PAPER

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 (Knarreborg *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 (Knarreborg *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 O_2, CO_2, 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 urease 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.

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Materials and methods

Animals and diets

Six Landrace x Large White barrows were

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%, soybean meal 6.5%, 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 (VevoVitall®, DSM Nutritional Products, Basel, Switzerland) was added in substitution of equal amounts of maize meal. The vitamin/mineral supplement contained 15 g/kg Ca, 24 g/kg P, 14 g/kg Mg, 14 g/kg K, 12 g/kg S, 9 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 ($BW^{0.75}$) following the established protocol for the production of Parma and San Daniele Ham. The average $BW^{0.75}$ 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 (1965):

$$HP\ (kJ/d) = (16.175\ O_2) + (5.021\ CO_2) - (2.167\ CH_4) - (5.987\ N)$$

where O_2, CO_2 and 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 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 diets 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), stored at -20°C 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 Kjeltec, Foss Analytical, Hilleroed, Denmark) technique (method 984.13, AOAC, 1995). Ether extract was determined following the method 920.29 of the AOAC (1995). Phosphorus was determined by atomic absorption spectrophotometer (PYE-UNICAM 8600 UV/VS, Philips, Amsterdam, The Netherlands) (Method 965.17, AOAC, 1995). The gross energy of feeds, faeces and urine (freeze dried) was measured using an adiabatic bomb calorimeter (IKA 4000, Staufen, Germany). Retained carbon (g/d) was calculated as: retained energy (kJ/d) + retained N (g/d) x 19.40/51.83 (McLean and Tobin, 1987); retained energy was computed as metabolizable energy minus heat production and retained N as intake N minus N in faeces and in urine.

Protein and fat deposition (g/d) was calculated according to Brouwer (1965) as follows: protein deposition = retained N × 6.25; fat deposition = (retained C - retained N × 3.25) x 1.304, where N and C are expressed as g/d.

Ammonia emission

In the last 2 days of adaptation to metabolic cages faeces and urine were collected separately to determine ammonia emission. Urine was collected without the addition of sulphuric acid and stored at 4°C. Ammonia emission from slurries was measured as indicated by Derikx and Aarnink (1993). Briefly, 2 kg of fresh slurries was prepared maintaining the respective proportion of urine and faeces in the excreta, and placed in a 10 L bowl, 390 mm high and with a 210 mm diameter, covered by a lid connected to a tube system. Air entered the bowl through small holes at the edge of the lid and left the bowl from the centre. Ammonia was removed from the air by passing through 2 flasks, each containing 140 mL 0.5M HNO_3. The air left the system after passing a water trap, a flow controller (Model MR3000, Key Instruments div., Brooks Instrument, Hatfield, PA, USA) at the rate of 4.2 L/min, and a vacuum pump (KNF Italia s.r.l, mod. N 035_18, Milan, Italy). The first flask was replaced daily whereas the second was replaced after 7 days. The concentration of ammonia in the liquid was determined using an automatic analyser (2300 Kjeltec, 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;
μ = general mean;
S_i = square effect (i=1,2);
A_{ij} = effect of animal within each square (j=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 Nørgaand *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 for C, B05 and B10, respectively). The numerically higher N retention is consistent with the trend for a better growth performance observed by Bühler *et al*. (2006) using diets additioned with 1% benzoic acid in the grower and the finisher phase without any difference in N balance between control and benzoic acid treatment.

Concerning P balance, the effects of benzoic acid in pigs have been studied for long (Mroz *et al*. 1998, Partanen and Mroz, 1999), however uncertainties on its action remain. For example, the retention of P decreased in one study (Mroz *et al*., 1997), increased in another (Sauer *et al*., 2009), and was not different in a third study (Nørgaand *et al*., 2010). In our study no differences were observed between diets and the amounts retained were 40.7, 43.1 and 42.1% of P intake for C, B05 and B10, respectively (Table 5). These values, on average, are consistent with the value generally reported in literature (among others: Gutzwiller *et al*., 2011; Sauer *et al*., 2009) on P excretion in growing pigs: 50-60% of the ingested P, with faecal output representing more than 90% of the total P excretion.

