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Original Research Article

Growth performance of nursery and grower-finisher pigs fed diets supplemented with benzoic acid

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ABSTRACT

Two experiments were conducted to investigate the efficacy of benzoic acid on the growth performance of nursery and grower-finisher pigs. A randomized complete block design was used in both experiments with the initial body weight as the blocking factor. There were 3 treatments corresponding to 3 dietary levels of benzoic acid: 0, 0.3%, and 0.5%. In experiment 1, a total of 144 PIC L1050 barrows (initial body weight 7.1 ± 0.6 kg) were used with each treatment replicated 8 times. In experiment 2, a total of 288 PIC L1050 barrows (initial body weight 36.1 ± 3.6 kg) were used with each treatment replicated 16 times. There were 6 barrows in each replicate pen for both experiments. Experiments 1 and 2 lasted 28 and 70 days, respectively. In experiment 1, average daily gain (ADG) of all growth phases increased linearly ($P < 0.05$) with increasing supplementation of benzoic acid, which led to a linear improvement in average body weight on d 28 ($P < 0.05$). There was also an improvement in feed conversion ratio (FCR) of d 0 to 14 (linear effect: $P < 0.05$) and in average daily feed intake (ADFI) of d 14 to 28 and d 0 to 28 (linear effect: $P < 0.01$). In experiment 2, ADG during d 0 to 35 and d 35 to 70 and average body weight on d 35 improved linearly ($P < 0.05$) with increasing supplementation of benzoic acid. Average daily gain of d 0 to 70 and average body weight on d 70 increased significantly in a both linear and quadratic manner. There was a linear improvement in FCR in all growth phases ($P < 0.05$). In conclusion, the dietary inclusion of benzoic acid at the supplementation levels of 0.3% and 0.5% significantly improved the growth performance of nursery and grower-finisher pigs in the current study; the nursery pigs responded to the dietary supplementation of benzoic acid up to 0.5% linearly while the grower-finisher pigs achieved the optimal ADG at the calculated supplementation level of 0.36%.

1. Introduction

Benzoic acid was superior to other acids in terms of *in vitro* bactericidal effects on coliforms (Knarreborg et al., 2002) and enhanced growth performance of piglets by the strong antimicrobial effects in the gastrointestinal tract (Kluge et al., 2006).

In piglets, there is a plethora of studies with benzoic acid. Benzoic acid (0.5%) improved growth rate, feed intake, and feed utilization rate in piglets (Kluge et al., 2006; Torrallardona et al., 2007; Halas et al., 2010). This could be attributed to the improved gut health via decreasing digesta pH value, increasing the number of *Bifidobacterium* while decreasing *Escherichia coli*, promoting the development of intestinal morphology (Diao et al., 2014), enhancing jejunal antioxidant capacity (Diao et al., 2016), diversifying ileal microbiota (Torrallardona et al., 2007; Halas et al., 2010), and increasing utilization rates of nutrients (Guggenbuhl et al., 2007). There is, however, a lack of long-term studies to ascertain the positive effects of benzoic acid on pigs from weaning until

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slaughter (Halas et al., 2010). Moreover, the finding by Shu et al. (2016) that adding between 2.5% and 5.0% benzoic acid could lead to organ injury in piglets makes it imperative to study benzoic acid during the entire grower-finisher period at the recommended levels which are usually less than 1%.

In the current study, 2 experiments were conducted to validate the efficacy of benzoic acid on the growth performance of both nursery and grower-finisher pigs.

2. Materials and methods

The animal protocol for this research was approved by the Animal Welfare Committee of DSM (China) Animal Nutrition Research Center (AWCCAN).

2.1. Animals and facilities

2.1.1. Experiment one

A total of 144 PIC L1050 barrows (initial body weight 7.1 ± 0.6 kg and final body weight 20.0 ± 2.7 kg) were used. Six barrows were housed in a pen (3.0 m × 1.8 m) furnished with 2 nipple drinkers and one trough with a stocking density at 0.9 m^2 per pig. The study lasted for 28 days in two 14-day phases.

2.1.2. Experiment two

A total of 288 PIC L1050 barrows (initial body weight 36.1 ± 3.6 kg and final body weight 112.4 ± 8.4 kg) previously not exposed to benzoic acid were used. Six barrows were housed in a pen (4.9 m × 2.7 m) furnished with 2 nipple drinkers and one trough with a stocking density at around 2.2 m^2 per pig. The experiment lasted 70 days in two 35-day phases.

