

Effect of benzoic acid with or without a *Bacillus*-based direct-fed microbial on the performance and carcass merit of grow-finish pigs

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ABSTRACT

Two hundred and forty barrows and gilts (DNA 600 × 241, DNA Genetics, Columbus, NE) with an initial body weight (BW) of 35.5 ± 4.2 kg were sorted into split-sex pens, blocked by initial body weight, and randomly allocated to one of three dietary treatments with eight pigs per pen and ten pens per treatment. Dietary treatments included a standard diet (CON), CON plus 0.3% benzoic acid (BA; VevoVital, DSM Nutritional Products, Parsippany, NJ), and CON plus 0.3% BA and 0.025% *Bacillus*-based direct-fed microbial (BA+DFM; PureGro, DSM Nutritional Products, Parsippany, NJ). The experimental diets were fed in four feeding phases. Pigs were weighed and feed intake measured at the beginning and end of each phase for the calculation of average daily gain (ADG), average daily feed intake (ADFI), and feed efficiency (G:F). In addition, ultra-sound was utilized at the conclusion of the trial on day 81 for measurements of backfat and loin eye area. Data were analyzed as repeated measures in SAS 9.4 (SAS Institute, Cary, NC) with fixed effects of treatment, phase, sex, and block included in the model. Pen was the experimental unit, and results were considered significant if $P \leq 0.05$ and a tendency if $0.05 < P \leq 0.10$. Overall, pigs fed BA had increased ADFI compared to pigs fed CON (2.88 vs. 2.75 kg, $P = 0.015$), while pigs fed BA + DFM had similar ADFI compared to pigs fed CON or BA ($P \geq 0.279$). There was a tendency for an effect of dietary treatment on ADG ($P = 0.063$), where pigs fed BA tended to grow faster than pigs fed CON (1.11 vs. 1.08 kg, $P = 0.051$); however, there were no differences in feed efficiency between treatments ($P = 0.450$). Additionally, there was no evidence of an effect of dietary treatment on pig BF or LEA ($P \geq 0.334$). In conclusion, supplementing 0.3% benzoic acid to grow-finish pigs stimulated feed intake, but did not affect efficiency, or carcass merit.

Key words: benzoic acid, direct-fed microbial, grow-finish, swine

INTRODUCTION

With increasing feed costs and restrictions on the use of growth promoters, such as beta-agonists and subtherapeutic levels of antibiotics, producers must look towards alternative strategies for improving the growth and efficiency of swine.

Benzoic acid (BA) has been approved for use as an acidifier in swine diets at an inclusion rate of up to 0.5% in the United States since 2014 and 0.30% to 1.0% in the European Union since 2019. Benzoic acid is a carboxylic acid that is conjugated with glycine in the liver and rapidly excreted in the urine as hippuric acid (Kristensen et al., 2009). In weaned pigs, benzoic acid has been attributed to improvements in feed intake and growth rate (Gutzwiller et al., 2014). In grow-finish pigs, Zhai et al. (2017) observed improvements in growth rate and feed conversion, estimating that daily gain was optimized by supplementing 0.36% BA.

Direct-fed microbials (DFM) have gained recent interest for their ability to confer beneficial health effects on the host. Specifically, *Bacillus*-based DFMs have been observed to improve feed efficiency, growth rate, and nutrient digestibility in various ages of pigs (Cai et al., 2015; Jørgensen et al., 2016; Lewton et al., 2021). However, the response to DFMs is often

strain-specific, making comparisons between experiments difficult (Blavi et al., 2019).

Two studies have shown the benefits of feeding BA with a *Bacillus*-based DFM. Papatsiros et al. (2011) reported additive effects of BA with a DFM, where combined supplementation of BA and *Bacillus cereus* var. *Toyoi* resulted in increased gain and efficiency and decreased morbidity and diarrhea in weaned pigs compared to pigs fed each additive separately. Recently, Pu et al. (2020) reported that feeding 0.3% BA with *Bacillus coagulans* improved performance, intestinal integrity, and increased the abundance of beneficial bacteria in the gastrointestinal tract of nursery pigs. To the best of authors knowledge, no work has been done to understand if the response of grow-finish pig performance to BA can be improved further with the addition of a *Bacillus*-based DFM.

