



## Effects of Benzoic Acid and Dietary Calcium:Phosphorus Ratio on Performance and Mineral Metabolism of Weanling Pigs

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**ABSTRACT:** In a 2×2 factorial experiment the hypotheses tested were that the metabolic acid load caused by benzoic acid (BA) added to the feed affects bone mineralization of weanling pigs, and that a wide dietary calcium (Ca) to phosphorus (P) ratio in phytase-supplemented feeds with a marginal P concentration has a positive effect on bone mineralization. The four experimental diets, which contained 0.4% P and were supplemented with 1,000 FTU phytase/kg, contained either 5 g BA/kg or no BA and either 0.77% Ca or 0.57% Ca. The 68 four-week-old Large White pigs were fed the experimental diets *ad libitum* for six weeks and were then slaughtered. Benzoic acid increased feed intake ( $p = 0.009$ ) and growth rate ( $p = 0.051$ ), but did not influence the feed conversion ratio ( $p > 0.10$ ). Benzoic acid decreased the pH of the urine ( $p = 0.031$ ), but did not affect breaking strength and mineralization of the tibia ( $p > 0.10$ ). The wide Ca:P ratio decreased feed intake ( $p = 0.034$ ) and growth rate ( $p = 0.007$ ) and impaired feed the conversion ratio ( $p = 0.027$ ), but increased the mineral concentration in the fat-free DM of the tibia ( $p = 0.013$ ) without influencing its breaking strength ( $p > 0.10$ ). The observed positive effect of the wide Ca:P ratio on bone mineralization may be attributed, at least in part, to the impaired feed conversion ratio, i.e. to the higher feed intake and consequently to the higher mineral intake per kg BW gain. The negative impact on animal performance of the wide dietary Ca:P ratio outweighs its potentially positive effect on bone mineralization, precluding its implementation under practical feeding conditions. (**Key Words:** Benzoic Acid, Calcium, Bone Characteristics, Pig)

### INTRODUCTION

Pig slurry contributes to the environmental pollution by phosphorus (P) and nitrogen, part of which is evaporated as ammonia. In regions with a high pig density, pig feeds commonly contain low amounts of P in order to minimize P output and are supplemented with phytase to improve intestinal P absorption. Ammonia emission from pig slurry can be reduced by supplementing the feed with benzoic acid (BA). Benzoic acid inhibits microbial ammonia formation via its metabolite hippuric acid, which is formed in the liver and excreted in the urine (Hansen et al., 2007). Benzoic and hippuric acid contribute to the metabolic acid load and may therefore affect bone integrity, because chronic acidosis stimulates bone resorption by osteoclasts and compromises

bone mineralization (Arnett, 2003). In an experiment reported by Gutzwiller et al. (2011), BA intake had a negative effect on the bone markers alkaline phosphatase (AP) and crosslaps in the serum of pigs weighing 25 kg which were fed a phytase supplemented diet with a low P concentration, which indicates that BA disturbed bone metabolism, but later on, at 60 kg BW, neither the bone markers nor bone mineralization were affected by BA. The normal bone mineralization of the animals slaughtered at 60 kg BW does not preclude that bone mineralization at a younger age had been impaired because compensatory bone mineralization may occur during the growing period (Fammatre et al., 1977). In order to verify the hypothesis based on blood traits that BA intake impairs bone metabolism in weanling pigs fed a diet with a low P concentration, the animals of the present experiment were slaughtered at  $23 \pm 4$  kg BW, six weeks after weaning.

