Effects of benzoic acid on nitrogen, phosphorus and energy balance and on ammonia emission from slurries in the heavy pig

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Abstract

The effects of two dietary levels of benzoic acid on nitrogen, phosphorus and energy balance were evaluated in the typical Italian heavy pig during the last phase of growth. Six Landrace x Large White barrows of 125 kg body weight (BW) on average were used in a repeated 3×3 Latin square design and housed in metabolic cages to collect faeces and urine separately, in 3 collection periods of 7 days, after 14 days of adaptation. The animals were individually housed in open circuit respiration chambers to determine the energy metabolism. The dietary treatments were as follows [% on dry matter (DM)]: i) diet C (control): 14.2 crude protein (CP), 3.7 EE, 13.8 NDF; ii) diet B05: diet C plus 0.5% benzoic acid; iii) diet B10: diet C plus 1.0% benzoic acid. DM fed was fixed at 6.8% BW^{0.75}. Apparent digestibility was similar among treatments for all the parameters studied. Nitrogen (N) retention was 35.8, 37.4, 41.6% of intake N for C, B05 and B10, respectively, with no significant difference. Energy and phosphorus balances were not influenced by dietary treatments. Ammonia nitrogen emission from the slurry, expressed as a proportion of the initial slurry nitrogen, was decreased (P=0.049) by the inclusion of benzoic acid in the diet: 35.2, 28.1, 26.2% for C, B05, B10, respectively. The addition of benzoic acid to the diet determined a numerically decrease of the urinary pH. In conclusion, the inclusion of benzoic acid in the diet of the heavy pig is beneficial to the environment without effects on N, phosphorus (P) and energy balances.

Introduction

Benzoic acid is an organic carboxylic acid used in pig nutrition as antimicrobial growth promoter particularly for weanling piglets. An in vitro study has shown that benzoic acid has a strong antimicrobial effect (Knaareborg et al., 2002), but little is known about its efficacy in vivo. Torrallardona et al. (2007) observed that the inclusion of 0.5% benzoic acid into the diet of weanling piglets improved performance by influencing ileal and caecal microbiota of the piglets; particularly, benzoic acid was effective in killing coliformi bacteria (Knaareborg et al., 2002). Biagi and Piva (2007) found that benzoic acid can positively influence swine caecal microflora in vitro fermentation reducing ammonia concentration. In a study of Kristensen et al. (2009), adding 1% benzoic acid to a diet for growing pigs markedly acidified the urine pH (about 2 points), but only slightly reduced blood pH and no effects of benzoic acid supplementation were detected on inter-organ fluxes of O2, CO2, glucose, lactate and urea. Similarly, Kluge et al. (2010) found a decrease in urine pH with the addition of 0.5% benzoic acid to the diet of sows. The decrease of the urinary pH was observed also by Kluge et al. (2006), Sauer et al. (2009) and by Torrallardona et al. (2007) who states that the addition of 0.5% benzoic acid to the diet determines an increase of the hippuric acid concentration from 455 to 741 mg/100 mL and a decrease of the urinary pH of 0.5 points. The lower urine pH is attributable to the fact that the ingested benzoic acid, unlike the other organic acids, is not oxidized, but it is absorbed in the first tract of the little intestine and then transported to the liver where is converted into hippuric acid by reaction with the amino acid glycine. Hippuric acid is excreted rapidly by the urinary pathway (Bridges et al., 1970). The concentration of hippuric acid in the urine consequently lowers urinary pH and thus the pH of the slurry. Furthermore, Murphy et al. (2011) showed that increasing dietary benzoic acid concentration from 0% to 3% reduced nitrogen (N) excretion by about 20% in 64 kg body weight (BW) boars. As a consequence the urine activity of the micro-organisms in the slurry decreases as well as the ammonia release from slurry to the air. Furthermore, diet acidification with several organic acids (formic, butyric, lactic, fumaric, and citric) increased the apparent total tract digestibility of phosphorus (P) in pigs (Jongbloed et al., 2000), since the efficacy of microbial phytase is pH-dependent (Simons et al., 1990) and the highest activity was observed at two pH optima, i.e. 5.0 to 5.5 and 2.5. However, the results of diets added with benzoic acid or Ca-benzoate on P utilization by pigs are not always consistent, probably for the different mineral concentration of the diets, the presence of organic acid other than benzoic acid as well as the absence/presence of microbial phytase in the diets (Gutzwiller et al., 2011). Moreover, in a recent study on piglets (Halas et al., 2010), dietary supplementation with benzoic acid failed to reduce gastric pH. This further supports the notion that the dietary effect of benzoic acid is not necessarily linked to a lower gastric pH. In their experiment, Halas et al. (2010) speculate that the better utilization of available nutrients should be ascribed to the observed increase of the small intestinal weight: length ratio and to the increase of the villous height and of the villous height:crypt depth ratio.

