



## Effects of a reduced calcium, phosphorus and protein intake and of benzoic acid on calcium and phosphorus metabolism of growing pigs

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### ABSTRACT

In order to minimise environmental pollution, many pig feeds contain low phosphorus and protein concentrations as well as benzoic acid (BA), an additive which reduces ammonia formation in the slurry. Since both a low P intake and metabolic acidosis compromise bone mineralisation, the effect of a diet with a low concentration of calcium (Ca), phosphorus (P) and crude protein (CP) and the effect of BA on Ca and P metabolism were examined in a 2 × 2 two-factorial experiment using pigs from 13 to 64 kg body weight (BW). Compared to the control piglet and grower diets (8.7 and 6.9 g Ca; 6.9 and 5.3 g P; 172 and 156 g CP per kg, respectively), the intake of the low nutrient piglet and grower diets (5.3 g Ca; 4.3 and 4.0 g P; 154 and 147 g CP per kg, respectively, both supplemented with 1500 U/kg microbial phytase) reduced ( $P < 0.01$ ) Ca and P retention by 27% and 24%, respectively, reduced ( $P < 0.05$ ) the growth rate of the piglets by 7%, and decreased ( $P < 0.05$ ) the bone breaking strength and bone mineral content ( $P < 0.01$ ) by 5% in the animals which were slaughtered at 64 kg BW. Benzoic acid (5 and 10 g per kg piglet and grower diet, respectively) did not influence ( $P > 0.05$ ) the apparent digestibility of Ca, increased the apparent digestibility of P ( $P < 0.05$ ) by 5% and increased the urinary Ca and P output ( $P < 0.01$ ) by 70% and 83%, respectively, but had no effect ( $P > 0.05$ ) on the proportion of ingested Ca and P which was retained. Furthermore, BA increased ( $P < 0.01$ ) the serum activity of the bone formation marker alkaline phosphatase at 25 and 40 kg BW by 17% and 13%, respectively and decreased ( $P < 0.01$ ) the concentration of the bone resorption marker serum crosslaps at 25 kg BW by 12%, implying that BA affected bone metabolism at 25 and 40 kg BW. Since BA neither affected the blood variables at 60 kg nor the bone breaking strength and bone mineral content, any possible negative effect of BA on bone metabolism of the piglets and of the young growing animals thus seems to have disappeared during the last period of the grower period. In conclusion, the slight metabolic acidosis caused by BA had no lasting negative effects on the bones of the growing pigs.

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**Abbreviations:** ADG, average daily weight gain; ALT, alanine aminotransferase; AP, alkaline phosphatase; AST, aspartate aminotransferase; BA, benzoic acid; BMC, bone mineral content; BW, body weight; CP, crude protein; DE, digestible energy; dEB, dietary electrolyte balance; DM, dry matter; GGT, gamma-glutamyltransferase; Mc3, Mc4, 3rd, 4th metacarpal bone; N, Newton; NL, nutrient level; OC, osteocalcin; SCL, serum crosslaps (epitope of the carboxyterminal telopeptide of type I collagen).