The dietary calcium (Ca):P ratio influences bone mineralization, too. The National Research Council (NRC, 2012) suggests a Ca:P ratio for grain-soybean meal diets

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between 1.1:1 and 1.25:1 based on the argument that a wide Ca:P ratio lowers P absorption, growth performance and bone mineralization. The finding of Lantzsch et al. (1995) and Létourneau-Montminy et al. (2010) that increasing the Ca:P ratio from 1.3:1 to 1.9:1 in diets supplemented with a high amount of phytase increased P retention and bone mineralization in weanling pigs, is in contradiction to the NRC recommendation. In these two balance studies the digestibility of both Ca and P was 70%, resulting in a ratio of absorbed Ca to absorbed P which corresponded to the dietary Ca:P ratio. Because the ratio of Ca:P retained in the body of growing pigs is 1.65:1 (Crenshaw, 2001), Ca absorbed from the phytase supplemented diets with a Ca:P ratio of 1.3:1 presumably became the limiting factor for bone mineralization. The negative effects of the wide dietary Ca:P ratio on growth performance stated by the NRC (2012) could not be verified in the balance studies of Lantzsch et al. (1995) and of Létourneau-Montminy et al. (2010) because feed intake of the pigs had been restricted. In the present study the effects of two Ca:P ratios in phytase supplemented diets on growth performance and bone traits were therefore studied in *ad libitum* fed weanling pigs kept in groups. Because BA increases the absorption, but also the renal excretion of P (Gutzwiller et al., 2011) and may therefore interact with the effects of the dietary Ca:P ratio, the effects of both factors were studied together in a 2×2 factorial experiment.

## MATERIALS AND METHODS

The experiment was approved by the animal welfare department of the canton of Fribourg, Switzerland (approval number FR 4/10).

### Experimental design and diets

The effects of two factors, BA and dietary Ca concentration, were examined in a 2×2 factorial experiment using 32 female and 36 castrated male weanling pigs. Groups of four littermates of the same gender with a similar BW were blocked. Each pig within a block was randomly assigned to one of the four dietary treatments. The four experimental diets had the same P concentration, but either a high (HCa) or a low (LCa) Ca concentration. Diets HCa- and LCa- contained no BA. For the production of diets HCa+ and LCa+ containing 0.5% BA, 2.5 kg BA (VevoVitall, DSM Nutritional Products Ltd., Basel, Switzerland) plus the amount of ingredients necessary for the production of 500 kg diets HCa- and LCa- respectively were mixed in the feed mill during the feed blending process.

Based on the analyzed DM, CP, crude fat, crude fiber and ash content of the batches of ingredients available for blending the feeds plus feed table data of their mineral and

apparent digestible P (dP) content and their digestible amino acid composition the diets were formulated to have the same energy and nutrient content, except for Ca. The formulated dietary nutrient content corresponded to the Swiss recommendations for pigs weighing 15 kg (Agroscope Liebefeld-Posieux, 2004) except for their lower than recommended apparent total tract dP content of 0.27%, which was calculated using the tabulated P digestibility data of the ingredients (Agroscope Liebefeld-Posieux, 2004) and a dP equivalence of 1.2 g for the 1,000 FTU of added phytase/kg diet (Kornegay, 2001). The four experimental diets were mixed in the feed mill of the institute using ingredients of the same batch. The meal was pelleted at 70°C in order to prevent thermal inactivation of native and supplemented phytase. The dietary electrolyte balance (dEB), expressed as milliequivalents (mEq)/kg diet, was calculated by subtracting mEq chloride (Cl)/kg from the sum of mEq sodium (Na)/kg plus mEq potassium (K)/kg.

### Animals and husbandry conditions

The 68 Large White pigs weighing on average 9.7 kg entered the experiment on the day of weaning at the age of four weeks. They were equipped with transponders for individual identification by the computer controlled feeding station and were transferred to four identical pens (one per treatment) with 7 m<sup>2</sup> slatted floor and 10 m<sup>2</sup> concrete floor with straw bedding, which were situated in one room of a climate controlled building. Each pen was equipped with one feeding station (Schauer, Prambachkirchen, Austria) having one feed trough for the feeding of one pig per visit. Visiting piglets were identified and could eat from the trough standing on a scale which registered feed disappearance per visit. The pelleted diets were available *ad libitum* during the experiment lasting 41 days. Three nipple drinkers per pen provided tap water. The technical installations and the piglets were checked daily by the attendants.

