Journal of Animal Science, 2022, **100**, 1–11
https://doi.org/10.1093/jas/skac296
Advance access publication 3 September 2022
Non Ruminant Nutrition

The effect of benzoic acid with or without a direct-fed microbial on the nutrient metabolism and gas emissions of growing pigs

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Abstract

Twenty-four gilts (PIC 337 × 1050, PIC Genus, Hendersonville, TN) with an initial body weight (**BW**) of 33.09 ± 1.33 kg were used to investigate the effects of benzoic acid (**BA**) and a *Bacillus*-based direct-fed microbial (**DFM**) on the nutrient metabolism and manure gas emissions of growing pigs. Pigs were blocked by BW, placed into metabolism stalls, and randomly assigned to one of four dietary treatments: basal control (**PC**), low nitrogen (**NC**), PC plus 0.3% BA (PC+BA; VevoVitall, DSM Nutritional Products), and PC plus 0.3% BA and 0.025% DFM (PC+BA+DFM; PureGro, DSM Nutritional Products). Pigs were fed a common diet from day 0 to 14, and the experimental diets were fed in two phases (day 14 to 28 and day 28 to 53). The experiment consisted of four collection periods, with each period subdivided into two subperiods to collect samples for gas emissions and nutrient balance. Firstly, manure samples were collected for 72 h. Twice daily, urine and feces were weighed, and urine pH was measured. After each period, manure was subsampled and taken to the lab to measure gas emissions. Secondly, urine and feces were quantitatively collected for 96 h to allow for measurement of nutrient digestibility (**ATTD**) and retention. Data were analyzed as repeated measures in SAS 9.4 (SAS Inst., Cary, NC) with fixed effects of treatment, collection period, and block. Pig was the experimental unit, and results were considered significant at $P \leq 0.05$ and a tendency at $0.05 < P \leq 0.10$. Pigs fed PC+BA had the greatest ADG compared to pigs fed NC ($P = 0.016$), with intermediate ADG for pigs fed PC or PC+BA+DFM ($P \geq 0.148$). The ATTD of dry matter, gross energy, P, and N did not differ between treatments ($P \geq 0.093$). However, the ATTD of Ca was reduced in pigs fed PC+BA+DFM compared to pigs fed PC+BA ($P = 0.012$). Pigs fed PC+BA or NC excreted less urinary N compared to PC and PC+BA+DFM ($P \leq 0.034$), which contributed to greater nitrogen retention in PC+BA compared to PC ($P = 0.016$). Furthermore, decreased manure pH from pigs fed PC+BA or NC resulted in lower ammonia (NH_3) emissions compared to pigs fed PC+BA+DFM or PC. There was no effect of dietary treatment on manure hydrogen sulfide, methane, or carbon dioxide emissions. In conclusion, supplementing 0.3% BA improved N retention and reduced manure pH and NH_3 emissions, similar to feeding pigs low N, but improved the ADG of pigs when compared to feeding a low N diet.

Lay Summary

Diet formulation as a strategy to improve economic and nutritional efficiency may be combined with nonnutritive feed additives, such as organic acids, specifically benzoic acid, and direct-fed microbials, to further improve nutrient utilization in pigs. Therefore, the objective of this trial was to investigate the effect of supplementing benzoic acid with or without a direct-fed microbial on the nutrient metabolism and emissions of ammonia, hydrogen sulfide, carbon dioxide, and methane from the manure of growing pigs. Feeding a diet containing 0.3% benzoic acid did not affect nutrient digestibility but reduced urinary nitrogen excretion, which resulted in improved nitrogen retention compared to the basal diet. Furthermore, benzoic acid reduced urine and manure pH, contributing to reduced manure ammonia emissions. However, supplementing the direct-fed microbial alongside benzoic acid attenuated these effects.

Key words: benzoic acid, digestibility, direct-fed microbial, emissions, swine

Abbreviations: ADFI, average daily feed intake; ADG, average daily gain; ATTD, apparent total tract digestibility; BA, benzoic acid; BW, body weight; Ca:P, total calcium-to-total phosphorus ratio; CFU, colony-forming units; CP, crude protein; DE, digestible energy; DFM, direct-fed microbial; DM, dry matter; FTU, phytase units; G:F, gain-to-feed ratio; GE, gross energy; ICP, inductively coupled plasma-mass spectrometry; ME, metabolizable energy; OM, organic matter; SID, standardized ileal digestible; TN, total nitrogen; TS, total solids; TVS, total volatile solids; VTM, vitamin and trace mineral

Introduction

The swine industry is under continual public and regulatory pressure to minimize environmental impact, while still meeting the increasing demand for pork. In the last few decades, vertical integration within the industry has allowed producers to increase economic efficiency by producing a greater number of pigs within a smaller geographic footprint. However, as swine production becomes more concentrated, minimizing environmental impacts from nutrient excretion and emissions becomes increasingly important. It is evident that

Received July 1, 2022 Accepted September 2, 2022.

reducing the environmental impact of swine production through nutrition will require a multifaceted approach, combining precise diet formulation with the use of nonnutritive feed additives.

Feeding benzoic acid (**BA**) has been shown to provide numerous benefits to swine by improving performance through improved nutrient availability, intestinal morphology, and modulating gut microbial populations (Kluge et al., 2006; Halas et al., 2010). BA is not oxidized in the body but is conjugated with glycine in the liver and excreted in the urine as hippuric acid (Kristensen et al., 2009), consistently resulting in decreased urine pH in various ages of pigs (Kluge et al., 2006, 2010; Kristensen et al., 2009; Sauer et al., 2009; Nørgaard et al., 2010; Galassi et al., 2011; Gutzwiller et al., 2011, 2014; Murphy et al., 2011). Decreased urine pH coupled with increased nitrogen retention has resulted in reduced ammonia (NH_3) emissions from finishing pigs fed BA at inclusion levels ranging from 1.0% to 3.0% (Murphy et al., 2011).

Supplementing *Bacillus*-based direct-fed microbials (**DFM**) has been associated with improved production performance (Davis et al., 2008; Balasubramanian et al., 2016; Jørgensen et al., 2016). *Bacillus* species produce digestive enzymes (Gould et al., 1975; Latorre et al., 2016), which may explain their ability to improve nutrient digestibility and production performance in swine. Furthermore, feeding *Bacillus*-based DFM lowered both manure NH_3 (Wang et al., 2009, 2021; Liu et al., 2018) and hydrogen sulfide (H_2S) (Lan et al., 2017; Liu et al., 2018) emissions through either improved nutrient retention or alterations in manure properties including microbial populations.

Little is known about the effects of supplementing both BA and DFM in swine diets and what is known is contradictory. Pu et al. (2020) reported improved average daily gain (**ADG**), feed efficiency, and intestinal morphology in weaned pigs with the combined use of BA and DFM, but Pérez Alvarado et al. (2013) showed only improved pig performance in response to BA and not in combination. However, the use of BA with DFM reduced ammonium in slurry further than if each product was used alone (Pérez Alvarado et al., 2013). Evaluating the benefits of feeding BA in combination with a DFM should be more wholistic, combining both improved nutrient digestibility with the added environmental benefits of reduced gas emissions from growing pigs.

The hypothesis was that feeding BA would improve nutrient digestibility, resulting in decreased nutrient excretion and gas emissions from manure, and that including a *Bacillus*-based DFM with BA improve those effects of BA further. Therefore, the objective of this experiment was to investigate the effect of BA with or without a DFM on the nutrient metabolism and gas emissions of manure from growing pigs.

Materials and Methods

All experimental protocols 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 20-181).

Animals, housing, and experimental design

This experiment was conducted at the Iowa State University Swine Nutrition Farm (Ames, IA), utilizing 24 crossbred gilts (PIC 337 × 1050, PIC Genus, Hendersonville, TN) with an initial body weight (**BW**) of 33.1 ± 1.3 kg.

