Livestock Science 122 (2009) 162–168

Contents lists available at ScienceDirect

Livestock Science

journal homepage: www.elsevier.com/locate/livsci

Effect of dietary inclusion of benzoic acid on mineral balance in growing pigs

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ARTICLE INFO

Article history:
Received 24 March 2008
Received in revised form 6 August 2008
Accepted 8 August 2008

Keywords:
Benzoic acid
Digestibility
Excretion
Mineral
Pig
Retention

ABSTRACT

The objective of this study was to determine the effect of dietary inclusion of benzoic acid on utilization of the macrominerals Ca, P, Mg, K, Na, and Cl in growing pigs. Eighteen barrows, initial BW of 28 ± 1.7 kg, were assigned to 3 diets: a basal diet based on barley, wheat, soybean meal, corn, and field pea and formulated to contain 9.31 MJ NE kg^{-1} and 8.84 g^{-1} kg standardized ileal digestible lysine, or the basal diet containing 10 or 20 g kg^{-1} benzoic acid by replacing tapioca starch. The pigs were fed the experimental diets a rate of 2.7 times the maintenance requirement for ME for 21 days. Faeces and urine were collected quantitatively from days 11 to 21, and blood and plasma was collected on days 1, 10, and 21. On day 21, the pigs were killed and the left femur was removed. Benzoic acid linearly decreased ($P = 0.001$) the urine pH from 7.32 to 5.32, and quadratically increased ($P < 0.05$) blood pH on day 21. Benzoic acid linearly increased ($P < 0.05$) the apparent total tract digestibility (ATTD) of Ca, P, and Na from 65 to 72%, 46 to 55%, and 78 to 90%, respectively, linearly decreased ($P < 0.05$) the ATTD of Cl from 94 to 93%, and did not affect the ATTD of Mg and K. Benzoic acid linearly increased ($P < 0.05$) the retention of Ca, P, and K from 58 to 67%, 46 to 54%, and 31 to 38%, respectively, linearly decreased ($P < 0.05$) the retention of Na and Cl from 57 to 48% and 75 to 44%, respectively, and did not affect retention of Mg. On day 21, benzoic acid linearly increased ($P = 0.001$) plasma P and quadratically increased ($P < 0.05$) plasma K or tended to increase ($P = 0.05$) plasma Na. Benzoic acid linearly reduced ($P < 0.05$) the concentration of ash in femur but not the amount of ash, reduced ($P < 0.05$) the concentration of Ca and Cl in femur ash, and linearly increased ($P < 0.05$) the concentration of P in femur ash. In summary, benzoic acid increased the utilization of dietary Ca, P, and K, did not affect the utilization of dietary Mg, and reduced the utilization of dietary Na and Cl. During swine feed formulation, effects of benzoic acid on macromineral utilization should be considered to ensure that macromineral requirements are met and not exceeded following benzoic acid supplementation.

1. Introduction

Organic acids, such as benzoic acid, are used in the preservation of food and feedstuffs. In swine, dietary inclusion of organic acids usually improved growth performance and digestibility and retention of minerals, in particular of Ca and P (Partanen and Mroz, 1999). The inclusion of benzoic acid reduced the pH of urine (Cahn et al., 1998; Van der Peet-Schwering et al., 1999). The dietary inclusion of benzoic acid may influence the mineral balance of pigs adversely by mobilization of minerals (e.g., P) from bone required to maintain the acid–base balance in the body.

The effects of benzoic acid and its salts on mineral balance in pigs have been studied (Mroz et al., 1996, 1997, 1998); however, uncertainties on the action of benzoic acid remain. For example, the retention of P was decreased in one study (Mroz et al., 1996) using Ca benzoate, respectively, but increased in another study (Mroz et al., 1997) after Na benzoate

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doi:10.1016/j.livsci.2008.08.008

was included in the diet. Also, the daily urinary excretion of Ca increased at high dietary inclusion levels (24 g kg^{-1}) of Ca benzoate, but Ca retention was not affected due to an increase in Ca digestibility (Mroz et al., 1998). The role of dietary benzoic acid in the mineral balance of grower–finisher pigs should thus be clarified. Mineral excretion of swine remains a major concern that affects the long-term environmental sustainability of the pork industry, and nutrition and feed formulation are key factors to reduce its environmental footprint (Aarnink and Verstegen, 2007).

