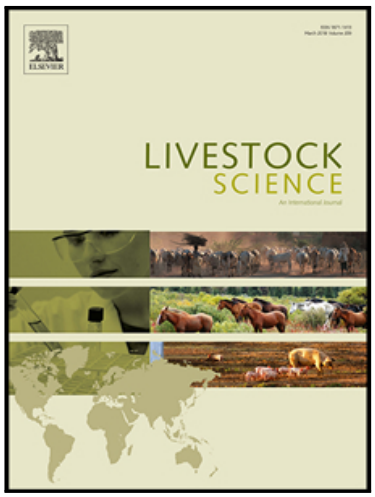

Effect of dietary inclusion of benzoic acid (VevoVitall®) on the microbial quality of liquid feed and the growth and carcass quality of grow-finisher pigs

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Highlights

- Benzoic acid limited lactic acid bacteria growth in liquid feed for finisher pigs
- The pH of liquid feed was stabilised with benzoic acid inclusion
- Benzoic acid limited spontaneous fermentation in liquid feed for finisher pigs
- Benzoic acid supplementation did not impact grow-finisher pig growth
- Supplementation of benzoic acid to grow-finisher pigs did not impact carcass traits

Effect of dietary inclusion of benzoic acid (VevoVitall®) on the microbial quality of liquid feed and the growth and carcass quality of grow-finisher pigs

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Abstract

Benzoic acid has long been used as a food preservative due to its antibacterial and antifungal effects. Supplementation to pig diets has also been shown to inhibit microbial free amino acid degradation and to control yeast growth in fermented liquid feed. However, the effect of dietary inclusion of benzoic acid (BA) in fresh liquid feed for grow-finisher pigs on feed quality and the resultant effects on pig growth remain unclear. The objective of the current study was to compare four inclusion levels of BA (VevoVitall®) on feed microbial quality and on the growth performance of grow-finisher pigs. Two-hundred and sixteen pigs with a starting weight of 30.0kg (± 7.43 SD) were used in the experiment. The four dietary treatments were as follows: (1) Basal diet + 0kg/t BA (0kg/t BA), (2) Basal diet + 2.5kg/t BA (2.5kg/t BA), (3) Basal diet + 5kg/t BA (5kg/t BA), (4) Basal diet + 10kg/t BA (10kg/t BA). Lactic acid bacteria (LAB) counts in the mixing tank were similar across treatments ($P>0.05$) but were lower in the troughs for the feed supplemented with 10kg/t BA than for all other treatments ($P<0.01$). The pH of the 10kg/t BA treatment was also lower than that of the other three treatments. However, this only occurred in the mixing tank ($P<0.01$), as in the trough, the basal diet had the lowest pH (lower than the other three treatments; $P<0.01$). Dietary BA inclusion did not affect average daily gain, average daily feed intake, feed conversion efficiency, final live-weight, carcass weight or carcass quality during the experimental period ($P>0.05$). In conclusion, while BA may limit the growth of LAB in liquid feed and stabilise feed pH, its inclusion in the diet did not improve the growth performance or carcass quality of grow-finisher pigs.

Key words: Dietary acidification; fattener; swine; wet feed

Introduction

Benzoic acid (BA) has been authorised as a feed additive for grow-finisher pigs at inclusion levels of 0.5% - 1% in the diet and is included in the functional group of 'other zootechnical additives' (EU regulation No. 1138/2007/EC; EFSA, 2007). The metabolic end product of BA is hippuric acid which can decrease urinary pH, so one of the main reasons for using BA is to reduce ammonia emissions from manure. Benzoic acid is a monocarboxylic acid which is used as an antibacterial and antifungal chemical preservative in the food industry (E-number: E210) (Mao et al., 2019). It has also been shown to reduce the loss of free amino acids (AA) in fermented liquid feed, which occurs via microbial degradation (Vils et al., 2018). This is presumably by inhibition of microbial growth, as the same study also showed an inhibition of yeast growth and a reduction in the amount of lactic acid produced in the benzoic-acid supplemented feed.