Table 6 shows the utilization of energy of the three diets. No significant difference was observed between treatments, with an average energy retention 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
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	0.48	0.543
Crude protein, %	86.0	86.6	86.7	0.59	0.606
Ether extract, %	89.8	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 $BW^{0.75}$	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 $BW^{0.75}$	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 $BW^{0.75}$	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	19.3	2.91	0.517
	g/kg $BW^{0.75}$	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 Nørgaand *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 $BW^{0.75}$) whereas in the other study the pigs weight 50 kg and were fed 3.6% of BW corresponding to 9.6% of $BW^{0.75}$. 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 NH_3-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 (NH_3-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
	g/kg $BW^{0.75}$	0.23	0.24	0.21	0.013	0.335
Phosphorus in faeces,	g/d	4.50	4.50	4.00	0.24	0.286
	g/kg $BW^{0.75}$	0.12	0.12	0.11	0.006	0.196
	% IP	53.5	51.7	52.4	1.54	0.633
Phosphorus in urine,	g/d	0.46	0.41	0.42	0.035	0.565
	g/kg $BW^{0.75}$	0.012	0.011	0.011	0.001	0.612
	% IP	5.7	5.2	5.4	0.68	0.788
Phosphorus retained,	g/d	3.40	3.90	3.30	0.32	0.398
	g/kg $BW^{0.75}$	0.09	0.11	0.09	0.009	0.408
	% IP	40.7	43.1	42.1	1.67	0.522

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
	kJ/$BW^{0.75}$	1042	1087	1032	63.5	0.856
Digestible energy,	kJ/$BW^{0.75}$	892	932	890	58.8	0.754
	% IE	85.9	85.4	86.3	0.57	0.525
Metabolizable energy,	kJ/$BW^{0.75}$	866	903	861	58.60	0.781
	% IE	83.3	82.6	83.4	0.76	0.795
Heat production,	kJ/$BW^{0.75}$	430	441	416	13.30	0.371
	% IE	42.9	42.8	40.7	2.31	0.817
Retained energy,	kJ/$BW^{0.75}$	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	0.486
Initial total N of slurry, mmol/kg	614	629	664	38.70	0.696
Initial NH_3-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 NH_3-N of slurry, mmol/kg	276	300	329	20.60	0.264
NH_3 emission°, mmol	419	348	343	39.70	0.362
NH_3-N emission/initial total N of slurry, %	35.2[a]	28.1[b]	26.2[b]	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; [a,b]values on the same row with different superscript differ significantly (P<0.05).

Conclusions

The present study demonstrated that in individually housed heavy fattening pigs, ammonia nitrogen emission from the slurry, expressed as a proportion of the initial slurry nitrogen, was decreased by the inclusion of benzoic acid in the diet. Dietary benzoic acid did not influence N, P and energy balance.

References

AOAC, 1995. Official Methods of Analysis. 15th ed., Association of Official Analytical Chemists, Washington, DC, USA.

Biagi, G., Piva, A., 2007. In vitro effects of some organic acids on swine cecal microflora. Ital. J. Anim. Sci. 6:361-374.

Bridges, J.W., French, M.R., Smith, R.L., Williams, R.T., 1970. The fate of benzoic acid in various species. Biochem. J. 118:47-51.

Brouwer, E., 1965. Report of sub-committee on constants and factors. pp 441-443 in K.L. Blaxter (ed.) Proc. 3rd Symp. on Energy Metabolism. EAAP Publication 11, Academic Press, London, UK.

Bühler, K., Bucher, B., Wenk, C., 2010. Apparent nutrient and mineral digestibility in growing-finishing pigs fed phosphorus reduced diets supplemented with benzoic acid and phytase. Livest. Sci. 134:103-105.

Bühler, K., Wenk, C., Broz, J., Gebert, S., 2006. Influence of benzoic acid and dietary protein level on performance, nitrogen metabolism and urinary pH in growing-finishing