At the start of the experiment, pigs were weighed and placed in metabolism stalls (0.7 × 1.5 m) equipped with a slatted floor, feeder, and nipple waterer. The metabolism stalls were in a temperature-controlled room, maintained at a temperature of approximately 21 °C. Pigs were blocked by initial BW and randomly assigned within a block to one of four dietary treatments.

Diets and feeding

Four dietary treatments were evaluated: a basal control diet (**PC**) formulated to represent a standard commercial diet with minimal crystalline amino acid supplementation, a low N diet (**NC**) formulated to reduce N excretion by lowering crude protein (**CP**) and amino acid levels, PC plus 0.3% BA (PC+BA; VevoVitall, DSM Nutritional Products, Parsippany, NJ), and PC plus 0.3% BA and 0.025% of a *Bacillus*-based DFM (PC+BA+DFM; PureGro, DSM Nutritional Products, Parsippany, NJ). The DFM supplied 1.47×10^8 colony-forming units (**CFU**) of *Bacillus* bacteria per gram of supplement, including two strains of *B. lichenformis* and one strain of *B. subtilis*. Four separate diets were milled, and the two feed additives were added to the basal diet at the expense of corn. Phytase (Ronozyme HiPhos 5,000 GT, DSM Nutritional Products, Parsippany, NJ) was included in all diets to provide 750 phytase units (**FTU**) per kg of diet and was assumed to release 0.12% available phosphorus.

All pigs were fed a common diet (Table 1) from day 0 to 14 for baseline measurements, as suggested by Jacobs et al. (2013). Following baseline collection, experimental diets were fed in two dietary phases, with phase one diets (Table 2) being fed from day 14 to 28 and phase two diets (Table 3) from day 28 to 53. Amino acid levels relative to lysine were held constant across diets within a phase. All diets were formulated to be isocaloric and met or exceeded NRC (2012) recommendations for vitamins, minerals, and amino acids, except for NC, which was formulated for reduced CP.

Feed allowance was determined based on the average ad libitum intake during the first acclimation period and set at 2.8 times the maintenance energy requirement ($197\,\text{kcal} \times \text{BW}^{0.6}$; NRC, 2012) of the average pig BW. Pigs were weighed at the end of each collection period, and feed allowance for the next period was adjusted based on the average BW. Feed allowance was split equally into two feedings at 0600 and 1600 hours daily. Feed remaining (orts) after 1 h was collected and weighed. Water was provided ad libitum throughout the entire trial.

Sample collection

Diet samples from each batch were collected at the time of mixing and stored at −20 °C for subsequent analysis. The 54-d experiment was separated into four collection periods, with the common diet (Table 1) being fed during collection period one, phase one experimental diets (Table 2) fed during collection period two, and phase two experimental diets (Table 3) fed during collection periods three and four. Each 8-d collection period was subdivided into two subperiods to facilitate studies on manure gas emissions and nutrient digestibility. The pigs were allowed 7, 6, 6, and 3 d of acclimation before collection periods one through four, respectively.

Table 1. Ingredients and nutrient composition of common diet (as-fed basis)

Ingredient, %	Common diet
Corn	61.590
Soybean meal	32.930
Monocalcium phosphate	0.930
Calcium carbonate	1.170
Sodium chloride	0.580
L-lysine HCl	0.230
L-threonine	0.080
DL-methionine	0.120
VTM premix[1]	0.350
Soybean oil	2.000
Phytase[2]	0.015
Total	100.000
Calculated composition	
Metabolizable energy, Mcal/kg	3.28
Crude protein, %	20.24
Calcium, %	0.74
Phosphorus, %	0.57
Available phosphorus, %	0.39
Ca:P	1.30
Total Lys, %	1.28
SID[3] Lys, %	1.14
SID Thr:Lys	0.61
SID Met + Cys:Lys	0.56
SID Trp:Lys	0.19
SID Val:Lys	0.67
SID Leu:Lys	1.25
SID Ile:Lys	0.63
Analyzed composition	
Gross energy, Mcal/kg	3.88
Dry matter, %	87.85
Ash, %	5.12
Crude protein, %	19.82
Total Lys, %	1.31
Calcium, %	0.77
Phosphorus, %	0.55
Ca:P	1.40

[1]Provided 4,594-IU vitamin A, 525-IU vitamin D, 37.5-IU vitamin E, 2.25-mg vitamin K, 8.25-mg riboflavin, 42-mg niacin, 20.25-mg pantothenic acid, 0.04-mg vitamin B12, 12-mg Cu (copper sulfate), 0.28-mg I (potassium iodate), 160-mg Fe (ferrous sulfate), 0.30-mg Se (sodium selenate), and 160-mg Zn (zinc sulfate) per kg of the diet.
[2]Ronozyme HiPhos 5,000 GT (DSM Nutritional Products); provided 750 FTU per kg diet, assuming 0.12% available phosphorus release.
[3]SID, standardized ileal digestible.

Manure gas emissions study

Each sub-period for manure gas emissions lasted 72 h. Urine and feces were collected twice daily at 0600 and 1600 hours. At each collection, urine and feces were weighed, and urine pH was measured using a pH probe (pH 150 Meter Kit, Oakton Instruments, Vernon Hills, IL), which was calibrated daily with certified pH 4, 7, and 10 buffer solutions (Fisher Scientific, Fair Lawn, NJ). Within each period, a constant weight of urine was retained, and feces were collected according to the excreta ratio (w/w) for each pig at each

Table 2. Ingredients and nutrient composition of phase one dietary treatments (as-fed basis)

Ingredient, %	Dietary treatment			
	PC	PC+BA	PC+BA+ DFM	NC
Corn	66.015	65.715	65.690	74.522
Soybean meal	28.853	28.853	28.853	20.408
Monocalcium phosphate	0.671	0.671	0.671	0.714
Calcium carbonate	1.076	1.076	1.076	1.140
Sodium chloride	0.579	0.579	0.579	0.579
L-lysine HCl	0.241	0.241	0.241	0.299
L-threonine	0.091	0.091	0.091	0.105
DL-methionine	0.111	0.111	0.111	0.090
L-tryptophan	–	–	–	0.015
L-valine	–	–	–	0.025
VTM premix[1]	0.350	0.350	0.350	0.350
Soybean oil	2.000	2.000	2.000	1.737
Phytase[2]	0.015	0.015	0.015	0.015
Benzoic acid[3]	–	0.300	0.300	–
Direct-fed microbial[4]	–	–	0.025	–
Total	100.000	100.000	100.000	100.000
Calculated composition				
Metabolizable energy, Mcal/kg	3.30	3.30	3.30	3.30
Crude protein, %	18.67	18.67	18.67	15.46
Calcium, %	0.65	0.65	0.65	0.65
Phosphorus, %	0.50	0.50	0.50	0.48
Available phosphorus, %	0.33	0.33	0.33	0.33
Ca:P	1.30	1.30	1.30	1.35
Total Lys, %	1.18	1.18	1.18	1.00
SID Lys, %	1.04	1.04	1.04	0.89
SID Thr:Lys	0.62	0.62	0.62	0.62
SID Met + Cys:Lys	0.57	0.57	0.57	0.57
SID Trp:Lys	0.18	0.18	0.18	0.18
SID Val:Lys	0.67	0.67	0.67	0.67
SID Leu:Lys	1.27	1.27	1.27	1.27
SID Ile:Lys	0.62	0.62	0.62	0.62
Analyzed composition				
Gross energy, Mcal/kg	3.91	3.87	3.90	3.85
Dry matter, %	88.07	87.79	87.73	87.95
Ash, %	4.34	4.17	4.47	4.12
Crude protein, %	17.93	17.23	17.50	14.31
Total Lys, %	1.17	1.10	1.27	1.06
Calcium, %	0.73	0.74	0.73	0.71
Phosphorus, %	0.48	0.48	0.48	0.45
Ca:P	1.52	1.54	1.52	1.58

[1]Provided 4,594-IU vitamin A, 525-IU vitamin D, 37.5-IU vitamin E, 2.25-mg vitamin K, 8.25-mg riboflavin, 42-mg niacin, 20.25-mg pantothenic acid, 0.04-mg vitamin B_{12}, 12-mg Cu (copper sulfate), 0.28-mg I (potassium iodate), 160-mg Fe (ferrous sulfate), 0.30-mg Se (sodium selenate), and 160-mg Zn (zinc sulfate) per kg of the diet.
[2]Ronozyme HiPhos 5,000 GT (DSM Nutritional Products); provided 750 FTU, assuming 0.12% available phosphorus release.
[3]VevoVitall (DSM Nutritional Products).
[4]PureGro (DSM Nutritional Products); provided 1.47×10^8 CFU of *Bacillus* bacteria per gram of supplementation.