To the best of our knowledge, no experiments have been conducted to evaluate the effect of benzoic acid on N and P balances in the heavy fattening pig. The aim of the present study was to test the effects of two dietary levels, 0.5% and 1%, of benzoic acid on N, P and energy balance and on the ammonia emission from slurry in the typical Italian heavy pig during the last phase of fattening.
used for the experiment. The animals were paired off to form three age- and weight-matched groups. The pairs were fed each of three different diets for 21 days in a repeated 3x3 Latin Square design, so that each animal received all dietary treatments throughout the experiment, in three consecutive periods. Each period lasted 21 days: after 14 days of adaptation, tests to investigate the digestibility of the diets were performed over the following 7 days (testing period). At the beginning of the first testing period the animals weighed on average 100 kg BW, at the end of the experiment they weighed on average 140 kg.

The composition of the three diets was the following:
- diet C (control): maize meal 50%, barley meal 30%, wheat bran 10%, soya bean meal 5.5%, calcium carbonate 1.8%, sodium chloride 0.5%, dicalcium phosphate 0.5%, vit/minerals 0.5%, L-Lys HCl 0.15%, DL-Met 0.05%;
- diet B05: diet C plus 0.5% benzoic acid;
- diet B10: diet C plus 1% benzoic acid.

Benzoic acid (Vetovital®; DSM Nutritional Products, Basel, Switzerland) was added in substitution of equal amounts of maize meal. The vitamin/mineral supplement contained 15 g/kg vitamin A, 210 KU/kg vitamin D, 3500 mg/kg Mn, 33 mg/kg Se, 37 mg/kg Co, 436 mg/kg I, 525 mg/kg Zn, 20808 mg/kg Cu, 1750 mg/kg Ca, 24 g/kg P, 14 g/kg Na, 12 g/kg Cl, 12793 mg/kg Fe, 20808 mg/kg Zn, 4270 mg/kg Cu, 1750 mg/kg Mn, 33 mg/kg Se, 37 mg/kg Co, 436 mg/kg I, 525 KU/kg vitamin A, 210 KU/kg vitamin D, 3500 U/kg vitamin E.

Feed supply was restricted at 6.8% metabolic body weight (BW) following the established protocol for the production of Parma and San Daniele Ham. The average BW during the experiment was 37.0, 37.1 and 36.9 for pigs fed diets C, B05 and B10, respectively. Drinkable water was always available. The animals were fed daily at 08:00 and 17:00 h.

The experiment was conducted according to the guidelines on animal welfare in animal research of the Italian Legislative decree no. 116/1992.

Measurements and analyses

The animals were housed individually in metabolic cages throughout the study period. During each 7 days test period animals were placed individually in an open-circuit respiration chamber to measure respiratory exchange over three 24-h cycles. For each Latin Square the three pigs were housed contemporaneously in three respiration chambers. Heat production (HP) for each animal was calculated from Brouwer’s equation (1963):

\[
HP \ (\text{kJ/d}) = (16.175 \text{ O}_2) + (5.021 \text{ CO}_2) - (2.167 \text{ CH}_4) - (5.987 \text{ N})
\]

where \( \text{O}_2, \text{ CO}_2 \) and \( \text{CH}_4 \) are the volumes (L/day) of the gases at standard temperature (0°C) and pressure (760 mmHg) conditions, consumed or produced during respiration and \( \text{N} \) is the urinary nitrogen (g/day).

Before feeding, orts were removed and weighed. Samples of each diet and orts were taken daily throughout the testing period to determine dry matter content after drying for 72 h in a forced ventilation oven at 60°C. During each testing period samples of each diet and individual orts were taken daily and pooled for chemical analyses. Faeces and urine were collected daily from each animal during the testing periods, sampled (20% of weight for faeces and 10% of weight for urine), and then pooled for animal and period and chemically analysed. One-hundred-fifty mL of 10% v/v sulphuric acid was placed in each animal’s urine collection vessel each day to keep the pH below 2.5 and avoid ammonia loss.