The hypothesis was that feeding benzoic acid combined with a direct-fed microbial would improve performance beyond feeding benzoic acid alone. Therefore, the objective of this experiment was to investigate the effect of benzoic acid with or without a *Bacillus*-based direct-fed microbial on the performance of growing and finishing pigs.

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MATERIALS AND METHODS

General

All procedures in this experiment adhered to guidelines for the ethical and humane use of animals for research according to the Guide for the Care and Use of Agricultural Animals in Research and Teaching (FASS, 2010) and were approved by the Institutional Animal Care and Use Committee at Iowa State University (IACUC 20-036). The experiment was conducted at Swine Research Services (SRS) in Summers, AR. Pigs were housed in pens that allowed 0.84 m² per pig with one two-space feeder and one nipple waterer, ensuring *ad libitum* access to feed and water.

Animals and experimental design

Two hundred and forty barrows and gilts (DNA 600 × 241, DNA Genetics, Columbus NE) were reared in the SRS nursery for 49 d. The pigs were *Mycoplasma hyopneumoniae*, porcine

reproductive and respiratory syndrome virus, porcine epidemic diarrhea virus, and *Actinobacillus pleuropneumonia* negative. Upon exiting the nursery, pigs were weighed, sorted into split-sex pens with eight pigs per pen, blocked by initial body weight with three pens of barrows and three pens of gilts per block, and fed a common diet for 11 d. Within blocks, pens were randomly assigned to one of three dietary treatments.

Diets

The dietary treatments consisted of a standard commercial diet, which served as the control (CON), CON plus 0.3% BA (BA; VevoVital, DSM Nutritional Products, Parsippany, NJ), and CON plus 0.3% BA and 0.025% DFM (BA+DFM; PureGro, DSM Nutritional Products, Parsippany, NJ). The DFM provided 1.47 × 10⁸ CFU of *Bacillus* bacteria per gram of product, including two strains of *Bacillus licheniformis* and one strain of *Bacillus subtilis*. Experimental diets were fed in four phases: days 0 to 18, days 18 to 39, days 39 to 60, and

Table 1. Ingredient and calculated nutrient composition of control diet for each feeding phase. Ingredients are listed as percent inclusion in the diet and reported on “as-fed” basis

Ingredients, %	Feeding phase			Phase 4 (days 60 – 81)
	Phase 1 (days 0 – 18)	Phase 2 (days 18 – 39)	Phase 3 (days 39 – 60)	
Corn	59.04	72.14	78.74	83.75
Soybean meal	37.32	24.13	17.82	13.05
Monocalcium phosphate	0.50	0.32	0.14	0.05
Calcium carbonate	1.06	1.13	1.08	1.00
Sodium chloride	0.50	0.50	0.50	0.50
L-lysine HCl	0.11	0.28	0.29	0.30
L-threonine	0.03	0.10	0.10	0.10
DL-methionine	0.11	0.11	0.08	0.05
L-tryptophan	–	–	0.01	0.01
Benzoic acid ¹	–	–	–	–
Direct-fed microbial ²	–	–	–	–
Phytase ³	0.04	0.04	0.04	0.04
Soybean oil	1.00	1.00	1.00	1.00
VTM premix ⁴	0.30	0.25	0.20	0.15
Total	100.00	100.00	100.00	100.00
Calculated nutrients				
Metabolizable energy, Mcal/kg	3.23	3.27	3.29	3.30
Crude protein, %	21.93	16.97	14.52	12.66
Calcium, %	0.65	0.60	0.53	0.47
Available phosphorus, %	0.36	0.31	0.27	0.24
SID ⁵ Lys, %	1.15	0.96	0.82	0.71
SID Ile:Lys	0.69	0.60	0.57	0.56
SID Leu:Lys	1.34	1.27	1.31	1.36
SID Met+Cys:Lys	0.58	0.58	0.58	0.58
SID Thr:Lys	0.61	0.62	0.63	0.64
SID Trp:Lys	0.21	0.18	0.18	0.18
SID Val:Lys	0.73	0.66	0.65	0.65

¹VevoVital, DSM Nutritional Products, Parsippany, NJ; 0.30% inclusion at the expense of corn in the benzoic acid and benzoic acid plus direct-fed microbial diets.

