INTRODUCTION

Campylobacter is a microaerophilic, fastidious, Gram-negative bacterium, ubiquitous in the environment, and recognized as the primary cause of bacterial gastroenteritis (Facciolà et al., 2017). Annually, there are ~250,000 campylobacteriosis cases in the European Union (EU) costing an estimated €2.5 billion in healthcare and lost working days (EFSA, 2014, 2019). Campylobacter is known to colonize the intestinal tract of numerous animals and wild birds, with...
poultry a major source of human illness (Facciòl et al., 2017). In Europe, where on average 26% of broilers and 38% of broiler carcasses are contaminated, 80% of campylobacteriosis cases are attributed to the poultry industry (EFSA, 2011, 2019).

A significant correlation has been observed between Campylobacter concentrations in the broiler caeca and on processed chicken carcasses (Rosenquist et al., 2003). Broilers become infected with Campylobacter through horizontal transmission including carryover from previously infected flocks via contaminated feeders and drinkers and from the environment via the boots and equipment of harvesters during thinning (Battersby et al., 2016; Koolman, Whyte, and Bolton, 2014). Once colonized, Campylobacter rapidly disseminates within a flock with reports of intraflock prevalence reaching 100% 4 days after introduction (Koolman, Whyte, & Bolton, 2014). From there, colonized chickens can spread Campylobacter during slaughter and contaminate carcasses, slaughter lines and the surrounding area (Johnson et al., 2006; Reich et al., 2008). Despite the implementation of comprehensive control strategies to reduce the transmission of this foodborne pathogen throughout the food chain, Campylobacter remains a serious public health issue with approximately half of Irish retail chicken contaminated (FSAI, 2016). It has been suggested that a 2–3 log10 reduction of caecal Campylobacter concentration could potentially result in a 76%–100% reduction in campylobacteriosis cases (Meunier et al., 2016).

The incorporation of additives in feed and/or water is a potential preharvest strategy, aimed at reducing intestinal Campylobacter in broiler flocks, which has yet to be thoroughly investigated. In the EU, feed additives are legally controlled in regulation (EC) No. 1831/2003. The use of antibiotics as feed additives is prohibited and only additives that have been approved by the EU may be used (European Commission, 2003). This ban on antibiotics as feed additives (driven by the emergence of multidrug resistant bacteria) combined with consumer demand for naturally produced chicken has generated interest in the use of natural compounds as feed and/or water additives (Micciche et al., 2019).

Organic acids (OAs), medium chain fatty acids (MCFAs), and essential oils (EOs) have all been identified as potential natural antimicrobials. Previously reported OAs with anti-Campylobacter activity include lactic acid, formic acid and sorbate (Byrd et al., 2001; Skånseng et al., 2010). Undissociated OAs enter the cytoplasm and dissociate into charged anions and protons, altering the cellular hydrogen ion equilibrium and raising the pH, resulting in the inhibition of essential metabolic reactions and accumulation of toxic anions (Kim & Rhee, 2013; Koolman, Whyte, Bolton, Meade, et al., 2014). Combinations of 1.5%–2% formic acid and 0.1% sorbate in broiler feed inhibit Campylobacter colonization in broilers (Skånseng et al., 2010). Moreover, water containing lactic acid at a concentration of 0.44% may be used to reduce the number of Campylobacter positive birds (Byrd et al., 2001).

MCFAs, including capric acid, caprylic acid, caproic acid and lauric acid have also reduced Campylobacter and colonization susceptibility of broilers when used as a water additive, and can significantly reduce Campylobacter colonization when used as a feed additive (Gracia et al., 2016; Hermans et al., 2012). Although the mode of action of MCFAs is not completely understood, it is commonly suggested that they act as nonionic surfactants, imbedding themselves in the lipid bilayer and forming pores resulting in cell destruction and leakage (Kim & Rhee, 2013; Koolman, Whyte, Bolton, Meade, et al., 2014).