Improved feed conversion efficiency (FCE) has also been reported with dietary BA supplementation in grow-finisher pigs (Den Brok, 1999; Van der Peet-Schwering et al., 1999; Øverland et al., 2008) and improved growth rates have been found in weaner pigs (Partanen and Mroz, 1999; Kluge et al., 2006; Guggenbuhl et al., 2007; Torrallardona et al., 2007; Halas et al., 2010; Diao et al., 2016). This enhanced growth performance is most likely due to the antibacterial activity of BA in the pig gut, particularly against coliforms (Knarreborg et al., 2002; Kluge et al., 2006; Øverland et al., 2008; Papatsiros et al., 2011). However, previous work has shown no significant effect of benzoic acid supplementation on carcass quality parameters when supplemented to diets alone (Lenis et al., 1998) oras part of an acid blend (Den Brok, 1999).

The objective of the current study was to compare the effect of four dietary inclusion levels of BA (0kg/t, 2.5kg/t, 5kg/t and 10kg/t) on the microbial quality of liquid feed and on the growth of grow-finisher pigs. It was hypothesised that BA would have an antimicrobial

effect in liquid feed, thereby limiting spontaneous fermentation and improving feed microbial quality. Furthermore, it was hypothesised that increasing dietary BA inclusion would improve growth and feed efficiency in liquid fed grow-finisher pigs.

Materials and methods

Ethical approval

Ethical approval for this study was granted by the Teagasc Animal Ethics Committee (approval no. TAEC 107/2015). The experiment was conducted in accordance with Irish legislation (SI no. 543/2012) and the EU Directive 2010/63/EU for animal experimentation.

Experimental design and animal management

The experiment used 216 Danavil Duroc x (Landrace x Large White) female and entire male pigs with an initial live-weight (LW) of 30.0kg ± 7.43 SD. Pigs were penned in groups of 6 pigs with a total of 9 pen replicates/treatment. Pen groups were given a 7-day adaptation period to liquid feeding prior to the start of the experiment, during which all were fed a control diet (0 kg/t BA). Pen groups were blocked by sex and weight, with both sexes represented in each block, following which pens were randomly assigned to one of four dietary treatments, as follows; (1) Basal diet, 0kg/tonne VevoVitall® (0kg/t BA); (2) Basal diet + 2.5kg/tonne VevoVitall® (2.5kg/t BA); (3) Basal diet + 5kg/tonne VevoVitall® (5kg/t BA) ; and (4) Basal diet + 10kg/tonne VevoVitall® (10kg/t BA).

All pigs were assigned to dietary treatments on the same day of the experiment (day 0). The heaviest two blocks of pigs were on trial for 56 days and slaughtered on day 57 (108.1kg ± 5.39 SD), while the lighter pigs were on trial for 76 days and slaughtered on days 77 and 78 (118.1kg ± 8.95 SD). Pigs were slaughtered at a mean LW of 115.kg ± 9.2 SD.

Pen groups were housed in pens (2.37m × 2.36m) with concrete slatted floors and solid PVC partitions. Each pen group had access to a water bowl (DRIK-O-MAT, Egebjerg International A/.S, Egebjerg, Denmark) as per regulation Council Directive 2008/120/EC (2008). Air temperature was maintained at 20 to 22°C and was recorded daily. The room was mechanically ventilated with exhaust fans and air inlets controlled by a Steinen PCS

8100 controller (Steinen BV, Nederwert, The Netherlands). Pigs were observed closely twice daily and any pig showing signs of ill-health were treated appropriately. All veterinary treatments were recorded, including identity of pig, symptom, medication and dosage administered.

Each pen was equipped with one solenoid valve above a short liquid feeding trough fitted with an electronic sensor. The electronic sensors were checked, 4 times per day, increasing to 6times per day, after 4 weeks, and additional feed was dispensed into troughs where the residual feed in the trough was below the level of the sensor. Feeding was according to a feeding curve to provide *ad-libitum* access to feed. Feed level in the trough was manually inspected daily before and after feeding and feed allocation per pen increased or decreased accordingly. The short stainless-steel troughs (100 cm × 32.5 cm × 21cm) were located on top of a rubber mat (1.5 × 1 m) which helped to minimise liquid feed wastage.

Diet preparation and feeding

A common diet based on wheat, barley and soybean meal formulated to 9.8 MJ net energy/kg and 9.97g/kg standardised ileal digestible lysine was used. All other AA were supplied relative to lysine according to the ideal protein concept (NRC, 2012). A commercially available BA product (VevoVitall®, DSM Nutritional Products, Basel, Switzerland) was included in the diet at 0 kg/t, 2.5kg/t, 5kg/t and 10kg/t, for treatments 1, 2, 3 and 4 respectively, and directly replaced wheat in the diet. The diets were manufactured in meal form at the Teagasc feed mill (Moorepark, Fermoy, Co. Cork). Ingredient and chemical composition of the diet is shown in Table 1.

