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Effect of strategic administration of an encapsulated blend of formic acid, citric acid, and essential oils on *Salmonella* carriage, seroprevalence, and growth of finishing pigs

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ABSTRACT

Controlling *Salmonella* at farm level can act as the first line of defence in reducing salmonellosis from pork. This study investigated the efficacy of an encapsulated blend of formic acid, citric acid, and essential oils (FormaXOL™) administered to finisher pigs for 28 days prior to slaughter in controlling *Salmonella* shedding on a commercial farm with a history of high *Salmonella* seroprevalence.

Fourteen pens of 8–10 pigs/pen were randomly assigned to a control (finisher diet without additive) or a treatment group (the same diet with 4 kg/t of FormaXOL™) for 28 days. Faeces were collected from each pig on days 0, 14, and 28, while on day 29 blood, caecal digesta and ileocaecal-mesenteric lymph nodes were collected at slaughter. Pigs were weighed at the start and end of the trial, feed intake was recorded, and carcass quality parameters were recorded at slaughter.

On day 14, *Salmonella* shedding was reduced in the treatment compared to the control group (27.9% versus 51.7% probability of detecting *Salmonella* in faeces, respectively; $p = 0.001$). However, on day 28, no reduction was observed (20.6% versus 35.9% probability of detecting *Salmonella* in faeces, respectively; $p = 0.07$). Interestingly, *Salmonella* shedding rates in the treated pigs remained stable throughout the trial compared to the control group. This suggests that the feed additive prevented additional pigs from acquiring the *Salmonella* infection. A lower *Salmonella* seroprevalence was detected at slaughter in the treatment compared to the control group using the 40% optical density cut-off (64.5% versus 88.5%, respectively; $p = 0.01$). However, no significant differences in *Salmonella* recovery rates were observed in the caecal digesta or lymph nodes between treated and control groups. Treated pigs had a lower feed intake than pigs fed the control diet ($p = 0.001$); however, average daily gain and feed conversion efficiency were not affected by treatment ($p = 0.45$ and 0.55, respectively). Consequently, supplementing the diet with FormaXOL™ for 28 days increased the feed cost per kg of live-weight gain by €0.08.

Overall, results suggest that strategic administration of an encapsulated blend of formic acid, citric acid, and essential oils, to finishing pigs for 28 days prior to slaughter has potential to prevent increased *Salmonella* shedding at certain time points as well as seroprevalence. However, this additive did not lower intestinal carriage, nor did it reduce seroprevalence to below the cut-off used for the high *Salmonella* risk category in Ireland (50%) or improve growth performance.

Abbreviations: NPSCP, 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; SCFA, short chain fatty acid; MCFA, medium chain fatty acid; ATP, adenosine triphosphate; AMR, antimicrobial resistance; TSB, tryptone soya broth; PBS, phosphate buffered saline; GIT, gastrointestinal tract; BPW, buffered peptone water; MSRV, modified semi-solid rappaport-vassiliadis; XLD, xylose lysine deoxycholate; BG, brilliant green; PCA, plate count agar; ELISA, enzyme-linked immunosorbent assay; SAS, statistical analyses system; OD, optical density; CI, confidence interval.

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1. Introduction

Globally, *Salmonella* is one of the most common causes of foodborne disease in humans and pork is considered an important source of human salmonellosis (EFSA, 2008). In the latest summary report on trends and sources of foodborne outbreaks within the European Union (EU), 225 foodborne outbreaks were linked to *Salmonella* (EFSA, 2015). Of these, 9.3% were linked to the consumption of pork, the third most commonly reported food vehicle after eggs and egg products and bakery products. The non-typhoidal *Salmonella* serotypes that cause human infection are usually carried asymptomatically in pigs, causing little or no clinical signs of disease (Callaway et al., 2008). As such, pigs become reservoirs for *Salmonella* contamination along the production chain (Rodriguez et al., 2006; Ojha and Kostrzynska, 2007; Dorr et al., 2009; Duggan et al., 2010). The most recent EU survey in slaughter pigs showed that *Salmonella* prevalence in intestinal lymph node samples was 10.3% and that 8.3% of carcasses were contaminated, indicating the extent of the problem (EFSA, 2008).