EOs, including thymol and carvacrol, are considered nonconventional and organic alternatives to antimicrobials (Micciche et al., 2019). They have been found to possess potent anti-Campylobacter activity, significantly reducing Campylobacter colonization of broilers at concentrations as low as 1% (carvacrol) and 2% (thymol) (Arsi et al., 2014). The antibacterial activity of EOs has been attributed to the presence of phenolic compounds, which alter cell membrane permeability, resulting in cellular leakage and death (Arsi et al., 2014). Thymol is also associated with altering the proteome, downregulating binding and chemotaxis proteins, and upregulating outer membrane proteins in Salmonella, while carvacrol may reduce cell motility in Campylobacter (Micciche et al., 2019; Navarro et al., 2015).

The aim of this study was to test the anti-Campylobacter activity of three OAs (lactic acid, formic acid, potassium sorbate), four MCFAs (sodium caprate, sodium caprylate, sodium caproate, sodium laurate) and two EOs (carvacrol, thymol) at various concentrations and exposure times in three different matrices (sterile distilled water [SDW], Mueller Hinton broth [MHB] and grower feed digestate [GFD]). The most effective chemicals in vitro would then be subject to preliminary evaluations (as OA, MCFa or EO mixtures) as potential water additives for broilers by investigating their effect on broiler performance.

**MATERIALS AND METHODS**

**Preparation of bacterial cultures**

The type strain, *Campylobacter jejuni* NCTC 11168, was obtained from the National Collection of Typed Cultures (Salisbury, UK) and used in this study. *Campylobacter jejuni* was stored in defibrinated horse blood at −70°C, and resuscitated in triple vented petri dishes (82.1473, Sarstedt) containing Mueller Hinton agar (CM0037, Oxoid) supplemented with 5% defibrinated sheep blood (SB054, Cruinn). Plates were incubated microaerobically (85% N₂, 10% CO₂, 5% O₂)
at 42°C for 48 h with Anaero Jars (AG0025A, Fannin) and Campygen atmosphere generation kits (CN025A, Oxoid).

Preparation of feed digestate

A commercial feed sample was digested as previously described (Annett et al., 2002; Greene et al., 2020). Briefly, 180 g of feed and 360 ml of 0.03 M HCl were combined, mixed vigorously and the pH was recorded (pH 5.19–5.22). The solution was incubated at 42°C in a shaking incubator for 30 min. Following this, 30,000 U of pepsin (Sigma Aldrich) and 90 ml of 1.5 M HCl were added to the mixture (pH 1.37–1.96) and incubated as previously described for 45 min. After this 1.23 g of 8x pancreatin (P7545, Sigma Aldrich) and 117 ml of NaHCO₃ was added to the mixture (pH 6.3–6.7) and incubated for 2 h. Finally, samples were centrifuged at 2000 g for 30 min to remove any remaining solids.

Selection of antimicrobials and identification of minimum inhibitory concentrations