2.1.3. Facilities

The experiments were conducted at DSM (China) Animal Nutrition Research Center Co., Ltd. (Bazhou, China). Room temperature was controlled by a computer system to maintain an optimal environment according to the age of pigs. Water and feed in pellet form were supplied *ad libitum*. The lighting was set as bright during the day and as dim during the night.

2.2. Experimental design

Both experiments were in a randomized complete block design with the initial body weight of pigs as the blocking factor. There was a total of 3 dietary treatments with each treatment in 8 replicate pens in experiment 1 and 16 replicate pens in experiment 2. The 3 treatments corresponded to 3 dietary levels of benzoic acid: 0, 0.3%, and 0.5%. The ingredient and nutrition compositions of the basal diets are shown in Table 1. The analyzed nutrients and benzoic acid are shown in Table 2.

The nursery, grower, and finisher pigs' diets were formulated to meet or exceed the energy and nutrients requirements for 11 to 25 kg, 50 to 75 kg, and 75 to 100 kg pigs in NRC (2012), respectively. The feed was pelleted at 80 °C.

Benzoic acid with a purity of 99.5% and phytase were provided by DSM (China) Ltd. (Shanghai, China). Phytase at the supplementation level of 0.15 g/kg feed was assumed to provide 1.27 kg total P and 1.06 kg total Ca per ton of feed. The mineral and vitamin premixes were provided by DSM Vitamins (Shandong) Ltd. (Liaocheng, China).

2.3. Sampling and analyses

The pigs were weighed on d 0, 14, and 28 in experiment 1, and on d 0, 35 and 70 in experiment 2. Feed allowance and leftover were recorded in each 14- or 35-d period. The number of pig-days per period and pen was recorded.

After extraction in 1% acetic acid and anhydrous ethanol (3/2, vol/vol), benzoic acid was measured with HPLC (Agilent 1200 series, Agilent Technologies, Santa Clara, CA, USA) using benzoic acid as an external standard, a ZORBAX SB-C18 column, 0.5% formic acid/acetonitrile as the mobile phase, and a detection wavelength at 230 nm. Dietary samples were dried at 105 °C in an oven for 4 h for dry matter determination. Nitrogen content was determined by the Dumas method (LECO FP-528, LECO Corporation, St Joseph, MI, USA). Gross energy was determined using an adiabatic bomb calorimeter (C 2000 basic, IKA, Germany). Calcium and phosphorus were determined by Inductively Coupled Plasma-Optical Emission Spectrometry (Optima TM 8000, PerkinElmer, Shelton, USA).

Table 1
Ingredients and nutrient compositions of basal diets (as-fed basis).

Item	Nursery	Grower	Finisher
Ingredients, g/kg			
Corn (7.8%)	611.00	760.00	831.00
Soybean meal (47%)	245.00	–	–
Soybean meal (43%)	–	200.00	130.00
Fishmeal (65%)	50.00	–	–
Whey powder (3%)	50.00	–	–
Soybean oil	15.00	8.00	8.00
NaCl	3.00	3.50	3.50
Limestone	7.00	8.95	8.50
Dicalcium phosphate	2.75	4.00	3.05
L-lysine·HCl	4.00	3.70	4.00
DL-methionine	0.80	0.50	0.30
L-threonine	1.30	1.00	1.20
L-tryptophan	–	0.20	0.30
Phytase	0.15	0.15	0.15
Treatment premix[1]	5.00	5.00	5.00
Vitamins and minerals premix 4205[2] (0.5%)	5.00	–	–
Vitamins and minerals premix 4305[3] (0.5%)	–	5.00	–
Vitamins and minerals premix 4405[4] (0.5%)	–	–	5.00
Total	1,000.00	1,000.00	1,000.00
Calculated nutrients & energy, %			
Crude protein	20.2	15.5	13.0
ME, kcal/kg	3,401	3,325	3,329
Total Ca	0.84	0.63	0.57
Total P	0.69	0.53	0.48
True total tract digestible P	0.46	0.32	0.29
Standardized ileal digestible amino acid			
Lys	1.28	0.91	0.78
Met	0.39	0.27	0.22
Thr	0.76	0.56	0.49
Trp	0.20	0.16	0.14
Val	0.80	0.56	0.47

[1] Treatment premix is made of silicon dioxide replaced with intended levels of benzoic acid.