### Experimental procedures

Feed samples were collected at the end of each experimental week, and the six samples of each diet were pooled for nutrient analysis. The pigs, which had constant access to feed and water until the end of the experiment, were weighed weekly and at the end of the experiment. Within two hours after the last weighing they were killed at the slaughterhouse situated on the premises of the research institute. They were stunned with CO<sub>2</sub>, and at sticking blood samples were collected into tubes without anticoagulant. During evisceration urine samples were collected from the bladder of 33 animals (HCa+: 9 pigs; HCa-, LCa+, LCa-: 8 pigs per treatment); the bladders of 35 animals contained no urine. The blood samples were centrifuged within two hours after collection. Both tibiae

were collected within half a day after slaughter, manually cleaned of adhering tissue and packed in sealed plastic bags. The bones, serum and urine samples except the samples for urinary pH determination were stored at  $-20^{\circ}\text{C}$  until they were analyzed.

#### Laboratory procedures

Urine pH was determined within half an hour after collection using a Metrohm pH 691 meter (Metrohm, Zofingen, Switzerland). The left tibiae were transferred from the freezer to a refrigerator having a temperature of  $10^{\circ}\text{C}$  16 h before their breaking strength was determined using the three point bending test. The bones were held on a testing machine (Zwick Roell, Ulm, Germany) by two supports spaced 49 mm apart and were broken by a wedge lowered on the centre of the bone at a speed of 10 mm/min. The peak of maximum force was recorded. After autoclaving the right tibiae at  $121^{\circ}\text{C}$  and 1 atmosphere during 45 min, the adhering soft tissue was removed, the bones were crushed, bathed for four hours in acetone under constant stirring for defatting and ground through a 3 mm screen. Samples of defatted ground bone and of feed were dried at  $105^{\circ}\text{C}$  for the determination of the DM. Bone and feed samples were ashed in a muffle furnace at  $550^{\circ}\text{C}$ . The ashed bone and feed samples were solubilized in 10 molar nitric acid, and their Ca, P, magnesium (Mg), Na and K concentrations were analyzed according to EN 15510:2007 using an inductively coupled plasma optical emission spectrometer (ICP-OES, Optima 2000 DV, Perkin-Elmer, Schwerzenbach, Switzerland). Phytate phosphorus in the diets was analyzed using the kit K-Phyt 12/12 (Megazyme, Bray, Ireland). Dietary chloride concentration was analyzed using the argentometric titration method. CP was analyzed using the Dumas method on a Leco FP-2000 analyzer (Leco, Mönchengladbach, Germany). For amino acid determination, the feed samples were prepared according to the Commission Regulation (EC) 152:2009 and were analyzed by HPLC (Alliance 2695, Waters, Milford, MA, USA) as described in the manufacturer's manual (Waters AccQ Tag Chemistry Package 052874 TP, rev. 1). Crude fiber and crude fat were analyzed according to the VDLUFA methods 6.1.4 and 5.1.1. Phytase activity in the feeds was measured using the ISO 30024 method (Gizzi et al., 2008). One FTU corresponds to the amount of enzyme that releases 1  $\mu\text{mol}$  P from 5 mM phytate/min at pH 5.5 and  $37^{\circ}\text{C}$ . The serum and urine analytes were assayed on an Cobas Mira analyzer (Roche, Basel, Switzerland) at  $37^{\circ}\text{C}$ , using kit 1489216 for Ca (Roche, Basel, Switzerland), 61571 for P (BioMérieux, Marcy l'étoile, France), 11489291 for creatinine (CREA Roche) and 2172933 for alkaline phosphatase (AP; Roche).

#### Statistical analysis

The individual pig served as the experimental unit. The data were analyzed with the ANOVA procedure of the statistics package NCSS 2007 (Hintze, Kaysville, Utah, USA) using the general linear model. The model included BA (+, -), dietary Ca concentration (HCa, LCa) and the BA $\times$ Ca interaction as fixed factors, and block as random factor. The block effect was not included in the model for the analysis of the urine variables because of missing data. Because the pigs of treatments HCa had a significantly lower final BW and consequently also smaller, mechanically less resistant tibiae than the pigs of treatments LCa, the force necessary to break the tibia was divided by the BW of the corresponding animal, and the corrected data as well as the actually measured data were statistically analyzed. Differences at  $p \leq 0.05$  were considered statistically significant, whereas differences with  $0.10 \geq p > 0.05$  were considered as tendency.