Table 3. Ingredients and nutrient composition of phase two dietary treatments (as-fed basis)

Ingredient, %	Dietary treatment			
	PC	PC+BA	PC+BA+ DFM	NC
Corn	70.865	70.565	70.540	78.584
Soybean meal	23.921	23.921	23.921	16.251
Monocalcium phosphate	0.763	0.763	0.763	0.803
Calcium carbonate	1.087	1.087	1.087	1.145
Sodium chloride	0.579	0.579	0.579	0.579
L-lysine HCl	0.243	0.243	0.243	0.301
L-threonine	0.093	0.093	0.093	0.107
DL-methionine	0.084	0.084	0.084	0.068
L-tryptophan	–	–	–	0.015
L-valine	–	–	–	0.025
VTM premix[1]	0.350	0.350	0.350	0.350
Soybean oil	2.000	2.000	2.000	1.757
Phytase[2]	0.015	0.015	0.015	0.015
Benzoic acid[3]	–	0.300	0.300	–
Direct-fed microbial[4]	–	–	0.025	–
Total	100.000	100.000	100.000	100.000
Calculated composition				
Metabolizable energy, Mcal/kg	3.30	3.30	3.30	3.30
Crude protein, %	16.72	16.72	16.72	13.81
Calcium, %	0.65	0.65	0.65	0.65
Phosphorus, %	0.50	0.50	0.50	0.48
Available phosphorus, %	0.35	0.35	0.35	0.35
Ca:P	1.30	1.30	1.30	1.35
Total Lys, %	1.05	1.05	1.05	0.89
SID Lys, %	0.93	0.93	0.93	0.79
SID Thr:Lys	0.63	0.63	0.63	0.63
SID Met+Cys:Lys	0.57	0.57	0.57	0.57
SID Trp:Lys	0.18	0.18	0.18	0.18
SID Val:Lys	0.67	0.67	0.67	0.67
SID Leu:Lys	1.30	1.30	1.30	1.30
SID Ile:Lys	0.61	0.61	0.61	0.61
Analyzed composition				
Gross energy, Mcal/kg	3.88	3.87	3.81	3.83
Dry matter, %	88.03	88.04	87.47	87.96
Ash, %	4.35	4.28	4.13	3.91
Crude protein, %	15.17	15.20	15.17	13.05
Total Lys, %	1.07	1.11	0.98	0.95
Calcium, %	0.74	0.75	0.75	0.74
Phosphorus, %	0.48	0.51	0.45	0.47
Ca:P	1.54	1.47	1.67	1.57

[1]Provided 4,594-IU vitamin A, 525-IU vitamin D, 37.5-IU vitamin E, 2.25-mg vitamin K, 8.25-mg riboflavin, 42-mg niacin, 20.25-mg pantothenic acid, 0.04-mg vitamin B_{12}, 12-mg Cu (copper sulfate), 0.28 mg I (potassium iodate), 160-mg Fe (ferrous sulfate), 0.30-mg Se (sodium selenate), and 160-mg Zn (zinc sulfate) per kg of the diet.
[2]Ronozyme HiPhos 5,000 GT (DSM Nutritional Products); provided 750 FTU, assuming 0.12% available phosphorus release.
[3]VevoVitall (DSM Nutritional Products).
[4]PureGro (DSM Nutritional Products); provided 1.47×10^8 CFU of *Bacillus* bacteria per gram of supplementation.

collection, as determined by weighing the total amount of urine and feces excreted. Urine and feces from each pig were stored together at room temperature (21 °C) in an 18.93-L plastic container, partially covered with a plastic lid. On day 5 of storage following each collection period, manure was homogenized and approximately 1,000 mL was stored at 4 °C for laboratory analysis. Manure remaining after sampling was combined with the manure from previous periods to determine the effects of extended storage on manure gas emissions and characteristics. The combined manure was stored at room temperature for 17 d after the addition of manure from period 3.

Laboratory analysis for manure gas emissions was conducted within 3 d of sampling at the farm. In brief, 500 mL of manure was added to 1.3-L bioreactors (New Brunswick Bioflo/ CelliGen 110/ 115, Eppendorf, Hamburg, Germany), maintained at 24 °C and continuously stirred (50 RPM), and purged with N_2 gas at 1-L/min. Headspace samples were collected from each bioreactor using sampling bags compatible with NH_3, H_2S, and methane (CH_4) gases (FlexFoil PLUS, SKC, Inc., Eighty Four, PA). Concentrations of NH_3, H_2S, and CH_4 were quantified using cavity ringdown spectrometers (Model G2103 and Model G2204, Picarro Inc., Santa Clara, CA). When gas concentrations in bags exceeded the instrument threshold, the bags were further diluted with N_2 gas. Headspace carbon dioxide concentrations (CO_2) were determined using a photoacoustic multigas analyzer (INNOVA Model 1312, California Analytical Instruments Inc., Orange, CA). Manure pH was measured at the time of gas measurement using a pH probe (Model 405-DPAS-SC-K85, Mettler Toledo, Columbus, OH).

Apparent digestibility and balance study

Each collection sub-period for digestibility samples lasted 96 h. Total quantities of urine and feces were collected twice daily at 0600 and 1600 hours and immediately stored at –20 °C. Urine was collected into stainless steel buckets containing 25 mL of 6 N HCl to prevent bacterial growth and N volatilization. After each period, urine was thawed, weighed, and a subsample retained and stored again at –20 °C for subsequent analysis.

Laboratory analytical methods

All orts were oven-dried at 75 °C to a constant weight and calculated back into dry matter (**DM**) intake. Before analysis, total feces from each collection period were oven-dried to a stable weight at 75 °C, ground, and subsampled. Diets and dried fecal samples were ground through a 1-mm screen using a Wiley Mill (Variable Speed Digital ED-5 Wiley Mill; Thomas Scientific, Swedesboro, NJ). Urine subsamples were thawed, homogenized, filtered through Whatman 41 filter paper (GE Healthcare Life Sciences, Chicago, IL), and stored in plastic screw-top containers at 4 °C until further analysis.

Diets and fecal samples were analyzed in duplicate for DM and ash. The percentage of DM and ash was calculated by the mass difference after oven drying for 24 h at 100 °C and 12 h at 600 °C, respectively. Diet, fecal, and urine samples were analyzed in duplicate for N by the Dumas combustion method using an automatic N analyzer (TruMac N; LECO Corp., St. Joseph, MI). Ethylenediaminetetraacetic acid (9.56% N) was used as the standard for calibration for N analysis and was determined to contain 9.56 ± 0.07% N. CP was calculated as N × 6.25. Total solids (**TS**), total volatile solids (**TVS**), and

total nitrogen (**TN**) of manure samples were determined using the same methods as DM, ash, and N in feed, respectively.