The antimicrobials used in this study were selected based on a short literature survey of recently published research. They included lactic acid (W261106-1KG-K), formic acid (27001-1L-R), sorbic acid (potassium sorbate [85520]), caprylic acid (sodium caprylate [C5038]), capric acid (sodium caprate [C4151]), lauric acid (sodium laurate [L9755]), thymol (T0501) and carvacrol (W224502), all supplied by Sigma Aldrich. The susceptibility of Campylobacter jejuni NCTC 11168 was evaluated using the microdilution assay method. MHB (CM0405, Oxoid) was inoculated with a single colony of Campylobacter jejuni NCTC 11168 to each antimicrobial was evaluated using the microdilution assay method. MHB (CM0405, Oxoid) was inoculated with a single colony of Campylobacter jejuni NCTC 11168 and incubated in a Whitley M85 Workstation (Don Whitley Scientific) at 42°C for 48 h under microaerobic conditions yielding a concentration of 7 log₁₀ CFU per ml. The inoculated broth was serially diluted 10-fold in sterile broth to achieve a final concentration of 5 log₁₀ CFU per ml. A suspension test, based on work published by Gutiérrez-Martín et al. (2011), was performed accordingly. Following resuscitation, C. jejuni NCTC 11168 was cultivated in 25 ml of MHB, incubated microaerobically at 42°C for 48 hours and subsequently centrifuged at 7500 g for 10 min. The supernatant was discarded, and the pellet was resuspended in 250 µl phosphate buffered saline (PBS) (BR0014, Oxoid) giving a final concentration of 9 log₁₀ CFU per ml (confirmed via serial dilution and enumerating as described below). Using a 24-well plate, with each four well column used to test each chemical four times, 100 µl of PBS-suspended C. jejuni was added to 900 µl of each antimicrobial suspended in each matrix, yielding the final concentration required. Controls for each test consisted of 900 µl of each matrix, without antimicrobial, and 100 µl of Campylobacter jejuni cells. Campylobacter jejuni was exposed to the antimicrobials for a set amount of time (60 s, as per the method used by Gutiérrez-Martín et al., 2011, and 60 min, to more accurately represent retention time in the broiler GIT) and the reaction was stopped by removing 100 µl from each well and diluting them 100-fold in 10 ml of PBS. Following this, 10-fold serial dilutions were performed by diluting 1 ml of the PBS/Campylobacter chemical mixture in 9 ml of maximum recovery diluent (MRD) (CM0733B, Oxoid) and dilutions were plated out, in duplicate, on modified Charcoal Cefoperazone Deoxycholate agar (mCCDA) (CM0739, Oxoid) supplemented with CCDA selective supplement (SR0155, Oxoid) and incubated microaerobically at 42°C for 48 h using Anaero Jars (AG0025A, Fannin, Dublin) and Campygen atmosphere generation kits (CN025A, Oxoid). Following incubation, the Campylobacter broth...
counts and any reductions arising from the OA, MCFA or EO treatments, were obtained.

**Testing of antimicrobials as potential water additives in vivo**

**Description of study design**

The trial took place in a commercial broiler house located on a farm in Co. Monaghan in the north-east of Ireland. Test birds were raised alongside a commercial broiler flock, where the only difference in rearing conditions was the water provided to the birds. In total, 208 1-day old broiler chicks were assigned to one of four treatments—sterile water (SW), OA blend (1.25% v/v lactic acid and 1.5% w/v potassium sorbate), EO blend (0.25% v/v thymol and 0.125% v/v carvacrol) or an MCFA treatment (1.5% w/v sodium caprylate), in a randomized block design. To achieve this, four large pens (8.5 m²), each one composed of four galvanized steel panels, bolted at the corners, with a slit to accommodate the rise and fall of the feeder lines, were constructed throughout the broiler house. Using a polyurethane film tied from end to end, each pen was subsequently subdivided into four smaller pens (2.8 m² each). On the day of chick placement, each pen was stocked with 13 birds (a total of 52 birds per pen). Each pen had access to the house feed line while each pen was stocked with 13 birds (a total of 52 birds per pen). The weights of two birds in each of the smaller pens as well as the weights of five birds from the general flock (2 × 10 faecal samples). From day 21 onwards, five cloacal swabs were also taken per pen and five cloacal swabs were taken from the general flock. Samples were processed using ISO methods 10272-1 and 10272-2 for the detection and enumeration of *Campylobacter* spp. (ISO, 2017a, 2017b).

**Statistical analysis**

The *in vitro* experiment was performed in triplicate on three separate occasions and microbial counts were converted to log_{10} CFU per ml. Data were analysed using one-way ANOVA, followed by Tukey’s multiple comparisons post hoc test using GENSTAT by ANOVA v. 14.1 (VSN International Ltd.). Significance was defined at the 5% (*p* ≤ 0.05) level. Bird weights were analysed using two-way ANOVA, followed by Tukey’s multiple comparisons test using Graphpad Prism ver. 7.2 (Graphpad Software Incorporated).