The hypotheses of the present study were that dietary benzoic acid increases mineral digestibility resulting in increased mineral retention, and that these effects would be more profound with a higher level of inclusion of benzoic acid. The objective was to investigate the effect of 10 or 20 g kg^{-1} inclusion of benzoic acid on balance, plasma concentration, and bone content (femur) of Ca, P, Mg, K, Na, and Cl. In addition, the effect of dietary inclusion of benzoic acid on the pH of urine and blood were determined.

2. Materials and methods

2.1. Animals and diets

The experimental procedures for care and treatment of the pigs were approved by the Animal Care Committee of the University of Alberta, in accordance with the guidelines established by the CCAC (1993).

Eighteen barrows (F2 Large White × Landrace; Genex Hybrid; Hypor, Regina, SK, Canada), with an average initial body weight of 26.5 ± 1.5 kg, were used at the University of Alberta Swine Research and Technology Centre. Pigs were housed individually in metabolism crates (height = 85 cm; length = 140 cm; width = 65 cm) that restricted pigs from turning around. The room temperature was maintained between 20 and 22 °C. Pigs were first fed the basal diet (Table 1) for a period of 7 days to adapt. Thereafter, the pigs were randomly allotted in a completely-randomized design to 1 of 3 experimental diets that were fed for 21 days in the experimental period, reaching 6 observations per diet. The average body weights of the pigs at the start and conclusion of the study were 28.1 ± 1.7 and 39.1 ± 2.3 kg, respectively.

The basal diet was based on barley, wheat, soybean meal, corn, peas, and canola meal (Table 1). Benzoic acid (C_6H_5COOH; DSM Food Specialties, Delft, The Netherlands) was included at 10 and 20 g^{-1} kg diet at the expense of tapioca starch. The basal diet was formulated to contain 9.31 MJ NE kg^{-1} (13.62 MJ ME kg^{-1}) and 8.84 g kg^{-1} standardized ileal digestible lysine, as fed, based on established nutrient values for feedstuffs (Sauvant et al. 2004). Lysine, threonine, methionine, and tryptophan were supplemented to meet or exceed NRC (1998) recommendations. Vitamins and minerals were included to meet or exceed NRC (1998) recommendations. Chromic oxide was included in the diet as indigestible marker for precautionary reasons in case problems occurred with total collection of faeces. The major dietary ingredients were ground through a 2-mm screen prior to incorporation into the diets. The three diets were processed in three separate batches as a mash.

Pigs were fed the three experimental diets at a rate of 2.7 times the maintenance requirement for ME (i.e., 443 kJ kg^{-1} $BW^{0.75}$; NRC, 1998) for 21 days, based on initial BW. The daily feed allowance was fed in two equal amounts at 0900 and 1700. Water was mixed with the feed at a ratio of 2.5 to 1. Faeces and urine were collected quantitatively for each pig for a period of 10 days, from 0900 on day 11 to 0900 on day 21. Faeces and urine were collected 3 times daily at 0900, 1500, and 2100. Faeces were immediately frozen at −20 °C after collection. The daily volume of urine from each pig was recorded and a sub-sample was taken (25% of total daily volume), filtered to ensure that solid particles were removed, and frozen at −20 °C. Prior to freezing, the pH of urine was measured on day 21. Approximately 20 mL of blood samples (2 samples of 10 mL) were taken from the jugular vein immediately prior to the morning feeding on days 1, 10, and 21 of the experimental period. The pH of blood was measured in one sample and the second sample was centrifuged at 3000 × g for 10 min. Plasma samples were stored at −20 °C. Pigs were killed 4 to 6 h after the morning feeding on day 21. The left femur was removed and stored at −20 °C.

Table 1
Composition of the basal diet (g kg^{-1}; as fed basis) [a].