Diet and fecal samples were analyzed in duplicate for gross energy (**GE**) using an isoperibolic bomb calorimeter (model 6200; Parr Instrument Co., Moline, IL). BA (6,318 kcal/kg) was used as the standard for calibration and was determined to contain 6321 ± 13 kcal/kg. To determine GE in urine, 3 ml of urine was added to 0.50 g of dried cellulose (Acros Organics, Geel, Belgium) and dried for 72 h at 50 °C. Dried urine plus cellulose samples were analyzed in triplicate, and urinary energy was calculated from the difference in energy determined in cellulose alone and the samples with both urine and cellulose.

Fecal and urine samples were submitted to the University of Missouri Agricultural Experiment Station Laboratories (Columbia, MO) to be analyzed in duplicate for Ca and P using inductively coupled plasma-mass spectrometry (ICP; method 993.14). Diet samples were also subject to complete amino acid profiling at the University of Missouri using cation-exchange chromatography coupled with postcolumn ninhydrin derivatization and quantification (method 982.30 E and 988.15; AOAC, 2006). Diet samples were submitted to Eurofins Scientific Inc. (Des Moines, IA) for ICP analysis of Ca and P (AOAC 984.27 mod, 927.02 mod, 985.01 mod, 965.17 mod).

Calculations and statistical analysis

Digestible energy (**DE**) was calculated by subtracting fecal energy from GE intake. Metabolizable energy (**ME**) was calculated by subtracting urinary energy from DE (CH_4 losses were omitted). Digestibility and nutrient balance values were calculated using the following equations:

$$\begin{aligned}\text{Apparent total tract digestibility (ATTD)} = \\ [\,(\text{nutrient in feed} \times \text{feed intake}) \\ -(\text{nutrient in feces} \times \text{fecal output})\,] \,/ \\ (\text{nutrient in feed} \times \text{feed intake}),\end{aligned}$$

$$\begin{aligned}\text{Total nutrient excretion} = \text{fecal nutrient excretion} \\ + \text{urinary nutrient excretion},\end{aligned}$$

$$\text{Nutrient retention} = \text{nutrient intake} - \text{total nutrient excretion},$$

$$\text{Retention (\% intake)} = \text{nutrient retention} \,/\, \text{nutrient intake},$$

$$\begin{aligned}\text{Retention (\% digestible)} = \text{nutrient retention} \,/ \\ (\text{nutrient retention} \times \text{ATTD coefficient}).\end{aligned}$$

Manure characteristics, gas emissions, and digestibility data were analyzed as repeated measures according to the following statistical model:

$$y_{ijkl} = \mu + T_i + B_j + C_{ijl} + P_k + (T * P)_{ik} + e_{ijkl},$$

where y_{ijkl} is the observed value for the first experimental unit within the i-th level of dietary treatment from the j-th block during the k-th period; μ is the overall mean; T_i is the fixed effects of the i-th dietary treatment (i = 1 to 4); B_j is the fixed effect of the j-th block (j = 1 to 6); C_{ijl} is the fixed effect covariate of the first experimental unit from the i-th dietary treatment and j-th block; P_k is the fixed effect of the k-thperiod (k = 1 to 3); $(T * P)_{ik}$ is the interaction between dietary treatment and period; e_{ijkl} is the random error associated with y_{ijkl}, assuming $e_{ijkl} \sim \mathbf{N}(0, \mathbf{R})$, where $\mathbf{R} = \mathbf{N}[0, \mathbf{I_n} \otimes \mathbf{ARH}(1)]$ for digestibility data and $\mathbf{R} = \mathbf{N}[0, \mathbf{I_n} \otimes \mathbf{AR}(1)]$ for manure characteristics and emissions. I_n is the identity matrix, **AR** (1) is the first-order autoregressive covariance matrix, and **ARH** (1) is the **AR** (1) covariance matrix with heterogeneous variances. The ratio of urine-to-feces of each pig was used as a covariate for the manure and gas emissions data, and the measurements from the baseline collection period were used as the covariates for the respective digestibility and balance response variables. Ammonia, H_2S, and CH_4 emissions were natural log-transformed to achieve normality. Manure gas emissions data were analyzed as headspace concentrations and reported as flux rates, assuming one atmosphere at 20 °C.

Aged manure data were analyzed according to the following statistical model:

$$y_{ijk} = \mu + T_i + B_j + C_{ij} + e_{ijk},$$

where y_{ijk} is the observed value for the k-th experimental unit within the i-th level of dietary treatment from the j-th block; μ is the overall mean; T_i is the fixed effect of the i-th dietary treatment (i = 1 to 4); B_j is the fixed effect of the j-th block (j = 1 to 6); C_{ij} is the fixed effect covariate on the urine-to-feces ratio of the k-th experimental unit from the i-th dietary treatment and j-th block; e_{ijk} is the random error associated with y_{ijk}, assuming $e_{ijk} \sim \mathbf{N}(0, \mathbf{I}\sigma_e^2)$.

The statistical models were implemented in SAS 9.4 (SAS Institute, Cary, NC) using the GLIMMIX procedure. The UNIVARIATE procedure was used to verify normality and homoscedasticity of the Studentized residuals. Statistical outliers were identified as Studentized residuals greater than three standard deviations from the mean and were excluded from the analysis. Data were reported as least squares means, and means separation was achieved using the probability of difference (**PDIFF**) option with Tukey adjustment for multiplicity. Results were considered significant if $P \leq 0.05$ and a tendency if $0.05 < P \leq 0.10$.

Results

General health

During initial acclimation (day 0 to 6), two pigs were observed with diarrhea and subsequently treated with tylosin phosphate (Tylan 200, Elanco Animal Health, Indianapolis, IN). Furthermore, during the final digestibility collection period (day 49 to 53), four pigs exhibited symptoms of a health challenge (i.e., low feed intake, fever, diarrhea). These pigs were treated with tylosin phosphate and flunixin meglumine. Fecal samples and nasal swabs were collected from affected pigs and submitted to the Iowa State Veterinary Diagnostic Laboratory (Ames, IA). The samples were not found to contain any pathogenic organisms; however, these four pigs (two from NC, one from PC+BA, and one from PC+BA+DFM) were removed from the trial because symptoms persisted for greater than 24 h and interfered with proper sample collection.

Diet analysis

Results of feed proximate analysis indicated that CP levels were slightly lower than expected across all treatments in both phases; however, as planned, PC, PC+BA, and PC+BA+DFM diets were similar in CP, and NC was 3% lower (Tables 2 and 3). Total lysine levels showed some variation but were not drastically different from formulated values in both phases (Tables 2 and 3). Analyzed Ca values were slightly higher than expected, resulting in a higher ratio of total Ca-to-total P (Ca:P); however, this was consistent across all treatments (Tables 2 and 3).

Growth performance

Pigs started the trial at an average BW of 33.09 ± 1.33 kg and ended on day 53 at an average BW of 77.13 ± 3.22 kg. There was no evidence for an effect of dietary treatment on BW (P = 0.548; Table 4), but as expected, BW increased over time (Period P < 0.001). ADG was significantly increased in pigs fed PC+BA compared to pigs fed NC (P = 0.016). Average daily feed intake (**ADFI**) was not different between treatments (P = 0.362; Table 4). Consequently, gain-to-feed ratio (**G:F**) tended to be increased in pigs fed PC+BA compared to pigs fed NC (P = 0.079). By design, feed intake increased from periods one to three (period P < 0.001; Table 4).

Manure characteristics and gas emissions

Manure from pigs fed NC had a lower NH_3 flux rate compared to manure from pigs fed PC (P = 0.006; Table 5) and PC+BA+DFM (P < 0.001). Furthermore, manure from pigs fed PC+BA had lower NH_3 emissions than manure from PC+BA+DFM fed pigs (P = 0.004) and tended to have lower NH_3 emissions than PC (P = 0.077). There was no evidence for an effect of dietary treatment on manure emissions of H_2S, CO_2, or CH_4 ($P \geq 0.200$).