Samples of feeds and faeces were ground through a 1 mm screen (Pulverisette, Fritsch, Idar-Oberstein, Germany) and analysed for DM and ash following the AOAC (1995) procedures (methods 945.15 and 942.05, respectively). Neutral detergent fibre content (NDF) of feed and faeces was determined according to Mertens (2002) and acid detergent fibre (ADF) according to the method of Van Soest et al. (1991) using an Ankom 200 fibre apparatus (ANKOM Technology Corporation, Fairport, NY, USA) with the addition of sodium sulphite. The N content of diets, faeces and urine were determined by the macro-Kjeldahl (2300 Kjtecl, Foss Analytical, Hilleroed, Denmark). Ammonia emission was determined daily for 14 consecutive days and the pH was detected at the beginning and at the end of this period.

Statistical analysis

The data were analysed by ANOVA using the SAS (2000) GLM procedure with the following model:

\[
Y_{ijk(t)} = \mu + S_i + A_{ij} + P_k + T(t) + e_{ijk}
\]

where:
- \( Y_{ijk(t)} \) = dependent variable;
- \( \mu \) = general mean;
- \( S_i \) = square effect (\( i = 1,2 \));
- \( A_{ij} \) = effect of animal within each square (\( i = 1,3 \));
- \( P_k \) = effect of period (\( k = 1,3 \));
- \( T(t) \) = effect of treatment (\( t = 1,3 \));
- \( e_{ijk} \) = residual error.

Results and discussion

The analyses of the experimental diets are shown in Table 1. DM intake was similar among treatments, with a slightly higher value for diet B05 in comparison with C and B10 diets (2043, 2138 and 2035 g/d for C, B05 and B10, respectively). Benzoic acid in our study did not hamper feed intake. This is consistent
with the experiment of Kluge et al. (2010) in sows where feed intake was decreased with a concentration of 2% of benzoic acid but not with lower concentrations. The amount of excreta are reported in Table 2; no significant difference between treatments was observed.

No significant difference among treatments was observed, in terms of digestibility, for any parameter studied (Table 3). This holds true also for P, whereas in literature the influence of benzoic acid and its salts on P utilization in growing pigs is inconsistent: the retention of P was decreased in one study (Mroz et al., 1996) using Ca benzoate, but increased in another study (Mroz et al., 1997) after Na benzoate was included into the diet. In another study, Sauer et al. (2009) found a significant increase in P digestibility adding 1 and 2% benzoic acid to the diet of piglets: 45.8, 50.5 and 54.8% for 0, 1 and 2% benzoic acid in the diet. Bühler et al. (2010) found no effect of 0.5% benzoic acid on P digestibility in the grower phase, but a positive effect in the finisher phase.

Nitrogen balance (Table 4) showed a slightly higher N intake for diet B05, due to the higher feed intake. No significant difference was observed between treatments, in accordance with Nørgaard et al. (2010), although the addition of benzoic acid was associated to a numerically lower urinary nitrogen excretion (50.4, 48.9 and 45.3% of N intake) and a higher N retention (35.8, 37.4 and 41.6% of N intake) and a higher N retention (35.8, 37.4 and 41.6% of N intake for C, B05 and B10, respectively). The numerically lower urinary nitrogen excretion of about 40%. This value is consistent with those obtained, even then in the Italian heavy pig, by Galassi et al. (2005). Protein deposition for treatments C, B05 and B10 was 105, 121 and 121 g/d, respectively (SEM=18.2; P=0.514), whereas fat deposition was 333, 350 and 340 g/d, respectively (SEM=38.7; P=0.795).

The pH of the excreta and the ammonia release from the slurries are reported in Table 7. The data obtained indicate a trend for a lower pH of excreta (faeces and urine) with the diets containing benzoic acid compared to the C diet, however because of the high residual variation of the values detected no signifi-

Table 1. Analytical composition of the experimental diets.

| Diet     | C   | B05 | B10 | SEM   | P   |
|----------|-----|-----|-----|-------|-----|
| Ash, % DM| 5.80| 5.50| 5.50|       |     |
| Crude protein, % DM | 14.10 | 14.10 | 14.50 |     |     |
| Ether extract, % DM | 3.70  | 3.60  | 3.80  |     |     |
| NDF, % DM | 13.70 | 13.50 | 14.00 |     |     |
| ADF, % DM | 5.00   | 5.00   | 4.40   |     |     |
| Phosphorus, % DM | 0.41   | 0.41   | 0.38   |     |     |
| Energy, MJ ME/kg | 15.38 | 15.44 | 15.56 |     |     |

C, control 0% benzoic acid; B05, 0.5% benzoic acid; B10, 1% benzoic acid; NDF, neutral detergent fibre; ADF, acid detergent fibre; DM, dry matter.