Ingredient	Basal diet
Barley	250.0
Wheat	200.0
Soybean meal, 48% CP	155.0
Corn	140.0
Field pea	75.0
Canola meal	50.0
Corn starch	35.5
Cane molasses	25.0
Tapioca starch	20.0
Tallow	17.5
Limestone	11.2
Monocalcium phosphate [b]	8.5
Chromic oxide	3.0
L-Lysine·HCl	2.2
Salt	1.9
Sodium bicarbonate	1.5
Mineral premix [c]	1.0
Vitamin premix [d]	1.0
DL-Methionine	0.6
Choline chloride [e]	0.5
L-Threonine	0.5
L-Tryptophan	0.1

[a] The basal diet was formulated to 9.31 MJ NE kg^{-1} and 8.84 g kg^{-1} standardized ileal digestible lysine. To create the diets containing 10 or 20 g kg^{-1} benzoic acid, tapioca starch was replaced (wt/wt) with benzoic acid.
[b] Contained 211 g^{-1} kg P and 170 g^{-1} kg Ca.
[c] Provided the following (kg^{-1} diet): Fe, 150 mg as ferrous sulfate; Zn, 150 mg as zinc carbonate; Mn, 40 mg as manganese sulfate; Cu, 25 mg as copper sulfate; I, 0.21 mg as potassium iodate; Co, 0.5 mg as cobalt sulfate; Se, 0.3 mg as sodium selenite; and ethoxyquin, 5.0 mg.
[d] Provided the following (kg^{-1} diet): vitamin A, 10,000 IU as vitamin A acetate; vitamin D_3, 1,000 IU; vitamin E, 80 IU as DL-α-tocopheryl acetate; vitamin K_3, 2.0 mg as menadione dimethylpyrimidinol bisulfite; riboflavin, 12 mg; niacin, 40 mg; D-pantothenic acid, 25 mg as calcium pantothenate; biotin, 0.25 mg; folic acid, 1.6 mg; thiamin, 3.0 mg; pyridoxine, 2.25 mg; and vitamin B_{12}, 0.03 mg.
[e] Contained 60% choline chloride.

2.2. Sample collection and chemical analyses

Samples of the diets were taken each time the meals were prepared and pooled for each dietary treatment. Faeces were pooled for each pig and freeze-dried. Samples of the diets and

Table 2
Analyzed content of crude protein and selected minerals and dietary electrolyte balance of the experimental diets (g kg^{-1}; DM basis).

Item	Benzoic acid, g kg^{-1}		
	0	10	20
Dry matter	880	900	880
Crude protein	183	190	186
Calcium	7.5	7.5	7.5
Phosphorus	6.3	6.2	6.4
Magnesium	1.7	1.7	1.7
Potassium	7.5	7.4	7.7
Sodium	2.1	2.1	2.2
Chloride	2.9	3.0	3.0
dEB (mEq kg^{-1}) [a]	200	196	208

[a] Dietary electrolyte balance (K + Na − Cl).

faeces were ground through a 1-mm mesh screen prior to analysis. The daily samples of urine were thawed, filtered, sub-sampled and pooled for each pig prior to analysis. The entire femurs were autoclaved for 15 min at 121 °C at 1.2 kg/cm^2 pressure to facilitate removal of soft tissue. Thereafter, the femurs were dried in an oven at 110 °C until the weight of the femurs remained constant for two consecutive days. The femurs were then ashed in a muffle furnace for 24 h at 600 °C, and immediately placed in a desiccator. The femurs were then ground through a 1-mm mesh screen.

Diet, faeces, and bone were analysed for DM according to the AOAC (2000). Bone was analysed for ash using AOAC (2000). The protein content (N × 6.25) of the diets was determined by combustion using a N analyzer (LECO FP-248, LECO Corporation, St. Joseph, MI). The diets, faeces, urine, plasma, and bone were analysed for Ca, P, Mg, K, and Na according to the AOAC (2000) except for P in plasma, which was determined using an analytical kit (360-UV, Sigma Diagnostics Inc., St. Louis, MO). Insufficient sample remained to measure plasma Cl. An atomic absorption spectrophotometer (Perkin Elmer 4000, The Perkin-Elmer Corporation, Norwalk, CT) was used for analyses of Ca, Mg, K, and Na. An array spectrophotometer (Spectronic 3000, Milton Roy Company, Ivyland, PA) and a microplate spectrophotometer (SpectraMax 190, Molecular Devices Corporation, Sunnyvale, CA) were used for the analyses of P in diets, faeces, urine, and bone, and plasma P, respectively. Diets, faeces, urine, and femur samples were analysed for Cl by a commercial laboratory (Norwest Labs, Edmonton, AB, Canada).