pigs. Arch. Anim. Nutr. 60:382-389.
Derikx, P.J.L., Aarnink, A.J.A., 1993. Reduction of ammonia emission from slurry by application of liquid top layers. pp 344-349 in Proc. 1st Int. Symp. Nitrogen Flow in Pig Production and Environmental Consequences. EAAP Publication 69, Wageningen, The Netherlands.
Galassi, G., Colombini, S., Malagutti, L., Rapetti, L., Crovetto, G.M., 2010. Effects of high fibre and low protein diets on performance, digestibility, nitrogen excretion and ammonia emission in the heavy pig. Anim. Feed Sci. Tech. 161:140-148.
Galassi, G., Crovetto, G.M., Rapetti, L., 2005. Trend of energy and nitrogen utilization of high fibre diets in pigs from 100 to 160 kg bodyweight. Ital. J. Anim. Sci. 4:149-157.
Gutzwiller, A., Hess, H.D., Adam, A., Guggisberg, D., Liesegang, A., Stoll, P., 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. Tech. 168:113-121.
Halas, D., Hansen, C.F., Hampson, D.J., Mullan, B.P., Kim, J.C., Wilson, R.H., Pluske, J.R., 2010. Dietary supplementation with benzoic acid improves apparent ileal digestibility of total nitrogen and increases villous height and caecal microbial diversity in weaner pigs. Anim. Feed Sci. Tech. 160:137-147.
Jongbloed, A.W., Mroz, Z., van der Weij-Jongbloed, R., Kemme, P.A., 2000. The effects of microbial phytase, organic acids and their interaction in diets for growing pigs. Livest. Prod. Sci. 67:113-122.
Kluge, H., Broz, J., Eder, K., 2006. Effect of benzoic acid on growth performance, nutrient digestibility, nitrogen balance, gastrointestinal microflora and parameters of microbial metabolism of piglets. J. Anim. Physiol. An. N. 90:316-324.
Kluge, H., Broz, J., Eder, K., 2010. Effects of dietary benzoic acid on urinary pH and nutrient digestibility in lactating sows. Livest. Sci. 134:119-121.
Knarreborg, A., Miquel, N., Granli, T., Jensen, B.B., 2002. Establishment and application of an in vitro methodology to study the effects of organic acids on coliform and lactic acid bacteria in the proximal part of the gastrointestinal tract of piglets. Anim. Feed Sci. Tech. 99:131-140.
Kristensen, N.B., Nørgaard, J.V., Wamberg, S., Engbæk, M., Fernández, J.A., Zacho, H.D., Poulsen, H.D., 2009. Absorption and metabolism of benzoic acid in growing pigs. J. Anim. Sci. 87:2815-2822.
McLean, J.A., Tobin, G., 1987. Animal and human calorimetry. Cambridge University Press, Cambridge, UK.
Mertens, D.R., 2002. Gravimetric determination of amylase-treated neutral detergent fiber in feeds using refluxing in beakers or crucibles: collaborative study. J. AOAC Int. 85:1217-1240.
Mroz, Z., Jongbloed, A.W., Partanen, K., Diepen, J.Th.M., van Vreman, K., Kogut, J., 1998. Ileal digestibility of amino acids in pig diets of different buffering capacity and with supplementary organic acids. J. Anim. Feed Sci. 7:191-197.
Mroz, Z., Jongbloed, A.W., Vreman, K., Canh, T.T., van Diepen, J.T.M., Kemme, P.A., Kogu,t J., Aarnink, A.J.A., 1996. The effect of different dietary cation-anion supply on excreta composition and nutrient balance in growing pigs. Report No. 96.028, ID-DLO Publ., Lelystad, The Netherlands.
Mroz, Z., Krasucki, W., Grela, E., 1997. Prevention of bacteriuria and ammonia emission by adding sodium benzoate to diets for pregnant sows. Page 294 in Proc. 6th Biennial APSA Conf., Canberra, Australia.
Murphy, D.P., O'Doherty, J.V., Boland, T.M., O'Shea, C.J., Callan, J.J., Pierce, K.M., Lynch, M.B., 2011. The effect of benzoic acid concentration on nitrogen metabolism, manure ammonia and odour emissions in finishing pigs. Anim. Feed Sci. Tech. 163:194-199.
Nørgaand, J.V., Fernandez, J.A., Sørensen, K.U., Wamberg, S., Poulsen, H.D., Kristensen, N.B., 2010. Effect of benzoic acid supplementation on acid-base and mineral metabolism in catheterized growing pigs. Livest. Sci. 134:116-118.
Partanen, K.H., Mroz, Z., 1999. Organic acids for performance enhancement in pig diets. Nutr. Res. Rev. 12:117-145.
SAS, 2000. User's guide: statistics, Version 8.01, edition 2000. SAS Inst. Inc., Cary, NC, USA.
Sauer, W., Cervantes, M., Yanez, J., Araiza, B., Murdoch, G., Morales, A., Zijlstra, R.T., 2009. Effect of dietary inclusion of benzoic acid on mineral balance in growing pigs. Livest. Sci. 122:162-168.
Simons, P.C.M., Versteegh, H.A.J., Jongbloed, A.W., Kemme, P.A., Slump, P., Bos, K.D., Wolters, M.G.E., Beudeker, R., Verfumaric, G.J., 1990. Improvement of phosphorus availability by microbial phytase in broilers and pigs. Brit. J. Nutr. 64:525-540.
Torrallardona, D., Badiola, J.I., Broz, J., 2007. Effects of benzoic acid on performance and ecology of gastrointestinal microbiota in weanling piglets. Livest. Sci. 108:210-213.
Van Soest, P.J., Robertson, J.B., Lewis, B.A., 1991. Methods of dietary fiber, neutral detergent fiber and non-polysaccharides in relation to animal nutrition. J. Dairy Sci. 74:3583-3597.