²PureGro, DSM Nutritional Products, Parsippany, NJ; 0.025% inclusion at the expense of corn in the benzoic acid plus direct-fed microbial diet.

³Ronozyme HiPhos 2,700, DSM Nutritional Products, Parsippany, NJ.

⁴Vitamin and trace mineral premix; Provided 3,528,000 IU vitamin A, 882,000 IU vitamin D3, 17,640 IU vitamin E, 15.44 mg vitamin B12, 1,764 mg menadione, 3,307.5 mg riboflavin, 11,025 mg D-pantothenic acid, 33,075 mg niacin, 73,000 mg Fe (ferrous sulfate), 73,000 mg Zn (zinc sulfate), 22,000 mg Mn (manganous oxide), 11,000 mg Cu (copper sulfate), 198 mg I (calcium iodide), and 198 mg Se (sodium selenite) per kg of supplementation.

⁵SID, Standardized ileal digestible.

days 60 to 81 (Table 1). For each phase, diets were formulated to be isocaloric and isonitrogenous with vitamin and mineral levels at or above NRC (2012) recommendations.

Sample collection and analysis

Individual pig body weights were captured at the beginning and end of each phase and on days 7, 28, 49, and 70. At the time of body weight (BW) collections, the weight of feed remaining in each feeder was determined volumetrically using the volume of the feeders and the bulk density of the feed. Body weights and feed intake data were used to calculate average daily gain (ADG), average daily feed intake (ADFI), and feed efficiency (G:F). On day 81, 10th rib back-fat (BF) and loin eye area (LEA) were measured via ultra-sound (Pie Medical Scanner 200; Pie Medical Equipment, Maastricht, The Netherlands) by a trained technician.

Diets were manufactured at the SRS feed mill (Van Buren, AR). Feed samples were collected from each batch at the mixer, and a composite sample representing each batch on an approximately equal weight basis was created for each dietary treatment in each phase.

Following completion of the study, feed samples were submitted to Whitbeck Labs (Springdale, AR) for proximate analysis of dry matter (method 930.15), crude protein (method 990.03), calcium, and phosphorus (method 993.14). Samples were also analyzed for gross energy at Iowa State University using an isoperibolic bomb calorimeter (model 6200; Parr Instruments Co., Moline, IL).

Statistical analysis

Average daily gain was calculated for each pig as the slope of the regression line of BW on day for each dietary phase and overall (days 0 to 81). Within a pen, pig ADG was aggregated to calculate mean ADG. Performance data were analyzed as repeated measures according to the following model:

$$y_{ijklm} = \mu + T_i + S_j + (T * S)_{ij} + B_k + P_l + (T * P)_{il} + (S * P)_{jl} + (T * S * P)_{ijl} + e_{ijklm}$$

where y_{ijklm} is the observed value for the m th experimental unit within the i th level of dietary treatment of j th sex in the k th block during the l th feeding phase; μ is the overall mean; T_i is the fixed effect of the i th dietary treatment ($i = 1$ to 3); S_j is the fixed effect of the j th sex ($j = 1$ or 2); $(T * S)_{ij}$ is the interaction between dietary treatment and sex; B_k is the fixed effect of the k th block ($k = 1$ to 5); P_l is the fixed effect of the l th feeding phase ($l = 1$ to 4); $(T * P)_{il}$ is the interaction between dietary treatment and feeding phase; $(S * P)_{jl}$ is the interaction between sex and feeding phase; $(T * S * P)_{ijl}$ is the three-factor interaction between dietary treatment, sex, and feeding phase; e_{ijklm} is the random error associated with y_{ijklm} , assuming $e_{ijklm} \sim N(0, R)$, where $R = N[0, I_n \otimes AR(1)]$ for ADG and $R = N[0, I_n \otimes ARH(1)]$ for BW, ADFI, and G:F. I_n is the identity matrix, $AR(1)$ is the first-order auto-regressive covariance matrix, and $ARH(1)$ is $AR(1)$ with heterogeneous variances.