## RESULTS

The formulation and the chemical composition of the experimental diets are shown in Table 1 and 2, respectively. The analyzed nutrient levels corresponded to the formulated levels. Phytase activity in diets HCa+ and HCa- was 23% and 32% higher than in diets LCa diets. The calculated dietary dP concentration amounted to 0.27%.

#### Growth performance

Benzoic acid increased feed intake ( $p = 0.009$ ) and ADG ( $p = 0.051$ ), but did not influence the feed conversion ratio (FCR,  $p > 0.10$ ; Table 3). The pigs fed the HCa diets had a reduced feed intake ( $p = 0.034$ ), a reduced ADG ( $p = 0.007$ ) and an impaired FCR ( $p = 0.027$ ).

#### Serum and urine variables

Benzoic acid lowered the urinary pH ( $p = 0.031$ ), but neither influenced ( $p > 0.10$ ) the other urine nor the serum variables (Table 4). The pigs fed the HCa diets had an increased serum Ca ( $p < 0.001$ ) and a decreased serum Mg ( $p = 0.002$ ) and P ( $p < 0.001$ ) concentration, whereas serum alkaline phosphatase activity was unaffected ( $p > 0.10$ ). The diets HCa increased the urinary Ca/creatinine ratio ( $p = 0.006$ ), but not the P/creatinine ratio ( $p > 0.10$ ).

#### Bone traits

Benzoic acid did not influence ( $p > 0.10$ ) any of the bone traits (Table 5). The mineral concentration in the bone DM was increased ( $p = 0.013$ ), and Mg concentration in the bone ash was decreased ( $p < 0.001$ ) in the pigs fed the HCa diets, whereas the breaking strength of their bones did not differ ( $p > 0.10$ ) from that of the pigs fed the LCa diets.

**Table 1.** Composition of the experimental diets with 0.77% calcium (HCa) and 0.57% calcium (LCa) respectively, as fed basis

Ingredients (%)	Diet HCa	Diet LCa
Corn, ground	42.8	43.7
Barley, ground	25.2	25.5
Oat flakes	3.0	3.0
Wheat middlings	0.4	0.4
Fat (tallow and lard mixture)	1.0	0.6
Expelled soybean meal (450 g CP/kg)	7.0	6.9
Corn gluten feed	1.0	0.9
Sodium caseinate	6.3	6.3
Whey powder, sweet	5.0	5.0
Apple pomace, dried	5.0	5.0
L-lysine-HCl (79%)	0.21	0.21
L-threonine (99%)	0.06	0.07
Dicalcium phosphate	0.42	0.40
Calcium formate	0.10	0.10
Calcium carbonate	0.61	0.01
Sodium chloride	0.28	0.30
Vitamin trace element premix <sup>1</sup>	0.40	0.40
Natuphos 5,000 G <sup>2</sup>	0.02	0.02
Pellan <sup>3</sup>	0.30	0.30

<sup>1</sup> Supplied per kilogram of diet: vitamin A, 8,000 IU; vitamin D<sub>3</sub>, 1,000 IU; vitamin E, 25 mg; menadione, 3 mg; thiamine, 2 mg; riboflavin, 5 mg; biotin, 0.1 mg; niacin, 20 mg; pantothenic acid, 15 mg; iron, 80 mg as iron sulfate; iodine, 0.15 mg as calcium iodate; copper, 6 mg as copper sulfate; manganese, 10 mg as manganese oxide; zinc, 75 mg as zinc oxide; selenium, 0.2 mg as sodium selenite.

<sup>2</sup> BASF (Ludwigshafen, Germany); provided 1,000 units *Aspergillus niger* phytase/kg diet; one phytase unit corresponds to the amount of enzyme that releases 1 μmol P from 5 mM phytate/min at pH 5.5 and 37°C.

<sup>3</sup> Pellan (Mikro-Technik, Bürgstadt, Germany) is a water soluble cellulose product used to facilitate feed pelleting.