Controlling the introduction, persistence, and transmission of *Salmonella* at farm level is therefore often the first line of defence in reducing human salmonellosis. Various control measures have been investigated in pigs to date, including dietary supplementation with organic acid feed additives (Berge and Wierup, 2012; De Busser et al., 2013; Walia et al., 2016). Generally, these organic acids are short- and medium-chain fatty acids (SCFA, MCFA), which, when used in an un-dissociated form ultimately disrupt vital metabolic processes within the bacterial cell, leading to cell death (Van Immerseel et al., 2006). Essential oils have also been shown to exhibit anti-*Salmonella* activity, mainly acting via membrane disruption, non-specific permeabilization of cell membranes, leakage of adenosine triphosphate (ATP) and potassium/hydrogen ions, inhibition of ATPase activity, and an increase in the fluidity of phospholipid bilayers (Burt, 2004; Oussalah et al., 2007; Bakkali et al., 2008; Barbosa et al., 2009; Berge and Wierup, 2012; Hyldgaard et al., 2012; Langeveld et al., 2014).

However, to our knowledge, only three in vivo studies to date have investigated essential oils as a dietary strategy for *Salmonella* reduction in pigs (Ahmed et al., 2013; Michiels et al., 2012; Rasschaert et al., 2016). Furthermore, despite the number of field studies that have evaluated in-feed organic acids for the control of *Salmonella* in pigs, only two of the studies above evaluated an essential oil in combination with organic acids and only one was conducted in finishers. Moreover, none of these studies performed a cost-benefit analysis. Additionally, no field trial to our knowledge, has evaluated the efficacy of an encapsulated blend of formic acid, citric acid, and essential oils as a dietary additive for *Salmonella* control in finishing pigs. Previous studies showed success in reducing *Salmonella* in finishing pigs when supplemented with various organic acid feed additives, i.e., potassium diformate, lactic-formic acid, formic-propionic acid for a minimum of 7 weeks (Creus et al., 2007; Visscher et al., 2009; Argüello et al., 2013a). Yet, few have evaluated a shorter duration of feeding (i.e., <30 days) as a low-cost approach to controlling *Salmonella* at farm level (Walia et al., 2016). Additionally, the economic value of administering a formic-citric acid and essential oil blend to finishing pigs for such a short period prior to slaughter, is absent from published literature. Therefore, given these knowledge gaps, the present study aimed to investigate the ability of targeted dietary supplementation with an encapsulated blend of formic acid, citric acid, and essential oils, during the last 28 days of the finishing period, to reduce faecal shedding, intestinal carriage, and *Salmonella* seroprevalence, together with an evaluation of its impact on growth performance.

2. Materials and methods

2.1. Animal ethics and experimental licensing

The feeding trial was performed on a commercial pig farm in the last 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

The feeding trial 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. One hundred and twenty four finisher pigs (70 males and 54 females; in 14 pens of 8–10 same gender pigs per pen), managed as a single all-in-all-out group, were used in the experiment. Each pig was ear tagged with a unique number for identification purposes. Each pen was 4.5 m × 2.8 m with concrete slatted floors and ad-libitum access to water was provided 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 National Pig *Salmonella* Control Programme (NPSCP)]; however, the prevalence of the batch of finishing pigs immediately prior to this trial had declined to 0%. In order to guarantee *Salmonella* carriage in the pigs, pens in the finishing house were artificially contaminated with a *S.* 4,[5],12:i:-, which had previously been isolated from sows in the same herd and had an antimicrobial resistance (AMR) profile of ASSuT. 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) to a final concentration of $\sim 5 \times 10^3$ CFU/mL. Five 25 mL vials (each containing $\sim 5 \times 10^3$ CFU/mL of *Salmonella*) were spread at five points in each pen: 3 in the defecation area, and 2 near the feeder. The final concentration of *Salmonella* at each inoculation point was therefore expected to be 2.5×10^4 CFU/mL. Contamination of the pens was performed 7 days before commencing the trial.

2.2.1. Diets

Approximately 4 weeks before the target slaughter date, pens of pigs were blocked (7 blocks) by sex and weight and randomly assigned within block, using a random number generator in Excel, to one of two dietary treatments: a standard finisher diet with no feed additive (control group) or the same finisher diet supplemented with 4 kg per tonne of an encapsulated blend of formic acid, citric acid, and essential oils from citrus fruit extract, cinnamon, oregano, thyme, and capsicum (FormaXOL™, Kemin Industries, Inc. Southport, Merseyside, UK). The composition of the trial diets is shown in Supplementary Table S-1. The pigs were fed the experimental diets for 28 days and were fasted for ~18 h prior to slaughter.

2.2.2. Blood and faecal sampling and measurement of production parameters

For serological analysis, blood was collected by jugular venipuncture, prior to feeding the experimental diets, and during exsanguination at slaughter. All samples were collected using plastic vacutainers 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 was stored at −20 °C until analysis.