**RESULTS**

The most effective OA treatments in SDW after 60 s exposure were LA (1.25%) and FA (3.1%) which achieved *C. jejuni* reductions of 5.4 and 4.5 log_{10} CFU per ml, respectively (Table 1). These were significantly (*p* ≤ 0.05) higher than FA (0.31%) and PS (1.5%) (2.7 and 2.2 log_{10} CFU per ml, respectively) while all other treatments were ineffective (<1.0 log_{10} CFU per ml). After 60 min, 6 log_{10} CFU per ml reductions were obtained with LA (1.25%), FA (0.31% and 3.1%) and PS (1.5%), which were significantly (*p* ≤ 0.05) higher than all other treatment combinations. *Campylobacter jejuni* reductions ranged from 0.1 to 4.1 log_{10} CFU per ml after 60 s in MHB, with LA (0.125% and 1.25%), FA (3.1%) and PS (1.5%) all achieving reductions of approximately 3–4 log_{10} CFU per ml. After 60 min these treatments, plus FA (0.31%), all achieved an approximate 7 log_{10} CFU per ml decrease. With the exception of PS (1.5%) (4.5 log_{10} CFU per ml) and FA (3.1%) (2.5 log_{10} CFU per ml) 60 s was not sufficient time to achieve any reduction in *C. jejuni* in GFD. After 60 min these treatment reductions were also significantly higher (6 log_{10} CFU per ml) than LA (1.25%) (4.8 log_{10} CFU per ml) and PS (0.15%) (1.6 log_{10} CFU per ml), with none of the other treatment combinations showing any effect.

The most effective MCFA treatments in SDW (>5 log_{10} CFU per ml) were CP (1.5%) and LU (0.15% and 1.5%) after 60 s and these treatments plus CR (1.5%) after 60 min (Table 2). *Campylobacter jejuni* reductions observed with all other treatments, including all of the concentrations tested for CO, were significantly lower, ranging from 0 to 2.2 log_{10} CFU per ml. When these treatments were tested in MHB, CP and LU (both 1.5%) rapidly killed *C. jejuni* (6.0 log_{10} CFU per ml reduction in 60 s) while CR (1.5%) and
LU (0.15%) required 60 min to achieve reductions of approximately 4 log_{10} CFU per ml. The CP treatment was equally as effective in GFD achieving a 5.8 log_{10} CFU per ml reduction after 60 s which was significantly (p ≤ 0.05) higher than all other treatments. However, after 60 min statistically similar (6 log_{10} CFU per ml) reductions were achieved in both the CP and CR mixtures while the LU treatments (maximum reduction of 1.9 log_{10} CFU per ml) were significantly less effective.

The EOs, TY (0.25% and 2.5%) and CA (0.125% and 1.25%) were also tested in SDW, MHB and GFD (Table 3). TY had potent anti- Campylobacter properties achieving reductions of 4.6–7.3 log_{10} CFU per ml at 0.25% regardless of exposure time or matrix. At the higher concentration (2.5%) statistically similar reductions were obtained. In contrast, CA (0.125%) required 60 min to achieve reductions of 1.9–6.4 log_{10} CFU per ml. However, at 1.25% concentration, CA was as effective as TY in SDW and GFD after both 60 s and 60 min but in MHB required the longer times to achieve statistically similar reductions.

Following the in vitro trials, the most effective chemicals were administered as blends (OA, EO) or single treatments (MCFA) into the drinking water of a commercial broiler flock. OA and EO blends were administered throughout the rearing period, and, due to cost the MCFA treatment (containing 1.5% sodium caprylate) was administered 5 days prior to slaughter to test its potential as a preharvest intervention. Water intake in birds treated with OA and EO blends was low compared to birds administered with SW and the general flock. Moreover, the birds in the OA and EO treated groups had significantly (p ≤ 0.05) decreased body weight gain (approximately half) compared to birds in the general flock (Table 4). Campylobacter was not detected in any of the treated birds or in the general flock during the rearing period.