## DISCUSSION

Although the low dietary phytate P content of 1.8 g/kg may have been the limiting factor for the release of P by the added phytase, the calculated P digestibility of 68% corresponds to previous P digestibility values using similar diets (Gutzwiller et al., 2011). The calculated dP concentration of 0.27% in the experimental diets, which corresponds to 0.19 g dP/MJ DE, is slightly below the 0.20 g dP/MJ DE required by pigs weighing 11 to 25 kg (NRC, 2012). The lower than recommended dietary dP concentration, which corresponds to levels used in Swiss pig feeds formulated to minimise P effluent, was chosen in order to detect possible dietary effects under the condition of a marginal P supply. It is known that the effect of different dietary Ca:P ratios on growth performance and bone characteristics is more pronounced at marginal compared to high dietary P levels (Reinhardt and Mahan, 1986; Hall et al., 1991). Despite the marginal dP supply, the bone ash concentration of the experimental animals

corresponds to the concentration of 50% to 53% ash in the fat-free DM reported by Koch et al. (1984), Traylor et al. (2005) and Adeola et al. (2006) in weanling and growing pigs fed diets containing adequate Ca and P levels.

The difference in phytase activity between diets HCa and LCa cannot be exclusively accounted for by the uncertainty of the analytical method used, which has a relative standard deviation for reproducibility of 15% (Gizzi et al., 2008). The reason for the larger than expected difference is unknown. Although BA had increased P digestibility in a previous experiment (Gutzwiller et al., 2011), and therefore might have modulated the effects of the Ca:P ratio on animal performance and mineral metabolism, no significant BA×Ca interaction on any of the tested parameters was observed in the present experiment. The effects of the two factors are therefore discussed separately.

### Effects of benzoic acid

Benzoic acid increased the growth performance of the pigs, confirming the results of previous studies (Guggenbuhl et al., 2007; Torrallardona et al., 2007). Although BA significantly lowered the urinary pH from 7.4 to respectively 7.1 and 6.5 in treatments HCa+ and LCa+, the values of the pigs receiving the BA supplemented diets corresponded to the urinary pH of pigs exposed to a physiological dietary acid load (Budde and Crenshaw, 2003) and therefore reflect an undisturbed acid-base balance. Torrallardona et al. (2007) and Gutzwiller et al. (2011) reported urinary pH values below 5.5 in weanling pigs which were fed diets containing 0.5% BA. The higher dEB (135 vs 82 mEq/kg) and the higher Ca concentration (5.7 and 7.7 g/kg vs 5.3 g/kg) in the diets of the present compared to those of the previous experiment account for the higher urinary pH in the present compared to our previous experiment because both a high dEB and a high dietary Ca concentration increase the urinary pH in pigs (Canh et al., 1998). The fact that BA neither affected the serum AP activity, which is in contrast to our previous finding (Gutzwiller et al., 2011), nor the bone traits, suggests that BA does not impair bone metabolism of weanling pigs unless its addition decreases urinary pH to below 6, a value associated with a reduced Ca retention in pigs (Patience and Chaplin, 1997).

The absence of any negative BA effect on bone characteristics confirms the findings of Sauer et al. (2009) and of Gutzwiller et al. (2011), who did not detect any negative effect of BA on bone ash concentration and breaking strength in pigs weighing 40 and 60 kg, respectively. The effects of BA on bone traits of growing-finishing pigs reported by Bühler et al. (2010) are equivocal: Benzoic acid tended to reduce ash concentration in the metatarsal bones, but did not affect the breaking