[2] Supplied per kilogram of diet: vitamin A, 9,750 IU; vitamin D_3, 3,000 IU; vitamin E, 63 mg; vitamin K_3, 3.0 mg; vitamin B_1, 3.0 mg; vitamin B_2, 9.6 mg; vitamin B_6, 4.5 mg; vitamin B_{12}, 36 μg; D-biotin, 240 μg; D-calcium pantothenate, 30 mg; folic acid, 1.8 mg; niacin, 36 mg; Cu (tribasic copper chloride), 190 mg; I (potassium iodate), 0.6 mg; Fe (ferrous sulfate), 120 mg; Mn (manganese sulfate), 60 mg; Zn (zinc sulfate), 120 mg; Se (sodium selenite), 450 μg; and choline (choline chloride), 300 mg.

[3] Supplied per kilogram of diet: vitamin A, 6,500 IU; vitamin D_3, 2,000 IU; vitamin E, 42 mg; vitamin K_3, 2.0 mg; vitamin B_1, 2.0; vitamin B_2, 6.4 mg; vitamin B_6, 3.0 mg; vitamin B_{12}, 24 μg; D-biotin, 160 μg; D-calcium pantothenate, 20 mg; folic acid, 1.2 mg; niacin, 24 mg; Cu (tribasic copper chloride), 125 mg; I (potassium iodate), 0.5 mg; Fe (ferrous sulfate), 100 mg; Mn (manganese sulfate), 50 mg; Zn (zinc sulfate), 100 mg; Se (sodium selenite), 250 μg; and choline (choline chloride), 250 mg.

[4] Supplied per kilogram of diet: vitamin A, 6,500 IU; vitamin D_3, 2,000 IU; vitamin E, 42 mg; vitamin K_3, 2.0 mg; vitamin B_1, 2.0; vitamin B_2, 6.4 mg; vitamin B_6, 3.0 mg; vitamin B_{12}, 24 μg; D-biotin, 160 μg; D-calcium pantothenate, 20 mg; folic acid, 1.2 mg; niacin, 24 mg; Cu (tribasic copper chloride), 30 mg; I (potassium iodate), 0.45 mg; Fe (ferrous sulfate), 80 mg; Mn (manganese sulfate), 40 mg; Zn (zinc sulfate), 80 mg; Se (sodium selenite), 150 μg; and choline (choline chloride), 250 mg.

Table 2
Analyzed nutrients of the experimental diets with different levels of benzoic acid (BA).

Item	Dry matter, %	Crude protein, %	Total Ca, %	Total P, %	BA, mg/kg
Experiment 1					
0 BA	88.2	19.4	0.74	0.55	0
0.3% BA	88.6	19.4	0.75	0.55	2,942
0.5% BA	88.9	19.9	0.74	0.55	4,206
Experiment 2: grower (1)[1]					
0 BA	87.5	16.0	0.58	0.40	0
0.3% BA	87.7	15.5	0.58	0.42	2,786
0.5% BA	87.9	15.3	0.56	0.40	4,699
Experiment 2: grower (2)[1]					
0 BA	87.3	15.5	0.57	0.39	0
0.3% BA	87.2	15.3	0.57	0.38	2,510
0.5% BA	87.6	15.7	0.59	0.40	5,156
Experiment 2: finisher (1)[2]					
0 BA	88.1	13.2	0.54	0.36	0
0.3% BA	88.3	13.1	0.52	0.35	2,860
0.5% BA	88.6	13.0	0.53	0.35	4,502
Experiment 2: finisher (2)[2]					
0 BA	87.8	13.1	0.55	0.36	0
0.3% BA	87.7	13.2	0.51	0.34	3,038
0.5% BA	88.0	13.1	0.52	0.36	4,597

[1] The first and second batches of diets for grower phase of experiment 2.
[2] The first and second batches of diets for finisher phase of experiment 2.

2.4. Statistical analysis

2.4.1. Calculations

Average daily gain (ADG) was calculated by adding the individual weight gains in each pen and then dividing by the number of pig-days of that pen. Feed intake was measured by pen, and average daily feed intake (ADFI) was calculated by dividing the feed intake of each pen by the number of pig-days of that pen. Feed conversion ratio (FCR) was calculated by dividing ADFI by ADG.

2.4.2. Statistical model

Data were analyzed with GLM procedure of SAS 9.3 (SAS Institute, Inc., Cary, NC, USA) using the following statistical model with pen-aggregated data:

$$Y_{ijk} = \mu + \alpha_i + \beta_j + \varepsilon_{ijk},$$

where μ is the overall mean, α_i is the effect of the ith block, β_j is the effect of the jth benzoic acid level, and ε_{ijk} is the error term.