Carcass data were analyzed according to the following statistical model:

$$y_{ijkl} = \mu + T_i + S_j + (T * S)_{ij} + B_k + e_{ijkl}$$

where y_{ijkl} is the observed value from the l th experimental unit within the i th level of dietary treatment of the j th sex in the k th

block; μ is the overall mean; T_i is the fixed effect of the i th dietary treatment ($i = 1$ to 3); S_j is the fixed effect of the j th sex ($j = 1$ or 2); $(T * S)_{ij}$ is the interaction between dietary treatment and sex; B_k is the fixed effect of the k th block ($k = 1$ to 5); e_{ijkl} is the random error associated with y_{ijkl} , assuming $e_{ijkl} \sim N(0, I\sigma_e^2)$.

All models were implemented in SAS 9.4 (SAS Institute, Cary, NC) using the GLIMMIX procedure. Covariance matrices were selected as the best fit for the repeated measures models according to Bayesian Information Criterion for each response variable. The normality of the studentized residuals was verified using the Shapiro–Wilk test from the UNIVARIATE procedure. Studentized residuals greater than three standard deviations from the mean were deemed statistical outliers and excluded from the analysis. Means separation was done using the PDIF option of the LSMEANS statement with a Tukey adjustment for multiplicity. Differences in the number of pigs administered therapeutics were tested using Fisher's Exact Test of the

Table 2. Analyzed nutrient composition experimental diets (as-fed basis)

Item	Dietary treatment		
	Control	Benzoic acid	Benzoic acid + DF ¹
Phase one (days 0 to 18)			
Gross energy, Mcal/kg	3.83	3.82	3.86
Dry matter, %	85.96	85.81	86.04
Crude protein, %	22.15	22.08	22.78
Calcium, %	0.94	0.86	0.88
Phosphorus, %	0.55	0.53	0.54
Total lysine, %	1.35	1.40	1.34
Phase two (days 18 to 39)			
Gross energy, Mcal/kg	8.84	8.88	8.86
Dry matter, %	86.52	86.79	86.68
Crude protein, %	15.28	16.67	16.89
Calcium, %	0.78	0.76	0.79
Phosphorus, %	0.42	0.43	0.41
Total lysine, %	1.03	1.09	1.18
Phase three (days 39 to 60)			
Gross energy, Mcal/kg	3.87	3.89	3.88
Dry matter, %	86.68	86.73	86.91
Crude protein, %	14.42	13.71	13.44
Calcium, %	0.69	0.69	0.69
Phosphorus, %	0.37	0.37	0.37
Total lysine, %	0.98	0.87	0.94
Phase four (days 60 to 81)			
Gross energy, Mcal/kg	3.84	3.87	3.87
Dry matter, %	86.44	86.32	86.39
Crude protein, %	12.39	12.06	12.39
Calcium, %	0.68	0.62	0.64
Phosphorus, %	0.32	0.31	0.32
Total lysine, %	0.79	0.88	0.84

¹DFM, Direct-fed microbial.

FREQ procedure. Results were considered significant if $P \leq 0.05$ and a tendency if $0.05 < P \leq 0.10$.

RESULTS

Diet analysis indicated that crude protein levels were similar to formulated values and gross energy values were similar across treatments within a phase, suggesting diets were isocaloric and isonitrogenous (Table 2).

