of *IL-22* in mesenteric lymph nodes of ANT, BV1, BV5 and BV10 were all lower than those of CON. As the dosage increased, mRNA expression levels of *IL-25* in mesenteric lymph nodes of BV1, BV5 and BV10 tended to be up-regulated.

4. Discussion

4.1. Effects of bamboo vinegar powder on growth performance of weaned piglets

Jessada (2014) added combined product of bamboo charcoal powder and bamboo vinegar at dosages of 0, 0.5%, 1% and 1.5% to the feed of Betong chickens and found that 1% group showed optimum growth performance. This researcher proposed that combined product of bamboo charcoal powder and bamboo vinegar could be applied in Betong chicken production to enhance growth. Choi et al. (2009) reported that ADG and feed conversion ratio of pigs fed 0.2% wood vinegar were evidently improved compared with control group and the improvement was only second to antibiotics group. Moreover, wood vinegar experimental group showed optimum ADFI. In our study, growth performances of weaned piglets in bamboo vinegar powder experimental groups were evidently improved, with no significant difference from antibiotics group. This manifested that bamboo vinegar powder showed growth-enhancing effects on weaned piglets, which could match antibiotics. Especially, F:G of BV5 was significantly lower than that of CON, indicating that 0.5% might be the proper dosage.

Wang et al. (2012) found that 0.4% bamboo vinegar added in feed of weaned piglets showed optimum growth-enhancing effects and the effect could also match antibiotics, which was consistent with our study.

4.2. Effects of bamboo vinegar powder on diarrhea situation of weaned piglets

Our experiment proved that bamboo vinegar powder at dosage of 0.1% and 0.5% could effectively improve the diarrhea situation of weaned piglets while bamboo vinegar powder at dosage of 1.0% showed poor effects on controlling diarrhea, which might be due to that balance of intestinal microflora was broken by the over-high dosage. *E. coli* was one of the major pathogenic bacterium of PWD. Benkeblia et al. (2005) revealed that phenolic compounds had favorable antibacterial effect and could be used as antibacterial additives for food. We mentioned in former part of experimental material that bamboo vinegar powder was rich in phenolic compounds. Yan et al. (2012) proved that bamboo vinegar added in feed of fattening pigs could significantly decrease the quantity of *E. coli* in faeces, resulting from the rich phenols, ketones and organic acids, etc. contained in bamboo vinegar product, which had potent antibacterial effect. The decrease of diarrhea rate and diarrhea score of bamboo vinegar powder groups might be mainly related to this, which also corresponded to the improvement of growth performance in these groups. On the other hand, Wang et al. (2012) found that richness and diversity index of fecal bacterial communities of pigs fed high level bamboo vinegar were reduced, indicating that buffering capacity of intestinal microflora might be decreased by bamboo vinegar at high dosage. This might be the reason that bamboo vinegar powder at dosage of 1.0% exerted poor effects on restrain diarrhea.

4.3. Expression levels of immunity-related genes

Even though mRNA changes do not always correlate with protein changes of cytokines due to post-transcriptional regulation, there are significant correlations between mRNA and protein expression changes of cytokines (Hiroyuki et al., 2014). Hence we measured mRNA expression levels of immunity-related cytokines by to reveal the effect on immunity of piglets by bamboo vinegar powder.

4.3.1. IL-10

Interleukin-10 was primarily called cytokine synthesis inhibiting factor (CSIF), discovered by Mosmannand in 1989 (Kim et al., 1992). Interleukin-10 was an important negative-regulating cytokine with relatively strong inflammation suppression capacity, mainly produced by monocytes, helper T cells 2 (Th2) and activated mast cells and B cells. Interleukin-10 could effectively block inflammatory response and immune regulation development and inhibit expression of cyclooxygenase and nitric oxide (NO) synthase induced by inflammation-related enzyme in macrophage. Furthermore, IL-10 could reduce activity of natural killer cell via inhibiting the production of interferon-γ (IFN-γ) and suppress the immunological effect of T cell via inducing immunosuppression and peripheral tolerance. In addition, IL-10 showed immune stimulation on mast cells and B cells (Swidsinski et al., 2002). The exact molecular mechanism of immunosuppressive action by IL-10 is still under research, e.g., El Kasmi et al. (2006) proved that activation of signal transducers and activator of transcription3 (STAT3) involved with IL-10 receptor was important for IL-10 anti-inflammatory process in cells derived from myeloid cells, by *in vitro* experiment and *in vivo* experiment using mice deficient in STAT3. In one research by Li et al. (2007), *IL-10* mRNA in pulmonary alveolar macrophage (PAM) of piglets was at high expression level after co-infection by porcine reproductive and respiratory syndrome virus (PRRSV) and porcine circovirus type2 (PCV2). This researcher argued that IL-10 might affect antigen presentation function of PAM through inhibiting the transcription of *IFN-γ* mRNA, and suppress immune-response capacity of helper T cell 1 (Th1), resulting in uncontrolled status of immune modulating function of PAM. Ma (2012) reported that chicken was under immunosuppression and serum IL-10, IFN-γ, interleukin-4 (IL-4) and transforming growth factor-β (TGF-β) were elevated after infection by infectious bursal disease virus (IBDV). After injection of immunopotentiator and smoked plum flavonoids, serum IL-10 content was decreased. Brice et al. (2013) reported that *Entada africana* extract could inhibit production NO while increase mRNA expression levels of *IL-10* and interleukin-13 (*IL-13*). Edmunds et al. (2011) found that kiwifruit extract, which was rich in phenols and organic acids, could decrease the production of IL-10 and NO in macrophages and intestinal epithelial cells. This study manifested expression levels of *IL-10* mRNA in liver and duodenum of ANT and bamboo vinegar powder groups were all lower than those of CON. This indicated that bamboo vinegar powder, as antibiotics, inhibited the expression of *IL-10* mRNA and relieved immunosuppression of liver and duodenum, strengthening immune-response ability and improving health status of weaned piglets.