Homogeneity of variance between treatment groups was checked by applying Levene's test. Normality was checked by applying Kolmogorov–Smirnov's method. To investigate the dose–response effect, orthogonal polynomial contrasts were used to test the linear and quadratic effects of benzoic acid. The contrast coefficients were generated using IML procedure of SAS considering the unequal spacing between the treatments. The statistical significance was defined at $P < 0.05$. The least square means are presented.

3. Results

3.1. Dietary analyses

The analytical results (Table 2) were in an acceptable range to the respective target levels (Table 1) and indicated that there were no errors in experimental diets that were prepared.

3.2. Model assumptions

Variance homogeneity was present in the data. For the normality test, only ADFI for the period of d 35 to 70 at the benzoic acid level of 0.3% in experiment 2 failed. Since the main measurements were all normally distributed, no transformation of the outcome was performed.

3.3. Growth performance

Three piglets in experiment 1 and 5 pigs in experiment 2 were culled out. The culled piglets were diagnosed of meningitis or enteritis, while the grower pigs were eliminated due to rectal prolapse, limping or enteritis.

In experiment 1, ADG of all growth phases increased linearly ($P < 0.05$) with increasing supplementation of benzoic acid, which led to a linear improvement in average body weight on d 28 ($P < 0.05$; Table 3). There was also an improvement in FCR of d 0 to 14 (linear effect: $P < 0.05$) and in ADFI during d 14 to 28 and d 0 to 28 (linear effect: $P < 0.01$) (Table 3).

In experiment 2, ADG during d 0 to 35 and d 35 to 70, and average body weight on d 35 were improved linearly ($P < 0.05$) with increasing supplementation of benzoic acid (Table 4). Average daily gain of d 0 to 70 and average body weight on d 70 increased significantly in a both linear and quadratic manner. There was a linear improvement in FCR of all growth phases ($P < 0.05$). The optimal dietary inclusion rate of benzoic acid was calculated at 0.36% by regressing ADG of d 0 to 70 against the supplemental levels of benzoic acid ($y = -483.33x^2 + 351.67x + 1{,}048$, $r^2 = 1$) (Table 4).

4. Discussion

Liver can efficiently extract 89% of the dietary benzoic acid to synthesize hippuric acid for clearance by kidneys with the urinary excretion of hippuric acid accounting for 85% of the benzoic acid intake, but the absorption of benzoic acid in the gut is a protracted process (Kristensen et al., 2009), which means a longer time for benzoic acid to act in the gastro-intestinal tract. Benzoic acid could improve the microbiota ecosystem in the gut and enhance the metabolic functions of the gut. Dietary supplementation of benzoic acid decreased the pH value of digesta inileum, cecum, and colon of piglets (Diao et al., 2014) and in crop, jejunum, and ceca of chickens (Olukosi and Dono, 2014), which could be a critical factor for the

Table 3
Growth performance of nursery pigs (Experiment 1).

Item	Benzoic acid, %			SEM	*P*-value	
	0	0.3	0.5		L[1]	Q[2]
Number of replicates	8	8	8			
Body weight, kg						
d 0	7.1	7.1	7.0	0.09	0.515	0.306
d 14	12.0	12.3	12.6	0.25	0.083	0.845
d 28	19.0	20.3	20.8	0.45	0.013	0.723
d 0 to 14						
ADG, g	349	369	403	15	0.024	0.503
ADFI, g	476	481	509	14	0.129	0.420
FCR	1.36	1.31	1.27	0.03	0.024	0.831
d 15 to 28						
ADG, g	507	571	583	17	0.004	0.387
ADFI, g	742	808	846	18	<0.001	0.875
FCR	1.47	1.42	1.45	0.02	0.555	0.138
d 0 to 28						
ADG, g	428	470	493	15	0.006	0.872
ADFI, g	609	645	678	15	0.005	0.774
FCR	1.42	1.38	1.38	0.02	0.118	0.439

SEM = standard error of the means; ADG = average daily gain; ADFI = average daily feed intake; FCR = feed conversion ratio, ADFI/ADG.
[1] Linear effect of benzoic acid.
[2] Quadratic effect of benzoic acid.

Table 4
Growth performance of grower-finisher pigs (Experiment 2).

