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Effect of feeding sodium butyrate in the late finishing period on *Salmonella* carriage, seroprevalence, and growth of finishing pigs

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ABSTRACT

Pork is an important source of human salmonellosis and low-cost on-farm control measures may provide a useful element in reducing the prevalence of this pathogen in food. This study investigated the effectiveness of dietary supplementation with sodium butyrate administered to finisher pigs for ~4-weeks prior to slaughter to control *Salmonella* shedding on highly contaminated farms.

Two trials (A and B) were conducted on two commercial pig farms, which had a history of high *Salmonella* seroprevalence. In both trials, pens (14 pens of 12 pigs/pen in Trial A and 12 pens of 12–17 pigs/pen in Trial B) were randomly assigned to a control (finisher feed without additive) or a treatment group (the same feed with 3 kg sodium butyrate/t) for 24–28 days, depending on the trial. Faeces were collected from each pig on days 0, 12 and 24/28, and blood, caecal digesta and ileocaecal/mesenteric lymph nodes were collected from the slaughterhouse. Pigs were weighed at the start and end of the trials, feed intake was recorded, and carcass quality parameters were recorded at slaughter.

In Trial A, *Salmonella* shedding was reduced in the treatment compared to the control group at the end of the trial (30% versus 57% probability of detecting *Salmonella* in faeces, respectively; $p < 0.001$). This reflected the serology results, with detection of a lower seroprevalence in the treatment compared to the control group using the 20% optical density cut-off (69.5% versus 89%; $p = 0.001$). However, no effect on faecal shedding or seroprevalance was observed in Trial B, which may be explained by the detection of a concomitant infection with *Lawsonia intracellularis*. No significant differences in *Salmonella* recovery rates were observed in the caecal digesta or lymph nodes in either trial. Furthermore, feed intake, weight gain, and feed conversion efficiency (FCE) did not differ between groups ($p > 0.05$) in either trial. Numerical improvements in weight gain and FCE were found with sodium butyrate treatment, which gave a cost benefit of €0.04/kg of live-weight gain.

Overall, results suggest that strategic feeding of sodium butyrate, at 3 kg/t of feed, to finishing pigs for 24–28 days prior to slaughter was effective in reducing *Salmonella* shedding and seroprevalance but perhaps only in the absence of co-infection with other pathogens. However, sodium butyrate supplementation at this rate did not influence intestinal carriage, nor did it reduce seroprevalence to below the cut-off used for the high *Salmonella* risk category in Ireland (50%), or significantly improve growth performance.

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1. Introduction

Asymptomatic intestinal carriage of *Salmonella* in pigs presented for slaughter can result in pork carcass contamination. An EU baseline survey conducted in 2006–2007, showed that Ireland had a high prevalence of *Salmonella* contamination on pork

Abbreviations: NPSC, national pig *Salmonella* control program; ILN, ileocaecal lymph nodes; MLN, mesenteric lymph nodes; ADFI, average daily feed intake; ADG, average daily gain; FCE, feed conversion efficiency.

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carcasses (20%) (EFSA, 2008). This can be linked to the relatively high prevalence of *Salmonella* in some Irish pig herds (McCarthy et al., 2013; Burns et al., 2015). In an attempt to reduce this prevalence, the National Pig *Salmonella* Control Program (NPSCP) was updated in 2010 (Department of Agriculture Food and the Marine (DAFM), 2010). Despite this, *Salmonella* herd prevalence has not declined (DAFM personal communication). This highlights a need to find low-cost control measures to reduce *Salmonella* shedding in pigs at primary production, especially finishing pigs (35–100 kg), as carriage rates are high during this stage of production (Burns et al., 2015) and finishers are a significant source of *Salmonella* in the abattoir (Duggan et al., 2010; Argüello et al., 2013a).

Dietary supplementation with organic acids or their salts is a potential strategy for the control of *Salmonella* in finishing pigs (Creus et al., 2007; Wales et al., 2010). Organic acids can decrease gastrointestinal pH, thus creating an environment, which is hostile to *Salmonella* while favouring the growth of beneficial bacteria such as lactobacilli. The un-dissociated form of various acids can also freely cross the bacterial cell membrane and enter the bacterial cell, causing cell death (Van Immerseel et al., 2006). In addition, some organic acids (e.g., butyric acid and propionic acid) also down regulate the expression of invasion genes (e.g., *hil*A) in *Salmonella*, thereby suppressing its ability to invade intestinal epithelial cells (Boyen et al., 2008).

Dietary supplementation with sodium butyrate has previously been shown to reduce *Salmonella* shedding and intestinal colonization in weaner pigs which were deliberately infected with *Salmonella* (Boyen et al., 2008). However, to our knowledge, no field trial has evaluated the effectiveness of sodium butyrate as a *Salmonella* control measure in finishing pigs on farms with historically high levels of the pathogen. In addition, despite the number of field trials that have evaluated organic acids for the control of *Salmonella* in pigs, few have investigated their use for a short targeted period prior to slaughter and the cost-benefit associated with their use (Gálfi and Bokori, 1990; Creus et al., 2007). Therefore, the objectives of the present study were to conduct a field study on two selected farms with a high *Salmonella* seroprevalence, to investigate the ability of dietary supplementation with sodium butyrate during the last month of growth pre-slaughter to: (1) reduce faecal shedding and intestinal carriage of *Salmonella*, and (2) impact growth performance in finisher pigs. Based on the findings, a cost-benefit analysis was also conducted.