During the experiment, five pigs were removed because of mortality or morbidity, resulting in a removal rate of 2.1% across treatments. The herd had a history of *Streptococcus suis* in finishing, which was the reason for removal for most of the pigs based on herd veterinarian observations. Morbidity was not deemed significant enough to require whole-barn treatment; therefore, affected pigs identified by animal caretakers were treated with either ceftiofur (Excede, Zoetis, Florham Park, NJ) or enrofloxacin (Baytril, Bayer HealthCare, Animal Health Division, Shawnee Mission, KS). There was evidence for differences in the number of animals administered therapeutics, where BA+DFM had the lowest number of pigs treated and CON has the greatest number of pigs treated ($P = 0.029$; Table 4).

Pigs began the trial at an average body weight of 35.6 ± 4.2 kg and ended on day 81 at an average body weight of 124.2 ± 9.8 kg. There was no effect of dietary treatment on pig BW for the entirety of the trial. However, there was a

significant sex by feeding phase interaction (Table 3), where barrows were heavier than gilts at all timepoints, with the exception of day 18, during which the two sexes were similar in weight. There was no evidence of an interaction between dietary treatment and phase for any of the response variables.

Overall (days 0 to 81), there was a significant effect of sex on BW, ADG, ADFI, G:F, and BF (Table 4; $P \leq 0.001$), where barrows ate more feed and gained faster than gilts but were consequently less efficient and had more backfat. Pigs fed BA had increased feed intake compared to pigs fed CON (Table 4; $P = 0.015$), while pigs fed BA+DFM exhibited similar ADFI compared to CON or BA fed pigs ($P \geq 0.279$). There was a tendency for differences in ADG, where pigs fed BA tended to have improved ADG compared to pigs fed CON ($P = 0.051$); however, there were no differences in feed efficiency, BF, or LEA between treatments ($P \geq 0.334$).

DISCUSSION

The response to diets supplemented with BA has been variable. Much of this variability can be attributed to differences in pig body weight, inclusion rates, health status, and diet composition. There has been relatively little work done investigating the effect of BA on the performance of growing and finishing pigs, especially at 0.3% inclusion level. Bühler et al., (2006) observed no differences in gain or efficiency in pigs fed 1% BA from 26 to 106 kg. However, pigs were fed restrictively

Table 3. Effect of dietary treatments and sex by phase on pig body weight (BW), average daily gain (ADG), average daily feed intake (ADFI), and gain-to-feed ratio (G:F)

Item	Dietary treatment			SEM	P-value	Sex		SEM	P-value	P-value ¹		
	Control	Benzoic acid	Benzoic acid + DFM ²			Barrow	Gilt			Trt ³	Trt X Sex	Trt X Sex X Phase
BW, kg				0.720	0.255			0.588	< 0.001	0.231	0.345	0.214
day 0	35.68	35.66	35.33			35.92 ^a	35.20 ^b					
day 18	52.53	53.76	52.98			53.70 ^a	52.47 ^a					
day 39	75.90	78.14	77.24			78.51 ^a	75.67 ^b					
day 60	99.51	101.82	100.97			102.95 ^a	98.58 ^b					
day 81	123.14	125.61	123.65			126.71 ^a	121.56 ^b					
ADG, kg				0.018	0.176			0.015	0.193	0.063	0.189	0.105
day 0–18	0.94	1.01	0.99			0.99	0.96					
day 18–39	1.11	1.16	1.16			1.18	1.10					
day 39–60	1.13	1.13	1.13			1.16	1.10					
day 60–81	1.12	1.14	1.09			1.13	1.10					
ADFI, kg				0.043	0.127			0.035	< 0.001	0.019	0.053	0.072
day 0–18	1.81	1.94	1.90			1.94 ^a	1.83 ^b					
day 18–39	2.65	2.84	2.78			2.90 ^a	2.61 ^b					
day 39–60	3.13	3.29	3.26			3.41 ^a	3.04 ^b					
day 60–81	3.42	3.47	3.33			3.59 ^a	3.22 ^b					
G:F				0.006	0.379			0.005	0.629	0.450	0.296	0.490
day 0–18	0.52	0.52	0.52			0.51	0.53					
day 18–39	0.41	0.41	0.42			0.41	0.42					
day 39–60	0.36	0.35	0.35			0.34	0.36					
day 60–81	0.33	0.33	0.33			0.31	0.34					

^{a,b} Within a row, means without a common superscript differ ($P \leq 0.05$).