Item	Benzoic acid, %			SEM	*P*-value	
	0	0.3	0.5		L[1]	Q[2]
Number of replicates	16	16	16			
Body weight, kg						
d 0	36.1	36.0	36.3	0.10	0.315	0.232
d 35	74.5	76.5	76.1	0.51	0.022	0.112
d 70	109.8	113.9	113.5	0.76	<0.001	0.048
d 0 to 35						
ADG, g	1,089	1,150	1,138	16	0.020	0.106
ADFI, g	2,490	2,544	2,524	34	0.415	0.425
FCR	2.29	2.21	2.22	0.02	0.014	0.207
d 36 to 70						
ADG, g	1,008	1,069	1,069	13	0.001	0.123
ADFI, g	3,085	3,176	3,163	34	0.084	0.296
FCR	3.07	2.97	2.96	0.04	0.025	0.435
d 0 to 70						
ADG, g	1,048	1,110	1,103	11	<0.001	0.051
ADFI, g	2,787	2,860	2,844	30	0.159	0.308
FCR	2.66	2.58	2.58	0.03	0.007	0.230

SEM = standard error of the means; ADG = average daily gain; ADFI = average daily feed intake; FCR = feed conversion ratio, ADFI/ADG.
[1] Linear effect of benzoic acid.
[2] Quadratic effect of benzoic acid.

selective suppression of the detrimental pathogens and the improved biodiversity of microbiota in the gut (Knarreborg et al., 2002; Torrallardona et al., 2007; Halas et al., 2010). Longer villi and higher activities of trypsin, lipase, and amylase in the jejunum of piglets were reported in response to supplementation of benzoic acid (Halas et al., 2010; Diao et al., 2014, 2016), which could support greater nutrient digestion and absorption by pigs as proved in the metabolism trials by Guggenbuhl et al. (2007), Sauer et al. (2009), and Murphy et al. (2011). The improved intestinal morphology could be related to the upregulation of mucosal glucagon-like peptide 2 (GLP-2) and activities of superoxide dismutase (SOD) and glutathione peroxidase (GSH-PX; Diao et al., 2016). In addition, the supplementation of benzoic acid reduced manure NH_3 emission (Halas et al., 2010; Murphy et al., 2011), which could help improve the environmental conditions for pigs.

For piglets in the current trial, the linear relationship between the growth performance and the supplemental levels of benzoic acid suggest the possibility of further improvement in growth performance of piglets with dietary benzoic acid supplementation greater than 0.5%. Kluge et al. (2006) showed that the dietary supplementation of 1% benzoic acid can further improve the growth performance of piglets when compared with the level of 0.5% benzoic acid. Previous trials (Kluge et al., 2006; Torrallardona et al., 2007; Halas et al., 2010) showed that 0.5% benzoic acid can improve ADG by 11% to 16%, ADFI by 8% to 11%, and FCR by 2% to 6% compared to the non-supplemented control, which compared with improvements of 15%, 11%, and 3% accordingly in the current study. It is worth noting that approximately 70% of improvement in ADG could be explained by the increase in ADFI in experiment 1, which is in agreement with the positive correlation between the effects of organic acid on ADG and ADFI (Partanen and Mroz, 1999).

In the literature, there is evidence that benzoic acid might be able to improve the growth performance of grower-finisher pigs. For example, benzoic acid (2%) increased apparent P and Ca digestibility, and reduced urinary pH and NH_3 emissions in grower pigs (Nørgaard et al., 2010; Murphy et al., 2011). Large-scale growth performance studies with grower-finisher pigs, however, are still lacking. In the current study, it was clearly shown that, compared to the non-supplemented control, both 0.3% and 0.5% benzoic acid can improve the ADG by 6%, ADFI by 3%, and FCR by 3% for the whole grower-finisher duration. The derived optimal supplemental level of 0.36% of benzoic acid for grower-finisher pigs in the current study indicates that grower-finisher pigs might need less benzoic acid than nursery pigs for the best growth performance. Furthermore, there was no sign of health problems with the grower-finisher pigs linked to the long time usage of benzoic acid at the supplemental levels in the current study, which is probably due to the efficient extraction of benzoic acid by the liver and clearance by the kidney (Kristensen et al., 2009).

5. Conclusion

In conclusion, the dietary inclusion of benzoic acid at the supplementation levels of 0.3% and 0.5% significantly improved the growth performance of both nursery and grower-finisher pigs in the current study; the nursery pigs responded to the supplementation level of benzoic acid up to 0.5% linearly while the grower-finisher pigs achieved the optimal ADG at the calculated supplementation level of 0.36%.

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