**DISCUSSION**

The public health significance of *C. jejuni* infection and emergence of multidrug resistance emphasizes the need to develop new control strategies in addition to farm biosecurity measures to lower the carriage of *C. jejuni* in poultry. Natural compounds that reduce populations and limit the spread of *C. jejuni* at preharvest production may be attractive alternatives to conventional antimicrobials. In this study, three OAs (lactic acid, formic acid and potassium sorbate), four MCFAs (sodium caprate, sodium caprylate, sodium caproate and sodium laurate) and two EOs (carvacrol and thymol) were tested for their in vitro efficacy against *C. jejuni* in three different matrices. The most cost effective treatments exhibiting the greatest efficacy at the lowest concentration were then tested in vivo as water additive blends/treatments (OA blend, EO blend and MCFA treatment) to determine how broilers would respond in terms of growth performance.

Prior to their use as antimicrobials in vivo, the MIC of each chemical was established. The MIC for LA and FA were much higher than PS and the MCFAs tested in this study, possibly because these acids can be utilized by *C. jejuni* as energy sources (Luethy et al., 2017; Shaw et al., 2012; Thomas et al., 2011). When tested in the three

**TABLE 1** The reduction in *Campylobacter jejuni* NCTC 11168 counts (log_{10} CFU per ml) observed in sterile distilled water, Mueller Hinton broth and grower feed digestate for lactic acid, formic acid and potassium sorbate at the different concentrations and exposure times

| [%]/time | Sterile distilled water | | Mueller Hinton broth | | Grower feed digestate |
|----------------|-------------------------|-----------------|----------------------|----------------------|------------------------|
|               | 60 s Mean ± SE | 60 min Mean ± SE | 60 s Mean ± SE | 60 min Mean ± SE | 60 s Mean ± SE | 60 min Mean ± SE |
| Lactic acid   | 0.125 0.3C ± 0.11 | 4.0B ± 1.95 | 2.7A ± 0.97 | 6.9A ± 0.53 | 0.0C ± 0.00 | 0.3D ± 0.32 |
|               | 1.25 5.4A ± 0.33 | 5.9A ± 0.11 | 4.1A ± 0.69 | 7.3A ± 0.36 | 0.0C ± 0.03 | 4.8B ± 1.13 |
| Formic acid   | 0.031 0.1C ± 0.09 | 0.1C ± 1.84 | 0.2B ± 0.05 | 0.1B ± 0.03 | 0.1A ± 0.06 | 0.1D ± 0.05 |
|               | 0.31 2.7B ± 1.11 | 6.2A ± 0.05 | 0.2B ± 0.22 | 6.6A ± 0.14 | 0.4C ± 0.01 | 0.0D ± 0.00 |
|               | 3.1 4.5A ± 0.20 | 6.3A ± 0.09 | 3.1A ± 0.32 | 6.6A ± 0.14 | 2.5B ± 1.28 | 6.0A ± 0.34 |
| Potassium sorbate | 0.015 0.1C ± 0.05 | 0.1C ± 0.02 | 0.1B ± 0.05 | 0.1B ± 0.03 | 0.2C ± 0.15 | 0.4C ± 1.58 |
|               | 0.15 0.1C ± 0.07 | 0.1C ± 0.07 | 0.2B ± 0.05 | 0.2B ± 0.10 | 0.2C ± 0.12 | 1.6D ± 0.20 |
|               | 1.5 2.2B ± 0.16 | 6.3A ± 0.09 | 3.4B ± 0.32 | 6.6A ± 0.14 | 4.5A ± 0.76 | 6.0A ± 0.34 |

The same capital letter indicates not statistically differed (p > 0.05) when comparing the Campylobacter reductions (log_{10} CFU per ml) obtained for a given compound at different concentrations and exposure times.

[^] [%] = concentration (v/v).
different matrices, LA and FA both exhibited strong efficacy towards _C. jejuni_ at the lower concentrations (0.125% LA and 0.31% FA) in SDW and MHB but could only achieve significant reductions in GFD at the highest concentrations (1.25% LA and 3.1% FA) tested. It is possible that the high organic matter content of the digestate interfered with the efficacy of LA and FA or that the bactericidal effect of both OAs resulted from a reduction in the pH of the medium as previously suggested (Skånseng et al., 2010; van Deun et al., 2008). In contrast, PS exhibited strong bactericidal activity in GFD at the median concentration selected for this study (0.15%) after 60 min and the