**Table 2.** Chemical composition of the four experimental diets, % as fed basis unless stated otherwise

Item	Ca concentration			
	High (HCa)		Low (LCa)	
	+	-	+	-
CP	17.1	16.8	17.1	17.0
Crude fat	3.5	3.8	4.0	3.5
Crude fiber	2.7	3.0	2.8	2.9
Ash	4.3	4.3	3.8	3.8
Calcium	0.76	0.78	0.55	0.58
Phosphorus	0.39	0.41	0.41	0.41
Phytate phosphorus	0.17	0.18	0.20	0.18
Magnesium	0.11	0.11	0.11	0.11
Potassium	0.52	0.53	0.54	0.55
Sodium	0.23	0.23	0.24	0.25
Chloride	0.36	0.36	0.36	0.37
Phytase activity (FTU/kg)	1,450	1,350	1,100	1,100
Lysine	1.10	1.10	1.08	1.08
Methionine	0.36	0.37	0.37	0.36
Cystine	0.27	0.27	0.28	0.28
Tryptophan	0.20	0.20	0.21	0.21
Threonine	0.74	0.74	0.72	0.72
DE (MJ/kg)	14.0	14.0	14.0	14.0
Calcium:phosphorus ratio	1.9	1.9	1.3	1.4
Dietary electrolyte balance (mEq/kg)	129	133	139	141

DE, calcium:phosphorus ratio and dietary electrolyte balance ( $\text{Na}^+\text{+K}^-\text{-Cl}$ , expressed in milliequivalents) were calculated while the other data represent analyzed values.

strength of the tibia, despite a significantly reduced tibial bone mineral density. In conclusion, the majority of published data do not show significant negative effects of BA on bone breaking strength and bone ash concentration, suggesting that the risk of reduced bone mineralization caused by this feed additive is low.

#### Effects of the dietary Ca:P ratio

The effects of the wide dietary Ca:P ratio in diets HCa on feed intake, ADG, FCR as well as serum Ca and P concentration confirm results of Lei et al. (1994) and Qian et al. (1996) on the effects of a wide Ca:P ratio in phytase-supplemented weanling pig diets on growth performance and serum clinical chemistry. According to Suttle (2010), maximum growth in pigs is associated with a serum P

concentration of at least 2.5 mmol/L. All but one of the pigs fed the LCa diets, but only two thirds of the pigs fed the HCa diets had a serum P concentration above that threshold concentration, which may explain the growth-depressing effect of the HCa diets. An increase in serum Ca concentration, as observed in the pigs fed diets HCa, is known to decrease serum parathyroid hormone concentration and to increase serum calcitonin concentration (Cooper et al., 1971), resulting in a reduced renal reabsorption of both Ca and Mg (Littledike and Goff, 1987). The increased urinary Ca:creatinine ratio observed in treatments HCa shows that urinary Ca excretion was increased in response to the increased serum Ca concentration. The decreased Mg concentration in the serum and in the bone ash of the animals on diets HCa may

**Table 3.** Effects of dietary calcium concentration and benzoic acid (BA) supplementation on growth performance from four to ten weeks of age (n = 17)

Item	Ca concentration and BA supplementation				SEM	p-value		
	HCa		LCa			BA	Ca	BA×Ca
	+	-	+	-				
Initial BW (kg)	9.7	9.6	9.8	9.7	0.29	0.793	0.657	0.977
Final BW (kg)	22.7	20.7	24.8	24.3	0.88	0.159	0.012	0.614
ADFI (g)	524	451	567	513	23.8	0.009	0.034	0.691
ADG (g)	329	284	370	343	17.6	0.051	0.007	0.628
FCR <sup>1</sup> (kg/kg)	1.60	1.64	1.55	1.50	0.044	0.885	0.027	0.334

<sup>1</sup> FCR = Feed conversion ratio (kg feed consumed per kg BW gain).

**Table 4.** Effects of dietary calcium concentration and benzoic acid (BA) supplementation on serum and urine parameters

Item	Ca concentration and BA supplementation				SEM	p-value		
	HCa		LCa			BA	Ca	BA×Ca
	+	-	+	-				
Serum (n = 17)								
Ca (mmol/L)	2.87	2.89	2.75	2.73	0.030	0.963	<0.001	0.356
Phosphorus (mmol/L)	2.60	2.69	3.47	3.30	0.089	0.648	<0.001	0.155
Magnesium (mmol/L)	1.20	1.21	1.29	1.27	0.022	0.869	0.002	0.458
Alkaline phosphatase (U/L)	329	332	328	352	17.9	0.434	0.611	0.567
Urine <sup>1</sup>								
pH	7.07	7.44	6.46	7.40	0.292 <sup>a</sup>	0.031	0.246	0.322
Ca/creatinine (mmol/mmol)	2.75	3.69	0.96	1.66	0.655 <sup>a</sup>	0.214	0.006	0.855
P/creatinine (mmol/mmol)	0.04	0.05	0.07	0.05	0.009 <sup>a</sup>	0.517	0.183	0.188

<sup>1</sup> Urine samples could be collected at slaughter from 33 animals only (9 HCa+, 8 of each other treatment).