2.3. Calculations and statistical analyses

Mass and concentrations of macronutrients in feed, faeces, and urine were used to calculate the balance for each macronutrient, and their digestibility and retention. Electrolyte balance was calculated as [Na + K − Cl] for each diet (Patience, 1989). The data were analysed according to a completely-randomized design using the GLM procedure of SAS (SAS Inst., Inc., Cary, NC). Diet was used as the main factor in the statistical model. For mineral balance (g day^{-1}), initial pig body weight was added to the model as a covariate. For blood pH and plasma minerals, initial blood pH and plasma mineral concentration was added to the model for values for days 10 and 21. Two orthogonal contrasts were used to test if a linear and quadratic effect of supplementing benzoic acid to the basal diet existed. Quadratic effects were only considered significant if linear effects were also significant. Probability levels of $P<0.05$ and $0.05<P<0.10$ were defined as significant differences and trends, respectively.

3. Results and discussion

All pigs remained healthy and usually consumed their meal allowances within 1 h after feeding throughout the experiment. Feed refusals were not observed. Minor differences in the mineral content and electrolyte balance existed among the experimental diets, likely due to the use of a small mixer and analytical errors for laboratory analyses (Table 2); however, these differences did not influence the results and interpretation of effects of benzoic acid for the present study.

3.1. pH of urine and plasma

Benzoic acid quadratically decreased ($P=0.001$) urine pH from 7.32 to 5.32 (Table 3). These results are in agreement with other studies that included Ca benzoate (Mroz et al., 1996; Mroz et al., 1998; Cahn et al., 1998) or Na benzoate (Mroz et al., 1997) in the diet. The reason for a quadratic response in urine pH is not clear; perhaps the kidney has a specific capacity to buffer blood pH that, once exceeded, results in accelerated reductions in urine pH. The lower pH is a combined result of the renal excretion of H^+ ions and hippuric acid in urine; modest amounts of benzoic and benzylglucuronic acids are also excreted (Kubota and Ishizaki, 1991). In the liver and to a lesser extent in the kidney, benzoic acid is conjugated with glycine to form hippuric acid or is conjugated with glucuronic acid to form benzoylglucuronic acid, both of which are rapidly excreted in urine (Kubota and Ishizaki, 1991). Hippuric acid is the end product of the two-step conjugation of benzoic acid with glycine (Hutt and Caldwell, 1990). In most animal species, this is the primary conjugation reaction unless glycine is limiting (Polonen et al., 2000). The conjugation reaction increases the water solubility of the acid, thereby improving renal clearance. A decrease in the pH of urine will decrease ammonia emission from manure (Cahn et al., 1998; Hansen et al., 2007).

Benzoic acid did not affect blood pH on day 10, but benzoic acid quadratically increased ($P<0.05$) blood pH from 7.51 to 7.65 on day 21 (Table 3). Although the change in blood pH may be a transient effect, the inclusion of 20 g kg^{-1} benzoic acid might have increased the capacity for renal acid clearance in conjunction with the possibility of improved renal bicarbonate reabsorption, and changes in urinary ammonium and phosphate excretion. The blood samples were taken prior to

Table 3
The pH values of urine and blood in growing pigs fed diets containing 0, 10, or 20 g kg^{-1} benzoic acid.

Item	Sampling day	Benzoic acid, g kg^{-1}			SEM [a]	*P*-value	
		0	10	20		Linear	Quadratic
Urine	21	7.32	6.55	5.32	0.043	0.001	0.001
Blood	1	7.56	7.52	7.60	0.017	0.237	0.010
	10	7.62	7.66	7.56	0.029	0.167	0.091
	21	7.51	7.53	7.65	0.014	0.001	0.044

[a] Standard error of least square means ($n=6$).

Table 4
Intake, faecal and urinary excretion and retention (g day^{-1}), and apparent total tract digestibility and retention (%) of Ca and P in pigs fed diets containing 0, 10, or 20 g kg^{-1} benzoic acid.