| % | Time/medium | Mean ± SE | Mean ± SE | Mean ± SE | Mean ± SE | Mean ± SE |
|---|-------------|-----------|-----------|-----------|-----------|-----------|
| Sodium caprate | 0.015 | 0.0^C ± 0.0 | 0.5^C ± 0.38 | — | — | — |
| | 0.15 | 0.1^C ± 0.03 | 1.2^B ± 0.24 | — | — | — |
| | 1.5 | 6.1^A ± 0.04 | 5.3^A ± 0.31 | 5.5^A ± 1.23 | 6.1^A ± 0.42 | 5.8^A ± 0.56 | 6.2^A ± 0.13 |
| Sodium caprylate | 0.015 | 0.1^C ± 0.09 | 0.1^C ± 0.07 | — | — | — |
| | 0.15 | 0.0^C ± 0.00 | 0.0^C ± 0.02 | — | — | — |
| | 1.5 | 2.2^B ± 0.93 | 4.2^A ± 1.74 | 1.3^B ± 0.18 | 4.5^B ± 1.01 | 3.5^B ± 0.06 | 5.9^A ± 0.18 |
| Sodium caproate | 0.03 | 0.0^C ± 0.04 | 0.0^C ± 0.03 | — | — | — |
| | 0.3 | 0.0^C ± 0.00 | 0.0^C ± 0.00 | — | — | — |
| | 3.0 | 0.5^C ± 0.50 | 0.5^C ± 0.06 | — | — | — |
| Sodium laurate | 0.015 | 2.1^B ± 2.01 | 2.2^B ± 1.56 | — | — | — |
| | 0.15 | 5.3^A ± 0.93 | 5.9^A ± 0.19 | 0.2^C ± 0.11 | 4.1^B ± 1.52 | 0.1^C ± 0.01 | 0.1^C ± 0.08 |
| | 1.5 | 5.3^A ± 0.61 | 6.0^A ± 0.23 | 5.9^A ± 0.92 | 6.1^A ± 0.53 | 0.3^C ± 0.09 | 1.9^B ± 0.33 |

The same capital letter indicates not statistically differed (p > 0.05) when comparing the _Campylobacter_ reductions (log_{10} CFU per ml) obtained for a given compound at different concentrations and exposure times.

TABLE 2 The reduction in _Campylobacter jejuni_ NCTC 11168 counts (log_{10} CFU per ml) observed in sterile distilled water (SDW), Mueller Hinton broth (MHB) and grower feed digestate (GFD) using medium chain fatty acids (MCFA) including sodium caprate (CP), sodium caprylate (CR), sodium caproate (CO) and sodium laurate (LU)

| % | Time/medium | Mean ± SE | Mean ± SE | Mean ± SE | Mean ± SE | Mean ± SE |
|---|-------------|-----------|-----------|-----------|-----------|-----------|
| Thymol | 0.25 | 5.7^A ± 0.35 | 5.9^A ± 0.11 | 4.6^A ± 1.19 | 7.3^A ± 0.36 | 6.7^A ± 0.15 | 6.4^A ± 0.26 |
| | 2.5 | 6.0^A ± 0.04 | 5.9^A ± 0.11 | 5.7^A ± 0.73 | 7.3^A ± 0.36 | 6.7^A ± 0.15 | 6.4^A ± 0.26 |
| Carvacrol | 0.125 | 1.9^B ± 0.80 | 5.9^A ± 0.11 | 3.2^B ± 1.67 | 5.3^B ± 2.21 | 0.5^B ± 0.27 | 6.4^A ± 0.26 |
| | 1.25 | 5.5^A ± 0.56 | 5.9^A ± 0.11 | 1.8^C ± 0.91 | 7.3^A ± 0.36 | 6.1^A ± 0.64 | 6.4^A ± 0.26 |

The same capital letter indicates not statistically differed (p > 0.05) when comparing the _Campylobacter_ reductions (log_{10} CFU per ml) obtained for a given compound at different concentrations and exposure times.