<sup>a</sup> SEM of the three treatments with eight replications.

be the result of either an increased urinary Mg excretion or of a decreased intestinal Mg absorption caused by the high dietary Ca concentration, as observed in the horse (Grace et al., 2003).

The increased bone ash concentration observed in the pigs fed diets HCa supports the finding of Létourneau-Montminy et al. (2010) that widening the dietary Ca:P ratio from 1.3 to 1.9 in a diet containing 0.56% P supplemented with 1,000 FTU/kg phytase had no negative effect on P digestibility and significantly increased bone ash concentration in weanling pigs. On the other hand Qian et al. (1996) reported a negative effect on P digestibility and bone mineralization of weanling pigs when the Ca:P ratio of a diet containing 0.45% P supplemented with 1,000 FTU/kg phytase was increased from 1.2 to 2. A meta-analysis of P utilization in pigs (Létourneau-Montminy et al., 2012) which shows that increasing dietary Ca negatively affects retained P when diets have a low concentration of non-phytate P, but increases retained P in diets having a high concentration of non-phytate P, explains the conflicting effects of a wide Ca:P ratio on bone mineralization reported by Qian et al. (1996) and by Létourneau-Montminy et al. (2010). The increased bone mineralization of the pigs in the present study fed low P diets with a high Ca concentration (diets HCa) is in contradiction to the results of this meta-

analysis. P digestibility was presumably less impaired than FCR by the wide Ca:P ratio of diets HCa, resulting in an increased amount of absorbed P per kg BW gain. The higher phytase activity analyzed in diets HCa, as compared to diets LCa, may have contributed to the positive effect on bone mineralization. However, this effect was presumably of minor importance, because the amount of phytate P released per unit of phytase markedly declines with increasing dietary phytase concentration (Paditz et al., 2004).

The hypothesis tested that the Ca:P ratio in phytase-supplemented feeds for growing pigs should be wider than 1.3:1 in order to maximise bone mineralization could not be verified in the present study because the effects of the increased Ca supply, of the reduced feed conversion ratio and the differences in dietary phytase activities on bone mineralization cannot be separated. However, the results show that a dietary Ca:P ratio of 1.9:1 in a low P diet reduces growth performance to such an extent that such a feeding regimen cannot be recommended for economic reasons. The question as to the effects of Ca:P ratios wider than 1.3:1 but lower than 1.9:1 in phytase supplemented low P diets on growth performance, Ca and P digestibility and bone mineralization merits further investigation.

**Table 5.** Effects of dietary calcium concentration and benzoic acid (BA) supplementation on characteristics of the tibia (n = 17)

Item	Ca concentration and BA supplementation				SEM	p-value		
	HCa		LCa			BA	Ca	BA×Ca
	+	-	+	-				
Breaking strength (N <sup>1</sup> )	1,329	1,266	1,377	1,341	46.7	0.298	0.192	0.779
Breaking strength (N/kg BW)	57.8	59.4	55.7	56.7	1.62	0.426	0.142	0.857
Ash (% in fat free DM)	53.1	53.3	51.4	51.2	0.74	0.952	0.013	0.776
Calcium (% in ash)	40.6	39.5	39.5	39.3	0.41	0.132	0.129	0.246
Phosphorus (% in ash)	19.1	19.1	19.4	19.0	0.20	0.361	0.548	0.283
Magnesium (% in ash)	0.90	0.92	0.98	0.98	0.02	0.833	<0.001	0.534

<sup>1</sup> N = Newton.

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