Urine pH was significantly increased in pigs fed PC compared to pigs fed PC+BA (P = 0.006) and NC (P = 0.042; Table 5). Urine from pigs fed PC+BA+DFM was intermediate in pH and was similar to all other treatments. Consequently, manure pH from pigs fed PC and PC+BA+DFM was significantly higher than manure from pigs fed PC+BA ($P \leq 0.001$) and NC (P < 0.001). Manure TN, TS, and TVS did not differ between treatments ($P \geq 349$).

There was no effect of dietary treatment on the aged manure gas emissions, TN, TS, or TVS; however, the pH of manure from pigs fed PC remained higher than all other treatments ($P \leq 0.04$; Table 6).

Apparent total tract digestibility

The ATTD of DM, ash, organic matter (**OM**), P, GE, and CP did not differ between dietary treatments ($P \geq 0.093$; Table 7). However, the ATTD of Ca was significantly reduced in the pigs fed BA+DFM compared to pig fed BA (P = 0.012), with intermediate Ca ATTD in pigs fed PC and NC. In general, ATTD decreased from periods one to three ($P \leq 0.011$).

Nitrogen balance

Small standard errors in the first period apparently allowed for the detection of biologically irrelevant differences in N

Table 4. Effect of dietary treatment and collection period on pig bodyweight, average daily gain, average daily feed intake, and gain-to-feed ratio

Item	Dietary treatment				SEM	*P*-value	Period				SEM	*P*-value	*P*-value
	PC	PC+BA	PC+BA+DFM	NC			0	1	2	3			Trt × Period
BW, kg	60.03	60.90	59.71	59.89	0.613	0.548	42.55^{z}	53.91^{y}	66.93^{x}	77.13^{w}	0.477	< 0.001	0.462
ADG, g/d	882.25^{a,b}	921.64^{a}	905.57^{ab}	848.60^{b}	16.566	0.023	–	811.13^{y}	930.25^{x}	927.17^{x}	14.699	< 0.001	0.681
ADFI, g/d	1826.52	1826.80	1834.27	1813.57	8.240	0.362	–	1597.71^{z}	1827.08^{y}	2051.07^{x}	11.00	< 0.001	0.391
GF	0.486	0.510	0.494	0.476	0.0117	0.095	–	0.508^{y}	0.510^{x}	0.457^{z}	0.0088	< 0.001	0.894

^{a,b}Treatment means without a common superscript differ ($P \leq 0.05$).
^{x,y}Period means without a common superscript differ ($P \leq 0.05$).

Table 5. Effect of dietary treatment and collection period on manure gas emissions and composition

Item	Dietary treatment				SEM	*P*-value	Period			SEM	*P*-value	*P*-value
	PC	PC+BA	PC+BA+DFM	NC			1	2	3			Trt × Period
NH_3, g/m^2/d	49.91^{b,c}	34.86^{a,b}	62.20^{c}	28.69^{a}	7.207	< 0.001	46.14	39.89	40.20	4.333	0.416	0.470
H_2S, mg/m^2/d	277.73	241.09	318.20	355.09	54.792	0.200	248.46^{x}	267.46^{x}	386.05^{y}	46.13	0.015	0.099
CH_4, mg/m^2/d	351.64	649.39	343.54	509.59	240.376	0.349	425.69^{x,y}	363.00^{x}	578.54^{y}	121.70	0.042	0.818
CO_2, g/m^2/d	203.35	201.46	200.29	196.68	9.630	0.965	191.91^{x}	188.47^{x}	220.97^{y}	7.483	0.006	0.106
Urine pH	7.73^{b}	7.30^{a}	7.44^{a,b}	7.40^{a}	0.080	0.008	7.63^{y}	7.37^{x}	7.40^{x}	0.046	< 0.001	0.086
Manure pH	8.59^{b}	8.25^{a}	8.47^{b}	8.11^{a}	0.040	< 0.001	8.54^{y}	8.22^{x}	8.32^{x}	0.043	< 0.001	0.343
TN, g/kg	5.18	5.08	5.38	5.21	0.374	0.947	5.29	4.87	5.48	0.241	0.047	0.308
TS, g/kg	77.53	78.54	74.92	83.94	5.522	0.720	76.67	77.32	82.21	3.401	0.314	0.053
TVS, g/kg	20.91	19.45	19.58	22.31	1.239	0.349	20.66	19.73	21.30	0.745	0.070	0.079

^{a,b,c}Treatment means without a common superscript differ ($P \leq 0.05$).
^{x,y}Period means without a common superscript differ ($P \leq 0.05$).

Table 6. Effect of dietary treatment on stored manure gas emissions and composition

Item	Dietary treatment				SEM	*P*-value
	PC	PC+BA	PC+BA+ DFM	NC		
NH_3, g/m²/d	18.45	14.38	15.74	15.14	2.924	0.774
H_2S, mg/m²/d	1255.47	1176.86	1139.39	939.77	632.970	0.981
CH_4, mg/m²/d	371.14	605.91	498.83	822.15	190.51	0.386
CO_2, g/m²/d	264.89	344.28	276.61	322.06	24.558	0.114
Manure pH	8.17[a]	7.65[b]	7.87[b]	7.73[b]	0.071	0.001
TN, g/kg	4.59	5.09	4.21	6.28	0.836	0.327
TS, g/kg	95.44	86.27	85.76	80.54	5.064	0.232
TVS, g/kg	24.64	23.52	23.31	21.31	1.289	0.327

[a,b]Treatment means without a common superscript differ ($P \leq 0.05$).

Table 7. Effect of dietary treatment and collection period on apparent total tract digestibility (dry matter basis)

ATTD, %	Dietary treatment				SEM	*P*-value	Period			SEM	*P*-value	*P*-value
	PC	PC+ BA	PC+ BA+ DFM	NC			1	2	3			Trt × Period
Dry matter	91.66	91.45	91.35	91.68	0.225	0.702	92.13[x]	91.44[x,y]	91.04[y]	0.273	0.007	0.096
Ash	71.84	73.07	70.24	71.06	0.910	0.141	73.27[x]	72.11[x,y]	69.28[y]	1.002	0.013	0.509
OM	92.69	92.38	92.43	92.66	0.201	0.645	93.09[x]	92.40[x,y]	92.13[y]	0.244	0.006	0.056
Calcium	66.21[a,b]	69.20[a]	60.97[b]	67.12[a,b]	1.741	0.012	67.85[x]	69.00[x]	60.78[y]	1.832	0.001	0.841
Phosphorus	65.10	69.23	64.70	65.53	1.408	0.100	66.82[x,y]	68.59[x]	63.01[y]	1.396	0.010	0.936
GE	90.44	90.22	90.54	90.33	0.249	0.781	91.07[x]	90.22[x,y]	89.86[y]	0.298	0.006	0.543
CP	89.46	90.23	89.32	88.66	0.426	0.093	90.33[x]	89.13[y]	88.79[y]	0.440	0.011	0.584

[a,b]Treatment means without a common superscript differ ($P \leq 0.05$).
[x,y]Period means without a common superscript differ ($P \leq 0.05$).