TABLE 3 The reduction in _Campylobacter jejuni_ NCTC 11168 counts (log_{10} CFU per ml) observed in sterile distilled water (SDW), Mueller Hinton broth (MHB) and grower feed digestate (GFD) using essential oils (EO) thymol (TY) and carvacrol (CA)
since it recorded minimal reductions in SDW (0.5 log10 CFU 1.5%) in MHB and GFD while CO was no longer considered est concentrations of CP, CR (both 1.5%) and LU (0.15% and was not significant. Thus, it was decided to use only the high-
C their efficacy in SDW against
for CP, CR, LU (all 0.015%) and CO (0.03%) were recorded,
tions than their acids (Hermans et al., 2010). While the MIC they react similarly and have better solubility in aqueous solu-
crobial activity (Sofos & Busta, 1980).
they treat and strengthen the mucosal barrier in broilers (Placha et al.,
with sorbate and the pH in the gut to increase the antimi-
the feed during the digestion process, act synergistically
that salts and sugars, which would have been released from
highest concentration (1.5%) after 60 s. It has been shown that salts and sugars, which would have been released from the feed during the digestion process, act synergistically with sorbate and the pH in the gut to increase the antimicrobial activity (Sofos & Busta, 1980).
The sodium salts of MCFAs were used in this study since they react similarly and have better solubility in aqueous solutions than their acids (Hermans et al., 2010). While the MIC for CP, CR, LU (all 0.015%) and CO (0.03%) were recorded, their efficacy in SDW against C. jejuni in the suspension test was not significant. Thus, it was decided to use only the highest concentrations of CP, CR (both 1.5%) and LU (0.15% and 1.5%) in MHB and GFD while CO was no longer considered since it recorded minimal reductions in SDW (0.5 log10 CFU per ml). CR, CP and LU are straight chain fatty acids with 8, 10 and 12 carbon atoms, respectively, and have high bacterial activity in weakly acidic environments and near-neutral pH environments such as SDW, MHB and GFD (Molatová et al., 2010).
The in vitro MIC assay for the EOs TY and CA showed strong anti-Campylobacter activity in all three matrices. TY and CA are structurally similar phenolic compounds and have previously been reported to exert a synergistic or additive antimicrobial effect when applied together (Bassolé & Juliani, 2012). CA also inhibits flagellar motility in C. jejuni (van Alphen et al., 2012) which may be useful to prevent C. jejuni from burrowing into the mucus layer of the intestinal crypts, thereby becoming difficult to reach for antimicrobial substances (Hermans et al., 2010). Dietary supplementation with TY has been reported to improve intestinal integrity and strengthen the mucosal barrier in broilers (Placha et al., 2014).

When the efficacy of these additives were tested in vivo, birds treated with OAs and EOs weighed significantly less than those in the general flock (p < 0.0001). One important aspect of this study is that these additives were tested on a commercial farm during a broiler rearing period rather than an experimental facility where feed and water intake can be tightly regulated. Several researchers have reported that growth performance was not affected in chicks housed in such facilities when feed or water supplemented with OAs were provided (Denli et al., 2003; Hernández et al., 2006; Vale et al., 2004). In contrast, studies similar to ours and carried out in commercial farms have identified a negative effect on the weight of broilers treated with EOs and OAs. Arsi et al., (2014) reported significant reduction in body weights of birds treated with thymol and carvacrol, while Skånseng et al. (2010) observed reductions of 16%–25% in the body weights of birds treated with formic acid and sorbate. While this study has identified potential feed/water additives that can reduce C. jejuni populations and can be readily used in the broiler industry, there are still many hurdles that must be overcome. In addition to the broiler performance issue identified in this study, other research has found that water additives that show strong efficacy in vitro are often less effective when tested in vivo (Hermans et al., 2010; van Deun et al., 2008). MCFAs, such as lauric acid, for example, are readily absorbed and incorporated into the muscles in the broiler GIT (Hankel et al., 2018) and sodium caprate, caprylate and caproate that are effective anti-Campylobacter treatments in laboratory trials do not reduce Campylobacter caecal concentrations in broilers (Hermans et al., 2010). A similar phenomenon has been observed with EOs such as TY and CA, but in addition to absorption they may be degraded.