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## 1. Introduction

Pig slurry contributes to the pollution of the environment with phosphorus (P). To minimise P output, the P concentration of feeds for growing pigs is frequently reduced to the lowest levels necessary for maximal growth rate. Since the P requirement for maximal bone mineralisation is higher than the requirement for maximal growth rate (NRC, 1998), such feeds may have an adverse effect on bone mineralisation. Feeds with a reduced P concentration usually contain a low Ca concentration as well, since a wide Ca/P ratio decreases P absorption (NRC, 1998). Ammonia emission from animal manure is an additional environmental problem challenging pig producers. The use of feeds with a low crude protein (CP) concentration and added benzoic acid (BA) reduces ammonia emission. After absorption, benzoic acid is transformed in the liver to hippuric acid which is excreted in the urine and reduces microbial urea decomposition. Benzoic and hippuric acid contribute to the metabolic acid load of the body. Chronic acidosis stimulates bone resorption by osteoclasts and may thus compromise bone mineralisation (Arnett, 2003). It is unclear whether a slight increase in dietary acid load is of practical relevance for bone mineralisation in pigs. Whereas Budde and Crenshaw (2003) failed to detect any negative effect of metabolic acidosis caused by chloride intake on the skeleton of piglets, the intake of BA significantly decreased the bone ash concentration in growing pigs in a balance study (Sauer et al., 2009), and tended to reduce bone ash concentration in growing-fattening pigs (Bühler et al., 2010). One objective of the present experiment was to determine if commercially available Swiss feeds formulated to minimise environmental pollution affect the bone mineralisation and thus compromise the welfare of growing pigs. For this purpose experimental piglet and grower diets with CP, P and phytase concentrations as found in these commercial feeds were formulated, and the effects of these diets were compared to the effects of diets which were formulated to contain the nutrient concentrations recommended in Switzerland by ALP (2004) and which did not contain added phytase. In addition, the hypothesis was tested that the increased dietary acid load provided by BA affects Ca and P metabolism, related blood serum parameters and bone mineralisation of pigs. The effects of dietary nutrient concentration and of BA on Ca and P metabolism, growth and bone traits of pigs were examined in a balance study and a feeding trial. As the focus was set on the period of rapid lean body mass accretion, i.e. the period of high Ca and P requirements and the highest risk of Ca and P deficiency, the animals were slaughtered after attaining 60 kg body weight (BW) in order to study the bone traits at the end of the grower period.

## 2. Animals, materials and methods

The balance study and the feeding trial were approved by the animal welfare department of the competent government authority (approval number FR 77/06).

### 2.1. Experimental design and feed formulation

The effects of two factors, dietary nutrient level (Ca, P and CP) and BA supplementation, were examined in a balance study using 16 pigs and in a feeding trial using 64 pigs. Groups of four littermates of the same gender with a similar body weight (BW) were blocked. Each piglet within a block was randomly assigned to one of the four dietary treatments.

The experimental diets contained either a high (H) or a low (L) Ca, P and CP concentration. Benzoic acid was added to the diets H+ and L+, whereas the diets H– and L– contained no BA. The pelleted piglet and grower diets (Table 1) were formulated according to the recommendations for piglets weighing 20 kg and for growing pigs weighing 40 kg (ALP, 2004), except for the Ca, P and CP content of diets L+ and L–, which corresponded to the levels found in commercially available Swiss pig feeds formulated with the aim to minimise P and nitrogen effluent. The Ca/P ratio of all diets was fixed at 1.3/1. The feed formulation programme Allix2 (A-Systems SA, Versailles, France) used to formulate the experimental diets contained the analysed dry matter, crude protein, crude fat, ash and crude fibre data of the ingredients and feed table data of their mineral content and their amino acid composition. Phytase (1500 U/kg feed; Natuphos 5000 G, BASF, Ludwigshafen, Germany) was added to diets L+ and L–. Five and 10 g BA (VevoVital, DSM Nutritional Products Ltd., Basel, Switzerland) were added per kg of the respective piglet and grower diets (H+ and L+), which corresponds to the dose recommended by the manufacturer.

### 2.2. Animals and husbandry

Large White piglets, which had been weaned at the age of five weeks and had received a piglet diet containing 180 g CP, 11 g Ca and 7 g P per kg feed during the first two to three weeks after weaning, were selected for the experiments at an average BW of 13 kg. They were housed in a climate controlled building and were fed the experimental piglet diets *ad libitum* until 25 kg BW. Thereafter they received the grower diets in amounts allowing for an average daily gain of 850 g during the growing-finishing period. Drinking water was constantly available.

The sixteen castrated male piglets weighing  $13.5 \pm 1.8$  kg at the start of the balance study were individually housed in pens with a surface of 2.6 m<sup>2</sup>.

At a BW of  $12.9 \pm 2.6$  kg the 36 female and 28 castrated male piglets used in the feeding trial were equipped with transponders and were transferred to four pens (one for each treatment) with 7 m<sup>2</sup> of slatted floor and 10 m<sup>2</sup> of concrete floor with straw bedding. Each pen was equipped with a computer controlled feeding station (Schauer, Prambachkirchen, Austria) which registered the amount of the experimental feed consumed by each animal and allowed for individual feed

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