The dietary treatments were prepared and liquid-fed using an automatic sensor feeding system (HydroMix, BigDutchman, Vechta, Germany). Diets were prepared in a mixing tank with a 6 pale agitator and agitated for ~5 minutes prior to feed-out. The high-pressure air system delivered liquid feed from the mixing tanks to troughs, each of which was fitted with

a solenoid valve and an electronic feed sensor. If feed was above the sensor in a trough, feed was not dispensed to that particular trough. If feed was below the level of the sensor, feed was dispensed to the trough and sensors were checked automatically before each scheduled feeding. A 12.5 litre rinse of the mixing tanks was carried out after feeding each treatment to prevent contamination from one mix to the next. The water-to-feed ratio used to prepare the liquid feed was 2:1 on a fresh matter basis (FM) or 2.4:1 on a dry matter (DM) basis.

Titrations

Titrations were carried out in order to determine the quantity of BA required to reduce the pH of the diet to 4 as described by Lawlor et al. (2005). Four samples of the basal diet were titrated in duplicate prior to the start of the experiment to determine the amount of acid required to bring the diet to pH 4. Briefly, a 0.5g sample of the diet was added to 50ml deionised water and continuously stirred using a magnetic stirrer. Hydrochloric acid (HCl, 0.1N) was added in 0.2ml increments every 3 minutes and the pH recorded (Mettler Toledo pH meter, Greisensee, Switzerland) prior to the addition of each increment. Four replicates of the VevoVitall® product were also titrated against sodium hydroxide (NaOH, 0.1N) in 1ml increments every 3 minutes to assess how much base would be required to raise the pH to 4. A Pearson square calculation was used to determine the proportions of feed and acid that would produce a diet of pH 4.

Records and sampling

All pigs were weighed on Day 0 and prior to slaughter at the end of the experiment (i.e. day 56 or day 76). Feed disappearance for each pen was recorded daily and average daily gain (ADG), average daily feed intake (ADFI) and FCE were calculated for the entire experiment.

The pH and temperature of liquid feed from each treatment from the mixing tank was recorded using a pH meter (Mettler Toledo) 3 times/week throughout the experiment. To do so, three ~100ml aliquots were removed from the mixing tank during agitation prior to feed-

out and the pH and temperature recorded immediately. The pH and temperature of liquid feed from all 36 troughs was recorded once/week during the experiment, provided feed was available.

Liquid feed samples (~50g) were collected on days 1, 42 and 70 into sterile containers from the mixing tank and 2 troughs/treatment and transported to the laboratory on ice for same-day microbiological analysis. Liquid feed samples for ethanol analysis were collected on day 42 and day 70 from the mixing tank and from 2 troughs/treatment and stored in ~20g aliquots at -20°C until analysis. Dry samples of each diet from each batch of feed produced in the feed mill were pooled into one diet sample per treatment for chemical analysis. Liquid feed samples (~250g) were also collected from the mixing tank (1/treatment) and troughs (2/treatment) on day 42 and day 70 and stored at -20°C for proximate analysis and AA determination.

During exsanguination at the slaughter house, blood samples were collected from 36 pigs (9 pigs/treatment) using Vacuette tubes (Labstock, Dublin, Ireland) for haematological analysis.

Slaughter

Pigs were fasted for ~12 hours prior to slaughter by CO_2 stunning followed by exsanguination in a commercial slaughterhouse. The following measurements were taken: hot carcass weight was recorded 45 minutes after stunning, and back-fat thickness and muscle depth measured at 6cm from the edge of the split back at the level of the 3rd and 4th last rib were determined using a Hennessy Grading Probe (Hennessy and Chong, Auckland, New Zealand). Lean meat content was estimated according to the following formula: Estimated lean meat content (%) = 60.3 – 0.847x + 0.147y where x = fat depth (mm); y = muscle depth (mm) (Department of Agriculture Food and Rural Development, 2001). Cold carcass weight was calculated as hot carcass weight (45 minutes after stunning) × 0.98. Kill-out percentage was calculated from final LW prior to slaughter and cold carcass weight. To calculate

carcass ADG, a kill-out percentage of 65% was applied to LW at the beginning of the experiment and the following equation used: ((carcass weight in kg – LW on day 0 × 0.65) × 1000) / number of days on treatment (Lawlor and Lynch, 2005).