Item	Benzoic acid, g kg^{-1}			SEM[a]	*P*-value	
	0	10	20		Linear	Quadratic
Calcium						
Intake, g day^{-1}	8.85	8.85	8.85	0.001	–	–
Faecal excretion, g day^{-1}	3.13	2.78	2.46	0.149	0.005	0.960
Urinary excretion, g day^{-1}	0.66	0.66	0.49	0.065	0.085	0.329
Retention, g day^{-1}	5.05	5.40	5.90	0.132	0.001	0.664
Digestibility, %	65.0	67.9	72.3	1.69	0.008	0.723
Retention, %	57.6	60.3	66.7	1.56	0.001	0.340
Phosphorus						
Intake, g day^{-1}	7.43	7.32	7.55	0.001	–	–
Faecal excretion, g day^{-1}	4.06	3.56	3.43	0.141	0.006	0.333
Urinary excretion, g day^{-1}	0.016	0.023	0.042	0.004	0.002	0.042
Retention, g day^{-1}	3.35	3.73	4.09	0.141	0.024	0.960
Digestibility, %	45.8	50.5	54.8	2.03	0.007	0.942
Retention, %	45.7	50.1	54.2	2.03	0.010	0.942

[a] Standard error of least square means ($n=6$).

morning feeding, a time point for an expected maximum renal clearance of acid and bicarbonate resorption. Increases in distal tubule cellular expression of carbonic anhydrase might have facilitated the reabsorption of bicarbonate into the vascular lumen with concomitant increases in transport of H^+ ions into the lumen of the distal tubules for urinary excretion, an adaptation that may have become most apparent at our last blood sampling on day 21 (Pitts, 1959). At 20 g kg^{-1} dietary inclusion of benzoic acid, plasma levels of benzoic acid might have increased sufficiently to induce an increase in the activity of carbonic anhydrase present in cells of the distal tubule, resulting in a decrease in urinary pH and elevated blood pH.

3.2. Mineral balance

Benzoic acid linearly increased ($P<0.01$) the apparent total tract digestibility (ATTD) and retention of Ca by 0.85 g day^{-1} in the present study (Table 4). Also, benzoic acid linearly increased ($P<0.05$) the ATTD and retention of P by 0.74 g day^{-1}. The very low urinary P excretion across diets indicates that pigs were not able to meet their P requirement (Ekpe et al., 2002). The reasons for the improvements in Ca and P digestibility and thus reduced faecal excretions are not clear. A relationship between the dose or type of acid and improvements in the ATTD of minerals might be lacking, but improvements in the ATTD of Ca and P with diet acidification have been observed previously. For example, diet acidification with formic, butyric, lactic, fumaric, and citric acid increased the ATTD of Ca and P in pigs (Jongbloed et al., 2000). Lactic acid (30 g/kg diet) increased the ATTD of Ca (Kemme et al., 1999). Citric acid added to a diet containing corn, soybean meal, and wheat middlings improved P utilization in growing pigs (Han et al., 1998). Similarly, 1.5% citric acid added to a corn–soybean meal diet improved availability of P and other minerals in young pigs (Höhler and Pallauf, 1993). Acidification may improve the absorption of minerals by increasing mineral solubility (Jongbloed, 1987), either directly by lowering the pH in the gastric contents or indirectly by reducing the rate of gastric emptying. In addition, compounds may be formed at a low pH between the acid group and various cations that act as chelating agents (Ravindran and Kornegay, 1993; Eidelsburger, 1997). Furthermore, acidification may have a positive effect on epithelial cell proliferation in the gastrointestinal mucosa, which allows for more efficient mineral absorption (Sakata et al., 1995).

Benzoic acid did not affect urinary excretion of Ca in the present study (Table 4), but benzoic acid quadratically increased ($P<0.05$) urinary excretion of P. At high inclusion levels of benzoic acid, P might be directed towards urine in an attempt to maintain the acid–base balance in the body. These results are in agreement with studies in which low dietary levels of Na benzoate were included in the diets (Mroz et al., 1997). In contrast, at high inclusion levels (≥2.4%) of Na benzoate, urinary excretion of Ca increased (Mroz et al., 1996, 1998), whereas urinary excretion of P was not affected. As reported by Mroz et al. (1996, 1998), this will not affect the retention of Ca due to an increase in the ATTD of Ca in the diets containing high levels of Ca benzoate.