¹Sex and phase main effects significant ($P \leq 0.001$).

²DFM, direct-fed microbial.

³Trt, dietary treatment.

Table 4. Effect of dietary treatment and sex on overall (days 0 to 81) average daily gain (ADG), average daily feed intake (ADFI), gain-to-feed ratio (G:F), 10th rib backfat (BF), and loin eye area (LEA)

Item	Dietary treatment			SEM	P-value	Sex		SEM	P-value	P-value
	Control	Benzoic acid	Benzoic acid + DFM ¹			Barrow	Gilt			
ADG, kg	1.08	1.11	1.09	0.010	0.063	1.12 ^x	1.07 ^y	0.008	< 0.001	0.189
ADFI, kg	2.75 ^b	2.88 ^a	2.82 ^{ab}	0.031	0.019	2.96 ^x	2.68 ^y	0.025	< 0.001	0.053
G:F	0.41	0.40	0.39	0.003	0.450	0.39 ^y	0.41 ^x	0.002	< 0.001	0.296
BF, cm	2.07	2.12	2.17	0.065	0.572	2.29 ^y	1.95 ^x	0.053	< 0.001	0.382
LEA, cm ²	43.67	43.33	42.36	0.631	0.334	42.80	43.43	0.515	0.398	0.624
Therapeutic interventions, %	16.25	8.86	3.75	–	0.029	11.76	7.50	–	0.282	–

^{a-b} Means without a common superscript differ ($P \leq 0.05$).

^{x-y} Means without a common superscript differ ($P \leq 0.05$).

¹DFM, direct-fed microbial.

²Trt, dietary treatment.

in metabolism stalls in this experiment, making it difficult to compare with the current study. Zhai et al. (2017) reported optimization of ADG at 0.36% BA and linear improvements in efficiency in grow-finish pigs up to approximately 110 kg. The current study observed a tendency for an improvement in ADG but no differences in efficiency in pigs fed 0.3% BA up to approximately 125 kg, indicating higher levels of BA may be required to show a more consistent response. Additionally, pigs in the current study experienced a health challenge, which may have blunted the response to BA by altering feed intake patterns. Based on this, further research utilizing BA at higher inclusion levels in growing and finishing pigs of varying health statuses is warranted.

It has been shown that 0.5% inclusion of BA can increase ADG and ADFI in nursery-aged pigs with no impact on efficiency (Diao et al., 2014; Gutzwiller et al., 2014). Diao et al. (2014) saw increased digestive enzyme activity and glucagon-like peptide 2 (GLP-2) concentration in the jejunal mucosa. Similarly, Halas et al. (2010) fed 0.5% BA and observed increases in ADG, ADFI, and CP digestibility, associated with improvements in gut morphology and reduced bacterial mass. These studies suggest that BA may improve growth by increasing enzyme activity and upregulating GLP-2, resulting in increased absorptive capacity.

In contrast to the hypothesis, feeding benzoic acid in combination with the *Bacillus*-based DFM did not result in further improvements in performance compared to feeding benzoic acid alone. Other studies feeding this *Bacillus*-based DFM have observed improvements in gain (Upadhaya et al., 2015) or efficiency (Davis et al., 2008) in growing and finishing pigs. These improvements were not observed in the current experiment when BA was added in conjunction with the DFM. Similarly, previous research in nursery pigs did not show improvements in performance when 0.5% BA was fed in combination with this DFM (Pérez Alvarado et al., 2013). Organic acids have been shown to prevent bacterial growth by disrupting cellular homeostasis through pH modification and suppressing enzymes and nutrient transport systems (Kluge et al., 2006). Benzoic acid has a relatively high dissociation constant, which may aid in its antimicrobial properties by allowing it to passively diffuse into bacterial cells and disrupt these homeostatic mechanisms. Specifically, feeding BA has been associated with decreased bacterial mass through reductions in lactic acid-forming bacteria, *Escherichia coli*, (Guggenbuhl et al., 2007), and total aerobic