Table 8. Effect of dietary treatment and collection period on nitrogen intake, excretion, and retention

Item	Dietary treatment				SEM	*P*-value	Period			SEM	*P*-value	*P*-value
	PC	PC+BA	PC+BA+ DFM	NC			1	2	3			Trt × Period
N intake, g/d	46.27	45.74	46.53	38.33	0.461	< 0.001	42.75	42.76	47.13	0.620	< 0.001	< 0.001
Fecal N, g/d	4.88	4.47	5.00	4.36	0.202	0.081	4.12[x]	4.62[y]	5.28[z]	0.211	< 0.001	0.286
Urine N, g/d	13.18[b]	10.64[a]	12.71[b]	9.02[a]	0.501	< 0.001	10.44[x]	10.37[x]	13.36[y]	0.382	< 0.001	0.576
Total N excretion, g/d	18.19[c]	15.28[b]	17.56[c]	13.28[a]	0.497	< 0.001	14.55[x]	15.10[x]	18.57[y]	0.419	< 0.001	0.112
Urinary N:fecal N	2.76[b]	2.48[ab]	2.61[ab]	2.18[a]	0.139	0.034	2.58	2.30	2.64	0.146	0.069	0.487
N retention, g/d	28.01[b]	30.29[a]	29.03[ab]	25.09[c]	0.567	< 0.001	28.19	27.56	28.57	0.652	0.044	0.120
N retention, % of N intake	60.67[b]	66.46[a]	62.51[ab]	65.33[a]	1.007	0.003	66.06[x]	64.60[x]	60.56[y]	0.884	< 0.001	0.625
N retention, % of dig. N	68.30[b]	73.97[a]	69.56[ab]	73.56[a]	1.218	0.007	73.09[x]	72.88[x]	68.07[y]	1.009	< 0.001	0.723

[a,b,c]Treatment means without a common superscript differ ($P \leq 0.05$).
[x,y,z]Period means without a common superscript differ ($P \leq 0.05$).

intake between all treatments (SEM = 0.033 g/d; Trt × period $P < 0.001$; Table 8). Aside from these differences in the first period, pigs fed NC had significantly lower N intake per day compared to all other treatments in all periods ($P < 0.001$).

Fecal N (g/d) did not differ between treatments ($P \geq 0.081$; Table 8) but increased with later collection periods ($P < 0.001$). Pigs fed PC+BA and NC excreted less urinary N compared to pigs fed PC and PC+BA+DFM, which resulted in pigs fed PC+BA and NC having less total N excretion (g/d) compared to pigs fed PC and PC+BA+DFM ($P \leq 0.034$). Furthermore, pigs fed NC had less total N output than pigs fed PC+BA ($P = 0.048$). The proportion of urine to fecal N was significantly impacted by dietary treatment ($P = 0.034$), with NC being lower than PC ($P = 0.024$) and PC+BA and PC+BA+DFM intermediate to PC and NC.

Lower N intake in the pigs fed NC decreased N retention (g/d) compared to all other treatments ($P \leq 0.005$). Furthermore, reduced urinary N in pigs fed PC+BA resulted in

increased N retention (g/d) compared to PC ($P = 0.028$), but retention in pigs fed PC+BA+DFM was similar compared to PC+BA and PC ($P \geq 0.400$). However, as a proportion of N intake, retention was similar between PC+BA, PC+BA+DFM, and NC fed pigs ($P \geq 0.076$) and lower in pigs fed PC than PC+BA and NC ($P \leq 0.016$). This relationship was also observed when retention was expressed as a proportion of digestible N intake.

Calcium and phosphorus balance

Small standard errors of intakes allowed for the detection of small differences of Ca and P intake per day (< 0.50 g; Trt × Period $P < 0.001$; Tables 9 and 10) in the first two periods, but not in the third period. There was no evidence for an effect of dietary treatment on fecal or urine *P* excretion. Consequently, there was no significant difference in total *P* excretion between treatments ($P = 0.581$; Table 9); however, numerical differences in total excretion contributed to the greater *P* retention (g/d) in PC+BA compared to PC+BA+DFM and NC ($P \leq 0.017$). These differences were not evident when retention was standardized on total or digestible P intake.

Total Ca excretion was significantly increased in pigs fed PC+BA+DFM compared to PC+BA (Table 10; $P = 0.014$), largely due to increased fecal Ca excretion in pigs fed PC+BA+DFM. Furthermore, pigs fed PC+BA retained more Ca than pigs fed PC+BA+DFM on both a grams per day and proportion of Ca intake basis ($P \leq 0.041$). There was a significant effect of collection period on all response variables analyzed ($P \leq 0.002$). Generally, Ca and P excretion increased, and retention decreased from periods one to three.

Energy value and efficiency

Similar other nutrients were investigated, extremely small standard errors allowed for the detection of biologically irrelevant differences in GE intake in the first two periods (Trt × period $P < 0.001$; Table 11), but not in the third period. However, DE and ME as a proportion of intake did not differ between treatments ($P \geq 0.496$), which resulted in similar ME efficiency across treatments ($P = 0.058$).

Discussion

Feeding BA alone increased ADG compared to NC, but, although ADFI was not different among treatments, there was no detectable difference in feed efficiency. Improvements in growth rate in response to feeding PC+BA is consistent with previous work, which has shown optimization of ADG at 0.36% BA in grow-finish pigs up to 110 kg (Zhai et al., 2017). Halas et al. (2010) fed nursery pigs 0.5% BA and observed increases in villous height, villous height-to-crypt dept ratio, and small intestine weight-to-length ratio. Therefore, it could be speculated that improvements in intestinal morphology and increased gastrointestinal mass contributed to the increased ADG in BA-fed pigs. This concept is

Table 9. Effect of dietary treatment and collection period on phosphorus intake, excretion, and retention

Item	Dietary treatment				SEM	*P*-value	Period			SEM	*P*-value	*P*-value
	PC	PC+ BA	PC+ BA+ DFM	NC			1	2	3			Trt × Period
Intake, g/d	8.67	9.01	8.45	8.30	0.098	0.001	7.50	8.73	9.60	0.132	< 0.001	< 0.001
Fecal, g/d	3.04	2.77	3.00	2.86	0.125	0.403	2.49^{x}	2.73^{x}	3.54^{y}	0.125	< 0.001	0.724
Urine, g/d	0.14	0.12	0.06	0.15	0.028	0.083	0.05^{x}	0.10^{y}	0.21^{z}	0.032	< 0.001	0.413
Total excretion, g/d	3.16	2.91	3.07	3.01	0.130	0.581	2.54^{x}	2.83^{y}	3.75^{z}	0.125	< 0.001	0.623
Retention, g/d	$5.51^{a,b}$	6.07^{a}	5.38^{b}	5.31^{b}	0.157	0.007	4.96^{y}	5.89^{x}	5.85^{x}	0.181	< 0.001	0.570
Retention, % of intake	63.79	67.76	63.81	63.82	1.495	0.149	66.08^{x}	67.51^{x}	60.79^{y}	1.434	0.002	0.920
Retention, % of dig. P	97.51	98.04	98.92	97.44	0.485	0.108	99.05^{x}	98.41^{y}	96.48^{z}	0.552	0.001	0.560

[a,b]Treatment means without a common superscript differ ($P \leq 0.05$).
[x,y,z]Period means without a common superscript differ ($P \leq 0.05$).

Table 10. Effect of dietary treatment and collection period on calcium intake, excretion, and retention

Item	Dietary treatment				SEM	*P*-value	Period			SEM	*P*-value	*P*-value
	PC	PC+ BA	PC+ BA+ DFM	NC			1	2	3			Trt × Period
Intake, g/d	13.37	13.50	13.65	13.02	0.149	0.041	11.65	13.56	14.93	0.201	< 0.001	< 0.001
Fecal, g/d	4.48^{ab}	4.21^{a}	5.39^{b}	4.33^{a}	0.237	0.004	3.74^{x}	4.20^{x}	5.87^{y}	0.172	< 0.001	0.267
Urine, g/d	0.589	0.514	0.714	0.623	0.141	0.554	0.896	0.600	0.413	0.096	< 0.001	0.039
Total excretion, g/d	$5.39^{a,b}$	4.81^{a}	6.00^{b}	$5.10^{a,b}$	0.269	0.020	4.71^{x}	4.89^{x}	6.38^{y}	0.185	< 0.001	0.103
Retention, g/d	$7.83^{a,b}$	8.74^{a}	7.63^{b}	$8.01^{a,b}$	0.286	0.051	6.94^{y}	8.66^{x}	8.56^{x}	0.310	< 0.001	0.520
Retention, % of intake	$58.74^{a,b}$	64.82^{a}	56.32^{b}	$61.22^{a,b}$	1.969	0.032	59.56^{y}	63.92^{x}	57.34^{y}	1.834	0.001	0.321
Retention, % of dig. Ca	91.54	93.09	90.80	91.19	1.028	0.393	87.92	92.67	94.38	0.594	< 0.001	< 0.001

[a,b]Treatment means without a common superscript differ ($P \leq 0.05$).
[x,y,z]Period means without a common superscript differ ($P \leq 0.05$).