Benzoic acid did not affect the ATTD and retention of Mg in the present study (Table 5). In contrast, lactic acid in the diet increased the ATTD of Mg (Kemme et al., 1999). Furthermore,

Table 5
Intake, faecal and urinary excretion and retention (g day^{-1}), and apparent total tract digestibility and retention (%) of Mg, K, Na, and Cl in pigs fed diets containing 0, 10, or 20 g kg^{-1} benzoic acid.

Item	Benzoic acid, g kg^{-1}			SEM[a]	*P*-value	
	0	10	20		Linear	Quadratic
Magnesium						
Intake, g day^{-1}	2.01	2.01	2.01	0.001	–	–
Faecal excretion, g day^{-1}	1.50	1.42	1.55	0.064	0.597	0.195
Urinary excretion, g day^{-1}	0.33	0.35	0.29	0.017	0.110	0.108
Retention, g day^{-1}	0.17	0.24	0.16	0.057	0.927	0.324
Digestibility, %	25.1	29.0	22.7	3.13	0.598	0.207
Retention, %	8.6	11.7	8.2	2.80	0.923	0.346
Potassium						
Intake, g day^{-1}	8.85	8.73	9.09	0.002	–	–
Faecal excretion, g day^{-1}	3.28	3.01	3.10	0.142	0.400	0.344
Urinary excretion, g day^{-1}	2.86	2.80	2.51	0.120	0.115	0.058
Retention, g day^{-1}	2.71	2.92	3.48	0.199	0.016	0.499
Digestibility, %	62.8	65.7	65.8	1.53	0.190	0.465
Retention, %	30.4	33.9	38.2	2.16	0.023	0.877
Sodium						
Intake, g day^{-1}	2.48	2.48	2.60	0.001	–	–
Faecal excretion, g day^{-1}	0.54	0.43	0.26	0.046	0.003	0.021
Urinary excretion, g day^{-1}	0.52	0.74	1.07	0.071	0.001	0.502
Retention, g day^{-1}	1.42	1.31	1.26	0.063	0.085	0.695
Digestibility, %	77.9	83.4	89.6	1.95	0.001	0.895
Retention, %	57.0	53.2	48.3	2.43	0.023	0.850
Chloride						
Intake, g day^{-1}	3.42	3.54	3.54	0.001	–	–
Faecal excretion, g day^{-1}	0.20	0.21	0.25	0.013	0.016	0.441
Urinary excretion, g day^{-1}	0.69	1.15	1.75	0.084	0.001	0.498
Retention, g day^{-1}	2.53	2.18	1.54	0.091	0.001	0.225
Digestibility, %	94.3	93.9	92.9	0.38	0.027	0.552
Retention, %	74.5	60.6	43.7	2.56	0.001	0.649

[a] Standard error of least square means ($n=6$).

citric acid added to a corn–soybean meal diet fed to young pigs improved the availability of Mg (Höhler and Pallauf, 1993). Benzoic acid did not affect urinary excretion of Mg and K. Similarly, urinary excretions of Mg and K were not affected by including Ca benzoate in the diet (Mroz et al., 1996, 1998), not even at a very high (4.8%) dietary concentration. In addition, benzoic acid did not affect the ATTD of K. Still, benzoic acid linearly increased ($P<0.05$) retention of K by 0.77 g day^{-1} in the present study, because the combined non-significant reductions in faecal and urinary resulted in a significant increase in retention.