and anaerobic bacteria (Kluge et al., 2006). Although this decrease in bacteria may contribute to improvements in performance, it could be speculated that BA interfered with the colonization of the *Bacillus* strains in the DFM by acting in a similar fashion as described above, thus diminishing the impacts of both additives in the current experiment. However, the mechanisms of this potential interaction were not characterized in this experiment. Therefore, further research should focus on investigating the viability of DFM in the presence of benzoic acid and potential changes in nutrient digestibility and absorption, which may occur with the combined use of these additives.

In conclusion, the results of the experiment indicate that supplementing 0.3% benzoic acid alone stimulates feed intake and improves average daily gain in grow-finish pigs from 35 to 123 kg. However, including a *bacillus*-based DFM in combination with benzoic acid did not influence performance.

Supplementary Data

Supplementary data are available at *Translational Animal Frontiers* online.

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Conflict of interest statement

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Literature Cited

- Blavi, L., J. N. Jørgensen, and H. H. Stein. 2019. Effects of *Bacillus amyloliquefaciens* and *Bacillus subtilis* on ileal digestibility of AA and total tract digestibility of CP and gross energy in diets fed to growing pigs. *J. Anim. Sci.* 97:727–734. doi:10.1093/jas/sky432
- Bühler, K., C. Wenk, J. Broz, and S. Gebert. 2006. Influence of benzoic acid and dietary protein level on performance, nitrogen metabolism and urinary pH in growing-finishing pigs. *Arch. Anim. Nutr.* 60:382–389. doi:10.1080/17450390600884369
- Cai, L., S. Indrakumar, E. Kiarie, and I. H. Kim. 2015. Effects of a multi-strain *Bacillus* species-based direct-fed microbial on growth performance, nutrient digestibility, blood profile, and gut health in nursery pigs fed corn–soybean meal–based diets. *J. Anim. Sci.* 93:4336–4342. doi:10.2527/jas.2015-9056
- Davis, M. E., T. Parrott, D. C. Brown, B. Z. de Rodas, Z. B. Johnson, C. V. Maxwell, and T. Rehberger. 2008. Effect of a *Bacillus*-based direct-fed microbial feed supplement on growth performance and pen cleaning characteristics of growing-finishing pigs. *J. Anim. Sci.* 86:1459–1467. doi:10.2527/jas.2007-0603
- Diao, H., P. Zheng, B. Yu, J. He, X. B. Mao, J. Yu and D. W. Chen. 2014. Effects of dietary supplementation with benzoic acid on intestinal morphological structure and microflora in weaned piglets. *Livest. Sci.* 167:249–256. doi:10.1016/j.livsci.2014.05.029
- FASS. 2010. *Guide for the care and use of agricultural animals in research and teaching*. 3rd ed. FASS Inc. https://www.fass.org/images/science-policy/Ag_Guide_3rd_ed.pdf.
- Guggenbuhl, P., A. Séon, A. P. Quintana, and C. S. Nunes. 2007. Effects of dietary supplementation with benzoic acid (VevoVital®) on the zootechnical performance, the gastrointestinal microflora and the ileal digestibility of the young pig. *Livest. Sci.* 108:218–221. doi:10.1016/j.livsci.2007.01.068