Table 11. Effect of dietary treatment and collection period on energy value and efficiency

Item	Dietary treatment				SEM	*P*-value	Period			SEM	*P*-value	*P*-value
	PC	PC+BA	PC+BA+DFM	NC			1	2	3			Trt × Period
GE intake, Mcal/d	7.03	7.01	7.04	6.83	0.080	0.243	6.19	7.02	7.72	0.108	< 0.001	< 0.001
DE, %	90.44	90.22	90.54	90.33	0.249	0.781	91.07^{x}	90.22^{x,y}	89.86^{y}	0.298	0.006	0.153
ME, %	86.96	86.73	86.68	87.25	0.290	0.496	87.53^{x}	86.77^{x,y}	86.41^{y}	0.324	0.028	0.221
ME/DE efficiency, %	96.12	96.05	95.88	96.64	0.203	0.058	96.11	96.18	96.23	0.149	0.825	0.933

^{x,y}Period means without a common superscript differ ($P \leq 0.05$).

further supported by a study conducted by Diao et al. (2016), which saw increases in jejunal mucosa glucagon-like peptide 2 concentration in weaned pigs in response to feeding BA. Glucagon-like peptide 2 is a hormone secreted by intestinal endocrine cells and has been shown to inhibit epithelial apoptosis and stimulate cell proliferation (Drucker, 2001).

Dietary supplementation of BA has been shown to improve ATTD of N, Ca, and P, but this has not been a consistent observation (Sauer et al., 2009; Nørgaard et al., 2010; Galassi et al., 2011; Gutzwiller et al., 2011; Murphy et al., 2011). The current experiment supports this lack of consistency, as no differences in ATTD were observed between PC+BA and PC-fed pigs.

It has been estimated that N excretion decreases by 8 to 10 percent for every one percent decrease of CP in the diet (Wang et al., 2018; Trabue et al., 2021). In the body, excess amino acids are degraded in the liver and excreted in the urine as urea; therefore, reducing CP in the diet will minimize the amount of excess amino acids that must be excreted, ultimately lowering N concentration in urine. This is supported by the current experiment results, where an approximate 3% decrease in CP in the NC diet resulted in an average 27% decrease in total N excretion per day. Decreased urinary N in pigs fed PC+BA resulted in approximately 16% lower total N excretion and increased retention compared to pigs fed PC. These results are supported by work conducted by Murphy et al. (2011), which saw a significant linear increase in N retention in response to feeding 0 to 3.0% BA.

Similar fecal N excretion and increased N retention in PC+BA-fed pigs could indicate that the pigs were absorbing similar amounts of CP from the diet, but pigs fed PC+BA had increased protein synthesis rates or decreased protein turnover. This relationship is further supported by the differences in growth observed in the experiment. However, measuring ATTD N is limited in that it ignores endogenous losses, making discernment of the origin of excreted N in the feces impossible to determine (Zhang and Adeola, 2017). Therefore, to further understand the cause of increased nitrogen retention, research investigating the true digestibility of nutrients in response to feeding BA is warranted.

BA is not included in calculations to determine metabolic acid-base load (Patience et al., 1987). However, the dominant route of BA metabolism is conversion to hippuric acid in the liver and subsequent renal excretion in the urine (Kristensen et al., 2009). Consequently, hippuric acid excretion from BA metabolism has been shown to significantly lower urine pH in growing and finishing pigs (Kristensen et al., 2009; Sauer et al., 2009; Nørgaard et al., 2010; Galassi et al., 2011; Gutzwiller et al., 2011; Murphy et al., 2011). Urine pH is a major determinant of manure pH; therefore, lowering urine pH by feeding BA has also been associated with decreased manure pH (Hansen et al., 2007; Galassi et al., 2011; Murphy et al., 2011; Pérez Alvarado et al., 2013). Comparable differences were observed in the current experiment, where PC+BA lowered urine and manure pH by approximately 0.43 and 0.34 units, respectively.

Ammonia emission from manure is a dynamic process influenced by numerous factors, including pH, temperature, and NH_3 concentration. In manure, NH_3 is in equilibrium with NH_4^+, and increasing pH favors the NH_3 species (Liu et al., 2013). In this experiment, at a constant temperature, NH_3 emissions were decreased from the manure of pigs fed PC+BA and NC diets compared to pigs fed PC or PC+BA+DFM. Furthermore, there were no differences in manure N content, indicating pH was the predominant factor influencing NH_3 volatilization. The balance portion of the experiment revealed that these two dietary treatments lowered total N excretion compared to PC or PC+BA+DFM, suggesting N in the manure would be lower. Rates of NH_3 emissions have been shown to increase with increasing manure ammoniacal nitrogen concentration (Canh et al., 1998). Based on this, it is possible that the rate of NH_3 loss was increased in manure from PC and PC+BA+DFM fed pigs during storage at the farm, causing manure total N content to be similar at the time gas emissions were measured in the lab. The aged manure further supported this, which showed similar NH_3 emissions and manure total N among dietary treatments.

In the present experiment, H_2S and CH_4 emissions were not affected by dietary treatment. Literature investigating the impacts of BA on H_2S and CH_4 emissions is limited. In one study, Eriksen et al. (2010) observed decreased H_2S and dimethyl trisulfide when BA was added at 2% in the diet; however, this was also associated with increased methanethiol emissions. In the current experiment, there were numerical decreases in H_2S from PC+BA manure, but considerable variation among these measurements may have hindered the detection of statistical significance.

Throughout the variables tested, the addition of the DFM diminished the significant changes caused by BA alone. Specifically, PC+BA+DFM failed to decrease urine N excretion, improve N retention, or lower manure pH and NH_3 emissions. Furthermore, PC+BA+DFM decreased Ca and P retention compared to pigs fed PC+BA. Undissociated organic acids can diffuse into bacterial cells in the gastrointestinal tract, inhibiting growth by disrupting pH homeostasis, enzyme activity, and nutrient transport systems (Kluge et al., 2006). BA has a dissociation constant of 4.2, leaving it in the undissociated form at physiological pH. Therefore, supplementation of BA may have interfered with DFM colonization in the gut, disrupted microbial turnover, and ultimately

altered the microbial community structure in the manure. However, because manure microbial populations were not investigated in this study, these mechanisms cannot be elucidated.

In conclusion, results of this experiment indicate that supplementing 0.3% BA without the *Bacillus*-based DFM to growing pigs from 42 to 77 kg improved N retention compared to the same diet without BA, and reduced manure pH and NH_3 emissions similarly to reducing N in the diet but improved the ADG of pigs when compared to feeding a low N diet.

Acknowledgments

The authors would like to acknowledge individuals at Iowa State University for their assistance and guidance to the project which include Spenser Becker and John Patience. Brian Kerr with the USDA-ARS for his guidance and discussion on manure gas emission evaluations. This project was fully funded by DSM Nutritional Products. The assigned grant numbers for the trial was GR-023500 and GR-21660.

Conflict of Interest Statement

Jon Bergstrom and Estefania Perez Calvo are employees of DSM Nutritional Products. DSM Nutritional Products provided financial support to this project.

Literature Cited

AOAC. 2006. *Official methods of analysis of AOAC International*. 17th ed. Gaithersburg (MD): AOAC International.