Microbiological analysis of liquid feed

Approximately 10g of each liquid feed sample was homogenized in a stomacher as a 10-fold dilution in maximum recovery diluent (MRD; Oxoid, Basingstoke, UK) and a 10-fold dilution series was performed in MRD. Relevant dilutions were plated in duplicate as follows; (1) pour-plated on de Man Rogosa & Sharpe, (MRS; Merck, Damstadt, Germany) agar, containing 50 U/mL nystatin (Sigma-Aldrich, Arklow, Co. Wicklow, Ireland), overlaid and incubated at 30°C for 72 hours for enumeration of lactic acid bacteria (LAB); (2) pour-plated on violet red bile glucose (VRBG; Oxoid) agar, overlaid and incubated at 37°C for 24 hours for *Enterobacteriaceae;* (3) pour-plated on ChromoCult tryptone bile X-glucuronide (CTBX; Merck) agar and incubated at 44°C for 24 hours for *E. coli*; and (4) spread-plated on yeast glucose chloramphenicol (YGC; Merck) agar and incubated at 25°C for 5 days for yeasts and moulds. Colonies were counted and the counts averaged and presented as $\log_{10}$CFU/g of the original sample.

Feed analysis

The four diets used in the experiment were ground through a 2mm Christy Norris mill and analysed for DM, ash, gross energy (GE) neutral detergent fibre (NDF) ether extract (EE), nitrogen (N) and AA concentration. The DM (AOAC.934.01), ash (AOAC.942.05), and EE concentration (AOAC.920.39) were determined according to methods of the Association of Official Analytical Chemists (AOAC, 2005). Gross energy was determined using an adiabatic bomb calorimeter (Parr Instruments, Moline, IL USA). The neutral detergent fibre (NDF) content was determined according to the method of Van Soest et al. (1991) using the Ankom 220 Fibre Analyser (Ankom Technology, Macedon, New York, USA). The N

content was determined using the LECO FP 528 instrument (Leco Instruments, UK Ltd., Cheshire, UK) (AOAC.990.0). Crude protein (CP) was determined as N × 6.25. Amino acid determination was carried out using cation exchange HPLC as previously described by McDermott et al. (2016) (AOAC 994.12).

Liquid feed samples collected from the mixing tank and troughs on day 42 and day 70 were oven-dried at 55°C for 72 hours and milled through a 2mm screen using a Christy Norris mill. These samples were pooled prior to analysis to give one mixing tank and one trough sample per treatment which were analysed for GE, N, CP, ash and AA as above.

Preparation of liquid feed samples for ethanol analysis was carried out as described by van Winsen et al. (2000). Briefly, feed aliquots were defrosted prior to centrifugation at 2000g for 10 minutes at 4°C. The supernatant was then centrifuged at 18 500g for 10 minutes. The resulting supernatant was filtered through a 0.2 µm filter and stored at -20°C until analysis. Samples were thawed slowly at room temperature prior to ethanol analysis by gas chromatography (Agilent 6890; Agilent Technologies, Waghaeusel-Wiesental, Germany) using a flame ionization detector. A 1 µL volume of each sample was injected by split injection 5:1 onto the column (AT-100 15 m × 0.53 mm i.d. × 1.2 micron) with a column flow rate of 3.4ml/min helium. The temperature programme was 40°C for 3 minutes, ramped at 10°C/min to 180°C and held at 180°C for 3 minutes.

Haematological analysis of blood samples

Blood samples for haematology were analysed on the day of slaughter using an Abbot Cell-Dyn 3700 analyser (GMI-Inc., Minnesota, USA). The following parameters were measured; white blood cells, neutrophil number and percentage, lymphocyte number and percentage, eosinophil number and percentage, monocyte number and percentage, basophil number and percentage, red blood cells, haemoglobin, packed cell volume, mean corpuscular volume, mean corpuscular haemoglobin and platelets.

Credit author statement

F.M. O ¶ 0 H D1 UD Investigation, formal analysis, writing - original draft

G.E. Gardiner: Supervision, writing - review & editing, methodology, validation

- 9 2 ¶ ' R:KHUW\ Supervision, writing – review & editing

P.G. Lawlor: Project administration, funding acquisition, methodology, writing - review & editing

Declarations of interest: none