Besides, mRNA expression levels of *IL-10* in spleen and mesenteric lymph nodes of BV10 (dosage of bamboo vinegar powder at 1.0%) were significantly decreased while those of ANT and the other two bamboo vinegar powder groups were all increased. Former results illustrated that diarrhea rate and diarrhea score of BV10 were higher. Gabriela et al. (2013) revealed that expression level of *IL-10* in colonic mucosa of patients during convalescence from ulcerative colitis (UC) was significantly higher than patients during active phase and non-inflammatory patients. Liu et al. (2011) pointed out that IL-10 secreted by antigen-presenting cell could regulate the self-balancing immune response to gut commensal bacteria by T cell. The function of IL-10, inflammation suppression or induction, depended on factors like the type and concentration of antigen for IL-10 in the microenvironment. Thus, the dosage of bamboo vinegar powder at 1.0% might be over-dosage and abnormally decrease mRNA expression level of *IL-10*, which then could not exert function of inflammation suppression sufficiently and result in an increase of diarrhea rate.

4.3.2. IL-22

Interleukin-22 was firstly discovered by Dumoutier et al. (2000), who researched on differentially expressed genes of T-lymphoma cell stimulated by IL-9. Interleukin-22 is a member of IL-10 cytokine family, mainly produced by activated helper T cell 22 (Th22), helper T cell 17 (Th17) and natural killer cell 22 (NK22). Interleukin-22 played important roles in inflammatory disease process through combination with IL-22 receptor complex consisting of IL-10 receptor 2 (IL-10R2) and IL-22 receptor 1 (IL-22R1) (Xu et al., 2001; Wolk et al., 2002). Wolk et al. (2007) found that expression level of *IL-22* in inflammatory bowel and mesenteric lymph nodes of colitis mouse model was increased while the constitutive expression of IL-22-binding protein was decreased. Moreover, the researcher reported that IL-22 levels were increased in the blood of Crohn's disease (CD) patients compared with normal subjects and the level was obviously related to the severity of the disease. At present, functional mechanism of IL-22 was further studied in the disease model of psoriasis. The mRNA expression level of *IL-22* in lesional skin area of psoriasis patient was up-regulated, however, mRNA expression level of *IL-22* in normal skin of healthy person was very low (Guilloteau et al., 2010). Hwang et al. (2009) found that gene expression level of *IL-22* was significantly up-regulated

and this stimulated cell inflammatory response in the gliocytes stimulated by lipopolysaccharide (LPS), using microarray analysis technology. Natsuko et al. (2012) reported that gene expression level of *IL-22* in Peyer's patch of mouse was increased after infusion with alliin. Jency et al. (2014) studied the effects of dietary resveratrol supplementation on gene expression in the hippocampus of streptozotocin-induced diabetic C57Bl/6 mice and discovered that resveratrol supplementation significantly decreased the expression levels of *IL-22* and most of other genes belonging to janus kinase-signal transducer and activator of transcription (Jak-Stat) pathway.

In our research, mRNA expression levels of *IL-22* in duodenum, mesenteric lymph nodes of ANT and bamboo vinegar powder experimental groups were all decreased, manifesting that bamboo vinegar powder might, like antibiotics, restrain expression of *IL-22* gene in gut system and thus suppress inflammation and control diarrhea. Our finding was consistent with Jency et al. (2014). Interleukin-22 had target distribution in digestive tract, skin and respiratory organs and played important roles in mucosal innate immunity (Arasteh et al., 2010). The mRNA expression levels of *IL-22* in liver and spleen were under the limit of detection in our study, consisting with the finding above.