Benzoic acid linearly increased the ATTD ($P<0.01$) of Na; however, benzoic acid linearly decreased ($P<0.05$) retention of Na by 9% in the present study (Table 5). Benzoic acid linearly decreased ($P<0.05$) the ATTD of Cl and linearly reduced ($P=0.001$) retention of Cl by 0.99 g day^{-1}. Benzoic acid linearly increased the urinary excretion of Na and Cl ($P=0.001$). The large increases in the urinary excretions of Na and Cl with the inclusion of benzoic acid explain their decreased retention. These results are not in agreement with previous studies (Mroz et al., 1996, 1998) indicating that, even at a dietary inclusion level of 4.8%, Ca benzoate did not increase the urinary excretions of Na and Cl. The form of dietary supplementation of benzoic acid, acid vs. salt, might have been responsible for the differences among studies. The reduced Na and Cl balance in conjunction with supplementation of benzoic acid might be a new phenomenon observed under the conditions of the present study. The importance relative to Na and Cl requirements was not studied in the present study, but extra salt might have to be supplemented in practical diet formulation. Overall, the improved utilization of dietary minerals might be one of the underlying mechanisms that explain the improved growth performance of weaned pigs following the inclusion of 5 g kg^{-1} benzoic acid in swine feeds (Torrallardona et al., 2007).

3.3. Plasma mineral concentrations

Benzoic acid linearly increased ($P<0.01$) plasma Ca on day 10 in the present study (Table 6), but did not affect plasma Ca on day 21. Benzoic acid linearly increased ($P<0.05$) the concentration of plasma P on days 10 and 21. Benzoic acid linearly increased ($P<0.01$) the plasma concentration of plasma Mg on day 10, but not on day 21. Benzoic acid quadratically increased ($P<0.05$) the concentration of plasma K on days 10 and 21. Benzoic acid tended to quadratically increase ($P<0.10$) plasma Na on day 21, but did not affect plasma Na on day 10.

Plasma mineral concentrations might be a valuable tool to measure mineral status in pigs. The plasma concentrations of the minerals reported in the present study are in the range of values reported by Kirchgessner (1997). The linear increase in plasma P caused by benzoic acid throughout the entire experiment coincided with increased ATTD of P and retention, similar to previous studies (e.g., Ekpe et al., 2002). Similarly, increased plasma K caused by benzoic acid on days 10 and 21 coincided with increased K retention. However, a trend for increased plasma Na on day 21 coincided with reduced Na retention and increased urinary excretion of Na in the benzoic acid-containing diets, indicating that the ability to use plasma concentrations of minerals as an indicator of mineral status differs among minerals. Plasma Na may not be useful as an indicator of Na status, whereas plasma P and K might be

Table 7
Weight of femur, concentration and total content of bone ash and selected minerals in growing pigs fed diets containing 0, 10, or 20 g kg^{-1} benzoic acid.

Item	Benzoic acid, g kg−1			SEM[a]	*P*-value	
	0	10	20		Linear	Quadratic
Weight of femur, g						
Fresh	68.7	74.3	70.3	0.74	0.130	0.001
Dry	45.0	47.6	46.4	0.33	0.007	0.001
Ash in femur, % DM	50.3	49.0	48.8	0.26	0.001	0.104
Total bone ash, g	22.6	23.3	22.7	0.25	0.928	0.049
Mineral concentration in ash, % DM						
Calcium	38.06	37.66	37.59	0.15	0.038	0.380
Phosphorus	19.11	19.64	19.75	0.14	0.005	0.229
Magnesium	0.75	0.75	0.79	0.01	0.006	0.022
Potassium	0.33	0.33	0.33	0.02	0.950	0.913
Sodium	1.06	1.08	1.05	0.01	0.582	0.016
Chloride	0.22	0.21	0.18	0.01	0.001	0.034

[a] Standard error of least square means ($n=6$).

Table 6
Plasma concentrations (mg^{-1} dL) of selected minerals in growing pigs fed diets containing 0, 10, or 20 g kg^{-1} benzoic acid.

Item	Sampling day	Benzoic acid, g kg^{-1}			SEM[a]	*P*-value	
		0	10	20		Linear	Quadratic
Calcium	1	11.98	11.33	12.30	0.23	0.347	0.012
	10	10.96	12.16	12.45	0.34	0.005	0.352
	21	11.86	12.15	12.77	0.53	0.223	0.823
Phosphorus	1	8.65	9.47	9.71	0.26	0.011	0.374
	10	10.53	11.09	11.40	0.21	0.024	0.616
	21	9.27	9.74	10.64	0.20	0.001	0.372
Magnesium	1	1.95	1.95	1.88	0.05	0.314	0.556
	10	1.87	1.93	2.07	0.05	0.017	0.529
	21	1.93	1.97	2.02	0.04	0.150	0.937
Potassium	1	17.92	20.82	18.67	1.01	0.609	0.060
	10	18.12	22.56	22.06	0.79	0.003	0.033
	21	17.89	22.33	21.10	0.88	0.019	0.029
Sodium	1	243.4	240.4	234.5	2.76	0.038	0.673
	10	237.9	234.1	241.6	5.73	0.679	0.415
	21	248.3	265.1	259.2	4.55	0.146	0.051

[a] Standard error of least square means ($n=6$).

useful as an indicator of P and K status at deficient or extreme high supplies, respectively.