Balasubramanian, B., T. Li, and I. Ho Kim; R. Brasileira de Zootecnia. 2016. Effects of supplementing growing-finishing pig diets with Bacillus spp. probiotic on growth performance and meat-carcass grade quality traits. *Bras. Zootec*. 45:93–100. doi:10.1590/S1806-92902016000300002

Canh, T. T., A. J. A. Aarnink, J. B. Schutte, A. Sutton, D. J. Langhout, and M. W. A. Verstegen. 1998. Dietary protein affects nitrogen excretion and ammonia emission from slurry ofgrowing-finishing pigs. *Livest. Prod. Sci*. 56:181–191. doi:10.1016/S0301-6226(98)00156-0

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., Z. Gao, B. Yu, P. Zheng, J. He, J. Yu, Z. Huang, D. Chen, and X. Mao. 2016. Effectsof benzoic acid (VevoVitall®) on the performance and jejunal digestive physiology inyoung pigs. *J. Anim. Sci. Biotechnol*. 7:32. doi:10.1186/S40104-016-0091-Y

Drucker, D. J. 2001. Glucagon-Like Peptide 2. *J. Clin. Endocrinol. Metab*. 86:1759–1764. doi:10.1210/jcem.86.4.7386

Eriksen, J., A. P. S. Adamsen, J. V. Nørgaard, H. D. Poulsen, B. B. Jensen, and S. O. Petersen. 2010. Emissions of sulfur-containing odorants, ammonia, and methane from pig slurry: effects of dietary methionine and benzoic acid. *J. Environ. Qual*. 39:1097–1107. doi:10.2134/JEQ2009.0400

FASS. 2010. *Guide for the care and use of agricultural animals in research and teaching*. 3rd ed. Champaign, IL: Federation of Animal Science Societies; p. 169.

Galassi, G., L. Malagutti, S. Colombini, L. Rapetti, and G. Matteo Crovetto. 2011. Effects of benzoic acid on nitrogen, phosphorus and energy balance and on ammonia emission from slurries in the heavy pig. *Ital. J. Anim. Sci*. 10:200–204. doi:10.4081/ijas.2011.e38

Gould, A. R., B. K. May, and W. H. Elliott. 1975. Release of extracellular enzymes from Bacillus amyloliquefaciens. *J. Bacteriol*. 122:34–40. doi:10.1128/jb.122.1.34-40.1975

Gutzwiller, A., H. D. Hess, A. Adam, D. Guggisberg, A. Liesegang, and P. Stoll. 2011. Effects of a reduced calcium, phosphorus and protein intake and of benzoic acid on calcium and phosphorus metabolism of growing pigs. *Anim. Feed Sci. Technol*. 168:113–121. doi:10.1016/J.ANIFEEDSCI.2011.03.015

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 oftotal 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

Hansen, C. F., G. Sørensen, and M. Lyngbye. 2007. Reduced diet crude protein level, benzoicacid and inulin reduced ammonia, but failed to influence odour emission from finishingpigs. *Livest. Sci*. 109:228–231. doi:10.1016/J.LIVSCI.2007.01.133

Jacobs, B. M., J. F. Patience, M. D. Lindemann, K. J. Stalder, and B. J. Kerr. 2013. The use of a covariate reduces experimental error in nutrient digestion studies in growing pigs. *J. Anim. Sci*. 91:804–810. doi:10.2527/jas.2011-4868

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

Kluge, H., J. Broz, and K. Eder. 2010. Effects of dietary benzoic acid on urinary pH and nutrient digestibility in lactating sows. *Livest. Sci*. 134:119–121. doi:10.1016/j.livsci.2010.06.116

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

Lan, R., H. Tran, and I. Kim. 2017. Effects of probiotic supplementation in different nutrientdensity diets on growth performance, nutrient digestibility, blood profiles, fecalmicroflora and noxious gas emission in weaning pig. *J. Sci. Food Agric*. 97:1335–1341. doi:10.1002/jsfa.7871

Latorre, J. D., X. Hernandez-Velasco, R. E. Wolfenden, J. L. Vicente, A. D. Wolfenden, A. Menconi, L. R. Bielke, B. M. Hargis, and G. Tellez. 2016. Evaluation and selection of bacillus species based on enzyme production, antimicrobial activity, and biofilmsynthesis as direct-fed microbial candidates for poultry. *Front. Vet. Sci*. 3(95). doi:10.3389/FVETS.2016.00095

Liu, W., S. Devi, J. Park, and I. Kim. 2018. Effects of complex probiotic supplementation ingrowing pig diets with and without palm kernel expellers on growth performance,nutrient digestibility, blood parameters, fecal microbial shedding and noxious gasemission. *Anim. Sci. J*. 89:552–560. doi:10.1111/ASJ.12965

Liu, M., D. Giard, and S. Barrington. 2013. Ammonium dissociation for swine and dairy cattle manures. *J. Environ. Protect*. 4:6–15. doi:10.4236/JEP.2013.45A002

Murphy, D. P., J. V. O'Doherty, T. M. Boland, C. J. O'Shea, J. J. Callan, K. M. Pierce, and M. B. Lynch. 2011. The effect of benzoic acid concentration on nitrogen metabolism, manure ammonia and odour emissions in finishing pigs. *Anim. Feed Sci. Technol*. 163:194–199. doi:10.1016/j.anifeedsci.2010.10.009

Nørgaard, J. V., J. A. Fernández, K. U. Sørensen, S. Wamberg, H. D. Poulsen, and N. B. Kristensen. 2010. Effect of benzoic acid

supplementation on acid-base status and mineral metabolism in catheterized growing pigs. *Livest. Sci.* 134:116–118. doi:10.1016/j.livsci.2010.06.115

NRC. 2012. *Nutrient Requirements of Swine*. 11th rev. ed. Washington, DC: Natl. Acad. Press.

Patience, J. F., R. E. Austic, and R. D. Boyd. 1987. Effect of dietary electrolyte balance on growth and acid-base status in swine. *J. Anim. Sci.* 64:457–466. doi:10.2527/jas1987.642457x

Pérez Alvarado, M. A., J. C. López, D. B. Varela, G. M. Landín, and J. A. Cuarón Ibargüengoytia. 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

Sauer, W., M. Cervantes, J. Yanez, B. Araiza, G. Murdoch, A. Morales, and R. T. Zijlstra. 2009. Effect of dietary inclusion of benzoic acid on mineral balance in growing pigs. *Livest. Sci.* 122:162–168. doi:10.1016/j.livsci.2008.08.008

Trabue, S., B. Kerr, K. Scoggin, D. Andersen, and M. van Weelden. 2021. Swine diets impact manure characteristics and gas emissions: part II protein source. *Sci. Total Environ*. 763:144207. doi:10.1016/j.scitotenv.2020.142528

Wang, Y., J. H. Cho, Y. J. Chen, J. S. Yoo, Y. Huang, H. J. Kim, and I. H. Kim. 2009. The effectof probiotic BioPlus 2B® on growth performance, dry matter and nitrogen digestibility and slurry noxious gas emission in growing pigs. *Livest. Sci.* 120:35–42. doi:10.1016/J.LIVSCI.2008.04.018

Wang, H., B. D. Ha, and I. H. Kim. 2021. Effects of probiotics complex supplementation in low nutrient density diet on growth performance, nutrient digestibility, faecal microbial, and faecal noxious gas emission in growing pigs. *Ital. J. Anim. Sci*. 20:163–170. doi:10.1080/1828051X.2020.1801358

Wang, Y., J. Zhou, G. Wang, S. Cai, X. Zeng, and S. Qiao. 2018. Advances in low-protein dietsfor swine. *J. Anim. Sci. Biotechnol*. 9:60. doi:10.1186/s40104-018-0276-7

Zhai, H., W. Ren, S. Wang, J. Wu, P. Guggenbuhl, and A. M. Kluenter. 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

Zhang, F., and O. Adeola. 2017. Techniques for evaluating digestibility of energy, amino acids, phosphorus, and calcium in feed ingredients for pigs. *Anim. Nutr*. 3:344–352. doi:10.1016/J.ANINU.2017.06.008