- Gutzwiller, A., P. Schlegel, D. Guggisberg, and P. Stoll. 2014. Effects of benzoic acid and dietary calcium:phosphorus ratio on performance and mineral metabolism of weanling pigs. *Asian-Aust. J. Anim. Sci.* 27:530–536. doi:10.5713/ajas.2013.13527
- Halas, D., C. F. Hansen, D. J. Hampson, B. P. Mullan, J. C. Kim, R. H. Wilson, and J. R. Pluske. 2010. Dietary supplementation with benzoic acid improves apparent ileal digestibility of total nitrogen and increases villous height and caecal microbial diversity in weaner pigs. *Anim. Feed Sci. Technol.* 160:137–147. doi:10.1016/J.ANIFEEDSCI.2010.07.001
- Jørgensen, J. N., J. S. Laguna, C. Millán, O. Casabuena, and M. I. Gracia. 2016. Effects of a *Bacillus*-based probiotic and dietary energy content on the performance and nutrient digestibility of wean to finish pigs. *Anim. Feed Sci. Technol.* 221:54–61. doi:10.1016/j.anifeedsci.2016.08.008
- Kluge, H., J. Broz, and K. Eder. 2006. Effect of benzoic acid on growth performance, nutrient digestibility, nitrogen balance, gastrointestinal microflora and parameters of microbial metabolism in piglets. *J. Anim. Physiol. Anim. Nutr. (Berl)* 90:316–324. doi:10.1111/j.1439-0396.2005.00604.x
- Kristensen, N. B., J. V. Nørgaard, S. Wamberg, M. Engbaek, J. A. Fernández, H. D. Zacho, and H. D. Poulsen. 2009. Absorption and metabolism of benzoic acid in growing pigs. *J. Anim. Sci.* 87:2815–2822. doi:10.2527/jas.2009-2003
- Lewton, J. R., A. D. Woodward, R. L. Moser, K. M. Thelen, A. J. Moeser, N. L. Trottier, R. J. Tempelman, and D. W. Rozeboom. 2021. Effects of a multi-strain *Bacillus subtilis*-based direct-fed microbial on weanling pig growth performance and nutrient digestibility. *Transl. Anim. Sci.* 5:1–12. doi:10.1093/TAS/TXAB058
- NRC. 2012. *Nutrient requirements of swine*. 11th rev. ed. Washington, DC: Natl. Acad. Press.
- Papatsiros, V. G., P. D. Tassis, E. D. Tzika, D. S. Papaioannou, E. Petridou, C. Alexopoulos, and S. C. Kyriakis. 2011. Effect of benzoic acid and combination of benzoic acid with a probiotic containing *Bacillus Cereus var. toyoi* in weaned pig nutrition. *Pol. J. Vet. Sci.* 14:117–125. doi:10.2478/v10181-011-0017-8
- Pérez Alvarado, M. A., J. C. López, D. B. Varela, G. M. Landín, and J. A. Cuarón Ibarguengoytia. 2013. Benzoic acid and a product based on *Bacillus* species to protect piglet productivity and the environment. *Rev Mex Cienc Pecu.* 4:447–468.
- Pu, J., D. Chen, G. Tian, J. He, P. Zheng, X. Mao, J. Yu, Z. Huang, J. Luo, Y. Luo, et al., 2020. Effects of benzoic acid, *Bacillus coagulans* and oregano oil combined supplementation on growth performance, immune status and intestinal barrier integrity of weaned piglets. *Anim. Nutr.* 6:152–159. doi:10.1016/j.aninu.2020.02.004
- Upadhaya, S. D., S. C. Kim, R. A. Valientes, and I. H. Kim. 2015. The effect of *Bacillus*-based feed additive on growth performance, nutrient digestibility, fecal gas emission, and pen cleanup characteristics of growing-finishing pigs. *Asian-Aust. J. Anim. Sci.* 28:999–1005. doi:10.5713/ajas.15.0066
- Zhai, H., W. Ren, S. Wang, J. Wu, P. Guggenbuhl, and A. M. Kluentner. 2017. Growth performance of nursery and grower-finisher pigs fed diets supplemented with benzoic acid. *Anim. Nutr.* 3:232–235. doi:10.1016/j.aninu.2017.05.001