2. Materials and methods

2.1. Animal ethics and experimental licensing

Two separate feeding trials (Trial A and Trial B) were performed on two commercial pig farms in the last quarter of 2014 and the first quarter of 2015. Ethical approval was obtained from the Waterford Institute of Technology ethics committee and an experimental license was obtained from the Irish Department of Health and Children (number B100/2982). All animals were handled in a humane manner and were slaughtered in a regulated abattoir.

2.2. Experimental procedure

2.2.1. Trial A farm

Trial A was conducted on a 90 sow farrow-to-finish farm. The finisher house in which the trial was conducted consisted of a barn with 14 pens. A total of 169 finisher pigs were used (72 males and 97 females; 12 pigs per pen). Each pig was ear tagged with a unique number for identification purposes. Pigs were housed in pens (each pen was 4.5 m × 2.8 m) with concrete slatted floors and provided with ad-libitum access to water from 2 nipple drinkers per pen. The temperature of the barn was maintained at ~20 °C. Ad-libitum access was provided to dry pelleted feed via single-spaced wet-dry feeders.

This herd had a historically high *Salmonella* seroprevalence (data extracted from the NPSCP); however, the prevalence of the batch of finishing pigs immediately prior to this trial declined to 0%. As a result, pens in the finishing house were artificially contaminated with a monophasic *Salmonella* Typhimurium (*S.* 4,[5],12:i:-) strain with an antimicrobial resistance (AMR) pattern of ASSuT, which had previously been isolated from sows in the same herd. Briefly, a single colony of *S.* 4,[5],12:i:- was inoculated into 90 mL of Tryptone Soya Broth (TSB, Oxoid, Basingstoke, UK), incubated overnight at 37 °C and then diluted in Phosphate Buffered Saline (PBS, Oxoid) to a final concentration of $\sim 5 \times 10^3$ CFU/mL. Five 25 mL vials per pen (each containing $\sim 10^3$ CFU/mL of *Salmonella*) were spread at five points: 3 in the defecation area, and 2 near the feeder. The final concentration of *Salmonella* at each inoculation point was 2.5×10^4 CFU. Contamination of the pens was performed 7 days before commencing the trial.

2.2.2. Trial B farm

Trial B was conducted on a 180 sow farrow-to-finish farm. The finisher house in which the trial was conducted consisted of a 2-room barn, each with 6 pens per room. A total of 177 finisher pigs were used (86 males and 91 females; 12–17 pigs per pen). Each pig was ear tagged with a unique number for identification purposes. Pigs were housed in pens (each pen was 3.2 m × 3.4 m) with concrete slatted floors and provided with ad-libitum access to water from 2 nipple drinkers per pen. The temperature of each room was maintained at ~20 °C. Ad-libitum access was provided to dry pelleted feed via single-spaced wet-dry feeders.

This farm had a historically high *Salmonella* seroprevalence (i.e. > 50% for 2014), and faecal shedding of *Salmonella* Typhimurium had been confirmed bacteriologically prior to commencement of the trial.

2.2.3. Treatment groups

Approximately 4 weeks before the target slaughter date, pigs in both trials A and B were blocked by sex and weight and randomly assigned to one of two diet groups: a standard finisher feed with no feed additive (control group) or the same finisher feed supplemented with 3 kg per tonne sodium butyrate (Adimix®, Nutriad, Kasterlee, Belgium; treatment group). The composition of the trial diets is shown in Table S-1 in the Supplementary material. In Trial A, the pigs were fed the experimental diets for 28 days and in Trial B, for 24 days. Pigs in both trials were fasted for ~18 h prior to slaughter.

2.2.4. Blood and faecal sampling and measurement of production parameters

For serological analysis, blood was collected during two occasions: (1) by jugular venipuncture, prior to feeding the experimental diets, and (2) during exsanguination at slaughter. All samples were collected using plastic tubes for whole blood (BD Vacutainer, Becton Dickinson, Oxford, UK). Serum was obtained after coagulation and centrifugation of the tubes (1500 rpm for 10 min) and stored at −20 °C until analysis.

On day 0 (the day prior to commencing experimental treatments), day 12 and either day 28 (Trial A) or day 24 (Trial B) (i.e., the final treatment day), faeces (~25 g) was collected into 100 mL sterile bottles (Sarstedt, Nümbrecht, Germany) from each pig by digital rectal stimulation. All samples were collected and handled aseptically to avoid cross-contamination.

Feed intake was recorded throughout each trial and individual body weights were recorded on day 0 and day 28 (Trial A) or day 24 (Trial B). These weights were used to calculate average daily feed

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