4.3.3. *IL-25*

Interleukin-25 was primarily identified and found by Fort et al. (2001) in 2001, which was also called interleukin-17E (IL-17E). Interleukin-25, belonging to interleukin-17 (IL-17) family, shared minimum homology with IL-17A among the cytokines in this family. Hence, biological function of IL-25 was rather different from other cytokines of IL-17 family. Therefore, researchers paid extensive attention to IL-25. Interleukin-25 could initiate, promote and enhance immunoreaction mediated by Th2 cells. It could also suppress the immunoreaction of Th1 and Th17 cells, which could then inhibit the immune-mediated diseases such as colonitis, experimental autoimmune encephalo myelitis and diabetes (Giovanni et al., 2010). Interleukin-25 gene knockout mice had serious intestinal inflammation since that it could not resist to *Trichuris muris*, of which secretions of IL-17A and IFN-γ were significantly increased (Owyang et al., 2006). A researcher found that IL-25 could restrain the inflammatory reaction mediated by toll-like receptor ligand in blood mononuclear cells and intestinal CD14+ cells. Further, IL-25 could also inhibit the increase of expression level of interleukin-23 (*IL-23*) in macrophages after stimulation by LPS. The researcher proposed that IL-25 could inhibit the production of cytokines related to Th1 and might help cure the colitis in mice caused by bacteria (Caruso et al., 2009). Chen (2012) proved that serum IL-25 contents of UC and CD patient were both significantly lower than those of control group, which was negatively correlated with the severity of the disease. This manifested that IL-25 might relieve inflammatory reaction in inflammatory bowel disease. Our study suggested that mRNA expression levels of *IL-25* in spleen and mesenteric lymph nodes of bamboo vinegar powder experimental groups tended to be up-regulated as the dosage increased, indicating that bamboo vinegar powder might inhibit the intestinal inflammation mediated by Th1 cell of weaned piglets via up-regulating *IL-25*. This might improve intestinal immunologic balance of weaned piglets, promoting growth and decreasing diarrhea rate.

Interleukin-25 mRNA could express in Th2 cells, heart, testis and brain while express rather lowly in small intestine, liver, uterus and lung of mice (Guo, 2009). In this experiment, mRNA expression levels of *IL-25* in liver and duodenum of piglets were under the limit of detection, consisting with the former findings on mice.

Under homeostatic, eubiotic conditions, intestinal microbe-associated molecular patterns (MAMPs) stimulate the secretion of cytokines such as IL-25 that promote development of tolerogenic macrophages and dendritic cells. Under invasion by pathogenic microbe, IL-22 will be up-regulated to protect the epithelial barrier (Craig et al., 2012). Function of IL-10 was mainly inflammation suppression as mentioned before. Based on the results above, up-regulation of IL-25 and down-regulation of IL-22 by bamboo vinegar powder might indicate that the intestinal microflora of piglets in these groups was under homeostatic, eubiotic conditions with less pathogen invasion. Down-regulation of IL-10 manifested that bamboo vinegar powder might strengthen immune response to pathogenic microbe of piglets.

Our research proved that bamboo vinegar powder exerted favorable regulatory effects on growth performance, diarrhea situation and expression of immunity-related genes. Researchers pointed out that rich active organic matters contained in bamboo vinegar powder, including phenols, aldehydes, ketones, organic acids etc., might have benign regulatory effects on immune system and intestinal microflora, which also had antioxidant activity (Wang et al., 2012; Chen et al., 2013). Moreover, plant extracts such as bamboo vinegar powder showed immune-modulatory effects different from chemical medicine, which was more synthetic adjustment with multi-link, multi-target and multi-level. Therefore, we speculated that growth-enhancing and immune-modulatory effects of bamboo vinegar powder on weaned piglets in our study might be related to its bioactive components such as phenolic compounds, aldehydes, ketones, organic acids, heterocycle compounds and alcohol as mentioned before.

5. Conclusion

Bamboo vinegar powder added in feed of weaned piglets could alleviate diarrhea and significantly decrease F:G of weaned piglets. Enhancement of growth performance by bamboo vinegar powder could match that of antibiotics. The mRNA expression levels of *IL-10* in liver and duodenum and mRNA expression levels of *IL-22* in duodenum and mesenteric lymph nodes of weaned piglets were down-regulated while mRNA expression levels of *IL-25* in spleen and mesenteric lymph nodes were up-regulated by bamboo vinegar powder. Moreover, the dosage of bamboo vinegar powder at 0.5% was appropriate for weaned piglets.

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