Table 2. Dry matter intake and faeces and urine production.

| Diet     | C   | B05 | B10 | SEM | P   |
|----------|-----|-----|-----|-----|-----|
| Dry matter intake, g/d | 2043 | 2138 | 2035 | 124 | 0.767 |
| Faeces, g/d | 860  | 880  | 731  | 50.2 | 0.122 |
| Faeces, g DM/d | 281  | 296  | 268  | 11.5 | 0.242 |
| Urine, g/d | 2146 | 1765 | 1646 | 383 | 0.586 |

C, control 0% benzoic acid; B05, 0.5% benzoic acid; B10, 1% benzoic acid.

Table 3. Apparent digestibility of the experimental diets.

| Diet     | C   | B05 | B10 | SEM   | P   |
|----------|-----|-----|-----|-------|-----|
| Dry matter, % | 86.4 | 85.9 | 86.8 | 0.55  | 0.491 |
| Ash, % | 55.1 | 51.5 | 55.8 | 1.81  | 0.196 |
| Organic matter, % | 88.3 | 87.9 | 88.6 | 0.08 | 0.543 |
| Crude protein, % | 80.0 | 86.6 | 86.7 | 0.59  | 0.606 |
| Ether extract, % | 88.9 | 85.3 | 89.2 | 2.07 | 0.221 |
| NDF, % | 55.0 | 50.5 | 56.6 | 2.23 | 0.158 |
| Phosphorus, % | 46.5 | 48.3 | 47.6 | 1.54 | 0.633 |
| Energy, % | 85.9 | 85.4 | 86.3 | 0.57 | 0.525 |

C, control 0% benzoic acid; B05, 0.5% benzoic acid; B10, 1% benzoic acid; NDF, neutral detergent fibre.

Table 4. Nitrogen balance.

| Diet     | C   | B05 | B10 | SEM   | P   |
|----------|-----|-----|-----|-------|-----|
| Nitrogen intake, g/d | 46.40 | 48.50 | 46.20 | 2.83 | 0.490 |
| g/kg BW0.75 | 1.28  | 1.33  | 1.26  | 0.077 | 0.510 |
| Nitrogen in faeces, g/d | 6.50  | 6.50  | 6.10  | 0.21 | 0.281 |
| g/kg BW0.75 | 0.18  | 0.18  | 0.17  | 0.008 | 0.381 |
| % IN | 13.8 | 13.7 | 13.1 | 0.59  | 0.606 |
| Nitrogen in urine, g/d | 23.10 | 22.60 | 20.80 | 1.57  | 0.526 |
| g/kg BW0.75 | 0.63  | 0.62  | 0.56  | 0.035 | 0.320 |
| % IN | 50.4 | 48.9 | 45.3 | 4.92  | 0.720 |
| Nitrogen retained, g/d | 16.70 | 19.40 | 19.30 | 2.91  | 0.517 |
| g/kg BW0.75 | 0.47  | 0.53  | 0.53  | 0.079 | 0.541 |
| % IN | 35.8 | 37.4 | 41.6 | 5.12  | 0.671 |

C, control 0% benzoic acid; B05, 0.5% benzoic acid; B10, 1% benzoic acid; IN, nitrogen intake; BW, body weight.
cant difference was observed among treatments. The decrease in urine pH (half point with diet B10) is much lower than that (two points of pH decrease) obtained by Kristensen et al. (2009) and by Nørgaard et al. (2010) on pigs of 50 kg body weight. However, it has to be considered that in our experiment pigs were heavy (120 kg BW) and fed restricted (6.8% of BW) whereas in the other study the pigs weight 50 kg and were fed 3.6% of BW corresponding to 9.6% of BW. Hence in our study the amount of benzoic acid ingested per unit of body weight was lower. Ammonia concentration in the slurries (Table 7) was similar at the beginning of the determination, but numerically higher at the end (14 days after) for the two test diets; this is due to the lower ammonia release from the slurries associated to the diets containing benzoic acid, which resulted significantly lower (P=0.049) when expressed as NH₃-N percentage of the total N of the slurries at the beginning of the determination. The addition of benzoic acid to the diet determined a numerically decrease of the urinary pH due to the conversion of benzoic acid into hippuric acid in the liver. Hippuric acid is then excreted with urine (Bridges et al., 1970) lowering urinary pH and it is well known that ammonia emission is affected by urinary pH. The value determined for C diet (NH₃-N percentage of the total N: 35.2%) is consistent with that obtained, for another control diet similar to that used in the present study and always in 14 days of ammonia emission study, in a previous experiment in the Italian heavy pig (Galassi et al., 2010). In the latter experiment, high fibre diets determined, in comparison with control, a decrease of ammonia emission from slurry in the same order of magnitude of that observed in the present study by the addition of benzoic acid in the diet.