3.4. Mineral content of the femur

Benzoic acid quadratically increased femur dry weight ($P=0.001$), and linearly decreased ash concentration in the femur ($P=0.001$), but did not affect the total amount of ash in bone in the present study (Table 7). Benzoic acid linearly increased ($P<0.01$) the concentration of P in bone ash and quadratically decreased ($P<0.05$) the concentration of Cl in bone ash. Benzoic acid linearly reduced ($P<0.05$) the concentration of Ca in bone ash and linearly increased ($P<0.01$) the concentration of Mg in bone ash. Benzoic acid did not affect the concentration of K and Na on bone ash. In chicks, bone ash and Ca concentration increased following 3% propionic acid inclusion in the diet (Ibardolaza et al., 1993), providing further evidence that dietary acid inclusion and mineral nutrition are related. Indeed, in rats the Ca concentration in bone increased following the inclusion of 1.6% acetic acid (Kishi et al., 1999) or 2.5% L-lactic acid (Chonan et al., 1998) in the diet.

The decrease in the concentration of Cl in the femur of pigs fed benzoic acid in the present study reflected the drastically increased urinary excretion of Cl. The decrease in the concentration of Cl in bone ash might be partially due to a repartitioning into other tissues and fluid spaces such as interstitial fluid or connective tissue. In contrast, metabolic acid load in growing swine can also alter Cl retention (Budde and Crenshaw, 2003), while not affecting the urinary excretion of Cl. The losses of Cl in urine observed in the present study but not in the study by Budde and Crenshaw (2003) may indicate an adaptive renal response to benzoic acid. More specifically, the influence of enhanced H^+ that is derived from benzoic acid may directly alter the renal compensatory mechanism driven towards maintenance of plasma pH. Given that bone weight and Ca concentration in bone ash were affected by benzoic acid, a change in bone resorption induced to buffer the increase in acid absorption is likely. If one considers that the normal resorption of AA from the glomerular filtrate is associated with cotransport of Na, a conjugation of free glycine with benzoic acid may have indirectly increased Na excretion in urine of the pigs fed the benzoic acid-containing diets. Furthermore, the increased urinary Cl excretion is likely a result of several other physiological adaptations including the increased urinary Na loss, the increased H^+ concentration in the distal tubules, and the increased bicarbonate resorption; however, the latter two were not measured in the present study. Therefore, the reduction in Cl concentration in the femur of pigs fed the benzoic acid-containing diets in this study is likely a direct reflection of the increased urinary excretion of Cl which in itself reflects the new homeorrhetic mineral balance established by these diets.

4. Conclusion

In summary, the dietary inclusion of benzoic acid affected the pH in urine and plasma, the ATTD, retention, and plasma concentrations of Ca, P, K, Na, and Cl, and the concentrations of Ca, P, and Cl in the femur. In conclusion, dietary benzoic acid improved utilization of dietary Ca, P, and K, did not affect utilization of dietary Mg, and reduced utilization of dietary Na and Cl. These results indicate that the dietary inclusion of benzoic acid affects the utilization and metabolism of the macrominerals Ca, P, K, Mg, Na, and Cl differently, suggesting that several underlying mechanisms are involved. During swine feed formulation, effects of benzoic acid on macromineral utilization should be considered to ensure that macromineral requirements are met and not exceeded following benzoic acid supplementation. In that case, benzoic acid can be used effectively as one of the tools to reduce the environmental footprint of impact of the pork industry.

Acknowledgement

The authors thank R. Engelbert, K. Sauer, and Y. Zhang for their technical assistance. The study was supported financially by DSM Food Specialties, Delft, The Netherlands.

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