### Table 5. Phosphorus balance.

| Diet      | C       | B05     | B10     | SEM     | P     |
|-----------|---------|---------|---------|---------|-------|
| Phosphorus intake, g/d | 8.40    | 8.70    | 7.70    | 0.51    | 0.373 |
| Phosphorus in faeces, g/d | 0.23    | 0.24    | 0.21    | 0.013   | 0.335 |
| Phosphorus in urine, g/d | 4.50    | 4.50    | 4.00    | 0.24    | 0.286 |
| Phosphorus in urine, % IP | 53.5    | 51.7    | 52.4    | 1.54    | 0.633 |
| Phosphorus retained, g/d | 0.06    | 0.11    | 0.11    | 0.001   | 0.612 |
| Phosphorus retained, % IP | 5.7     | 5.2     | 5.4     | 0.68    | 0.788 |

C, control 0% benzoic acid; B05, 0.5% benzoic acid; B10, 1% benzoic acid; IP, phosphorus intake; BW, body weight.

### Table 6. Daily energy utilization and partition.

| Diet      | C       | B05     | B10     | SEM     | P     |
|-----------|---------|---------|---------|---------|-------|
| Gross energy intake, MJ/d | 37.79   | 39.79   | 37.96   | 2.310   | 0.892 |
| Gross energy intake, kJ/BW⁰.⁷⁵ | 1042    | 1087    | 1032    | 63.5    | 0.856 |
| Digestible energy, kJ/BW⁰.⁷⁵ | 892     | 932     | 890     | 58.8    | 0.754 |
| % IE      | 85.9    | 85.4    | 86.3    | 0.57    | 0.525 |
| Metabolizable energy, kJ/BW⁰.⁷⁵ | 866     | 903     | 861     | 58.60   | 0.781 |
| % IE      | 83.3    | 82.6    | 83.4    | 0.76    | 0.795 |
| Heat production, kJ/BW⁰.⁷⁵ | 430     | 441     | 416     | 13.30   | 0.371 |
| % IE      | 42.9    | 42.8    | 40.7    | 2.31    | 0.817 |
| Retained energy, kJ/BW⁰.⁷⁵ | 435     | 462     | 445     | 52.60   | 0.846 |
| % IE      | 40.4    | 39.8    | 42.7    | 2.89    | 0.783 |

C, control 0% benzoic acid; B05, 0.5% benzoic acid; B10, 1% benzoic acid; IE, energy intake; BW, body weight.

### Table 7. Excreta pH and ammonia emission from the slurries.

| Diet                        | C       | B05     | B10     | SEM     | P     |
|-----------------------------|---------|---------|---------|---------|-------|
| Initial faecal pH           | 7.42    | 7.34    | 7.01    | 0.134   | 0.130 |
| Initial urine pH            | 8.99    | 8.65    | 8.49    | 0.277   | 0.453 |
| Initial pH of the excreta    | 8.89    | 8.51    | 8.43    | 0.259   | 0.437 |
| After 14 days pH of the excreta | 8.17    | 7.97    | 7.97    | 0.129   | 0.466 |
| Initial total N of slurry, mmol/kg | 614    | 629     | 664     | 38.70   | 0.696 |
| Initial NH₃-N of slurry, mmol/kg | 265    | 247     | 265     | 43.50   | 0.935 |
| After 14 days total N of slurry, mmol/kg | 479    | 521     | 557     | 31.70   | 0.272 |
| After 14 days NH₃-N of slurry, mmol/kg | 276    | 300     | 329     | 20.60   | 0.264 |
| NH₃ emission*, mmol          | 419     | 348     | 343     | 39.70   | 0.362 |
| NH₃-N emission/initial total N of slurry, % | 35.2² | 28.1³  | 26.2³   | 1.92    | 0.049 |

C, control 0% benzoic acid; B05, 0.5% benzoic acid; B10, 1% benzoic acid; *total emission from 2 kg of fresh slurries during 14 days determination; ²values on the same row with different superscript differ significantly (P<0.05).

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