| Day | Average weight (kg) OA blend | Average weight (kg) EO blend | Average weight (kg) MCFA treatment | Average weight (kg) Control (SW) | Average weight (kg) General Flock |
|-----|-----------------------------|-----------------------------|-----------------------------------|-------------------------------|----------------------------------|
|     | Mean ± SE                  | Mean ± SE                  | Mean ± SE                         | Mean ± SE                     | Mean ± SE                       |
| D1  | 0.045 ± 0.007              | 0.051 ± 0.003              | 0.051 ± 0.003                     | 0.056 ± 0.003                 | 0.055 ± 0.0                   |
| D4  | 0.08 ± 0.001               | 0.091 ± 0.002              | 0.105 ± 0.001                     | 0.111 ± 0.002                 | 0.109 ± 0.0                   |
| D7  | 0.121 ± 0.009              | 0.161 ± 0.009              | 0.193 ± 0.007                     | 0.173 ± 0.018                 | 0.183 ± 0.0                   |
| D11 | 0.169 ± 0.006              | 0.299 ± 0.017              | 0.355 ± 0.019                     | 0.379 ± 0.009                 | 0.35 ± 0.019                  |
| D14 | 0.265 ± 0.013              | 0.441 ± 0.015              | 0.541 ± 0.028                     | 0.584 ± 0.004                 | 0.591 ± 0.024                 |
| D18 | 0.324 ± 0.022              | 0.545 ± 0.04               | 0.728 ± 0.024                     | 0.822 ± 0.041                 | 0.827 ± 0.062                 |
| D21 | 0.445 ± 0.042              | 0.713 ± 0.064              | 1.001 ± 0.034                     | 1.015 ± 0.037                 | 0.897 ± 0.042                 |
| D25 | 0.612 ± 0.026              | 0.922 ± 0.098              | 1.478 ± 0.07                      | 1.568 ± 0.054                 | 1.391 ± 0.08                  |
| D28 | 0.826 ± 0.045              | 1.008 ± 0.104              | 1.707 ± 0.127                     | 1.898 ± 0.086                 | 1.66 ± 0.127                  |
| D32 | 0.933 ± 0.106              | 1.102 ± 0.067              | 2.019 ± 0.096                     | 2.19 ± 0.136                  | 2.08 ± 0.017                  |
| D35 | 1.087 ± 0.09               | 0.952 ± 0.049              | 2.324 ± 0.139                     | 2.616 ± 0.085                 | 2.057 ± 0.106                 |
| p value (compared to general flock) | <0.0001                   | <0.0001                     | 0.9062                            | 0.0358                        | N/A                             |
in the GIT before they reach the caeca (Arsi et al., 2014). Potential approaches to overcome such hurdles include the use of these compounds in combination or encapsulating the compounds in biodegradable material to enhance delivery to the caeca (Pham et al., 2020; Skånseng et al., 2010). In conclusion, this study identified potential natural compounds that are effective at reducing \textit{C. jejuni} populations \textit{in vitro} but are not suitable for application in broiler flocks as they adversely affect performance, highlighting the gap between laboratory based \textit{in vitro} success and practical application under real commercial conditions.

**ANIMAL ETHICS STATEMENT**

The study design and protocols used to study the effect of water additive treatments on broiler performance was approved by the Teagasc Animal Ethics Committee on April 2, 2020 (TAEC2020-254). This committee operates under the auspices of the Health Products Regulatory Authority (HPRA, Ireland) who protect and enhance public and animal health by regulating medicines, medical devices and other health products.

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**CONFLICT OF INTEREST**

No conflict of interest declared.

**AUTHOR CONTRIBUTIONS**

Genevieve Greene: Conceptualization, methodology, data curation, formal analysis, visualization, writing, original draft preparation, review and editing. Leonard Koolman: Data curation, formal analysis, visualization, writing, review and editing. Paul Whyte: Conceptualization, methodology, data curation, writing, review and editing, visualization, supervision, project administration, funding acquisition. Helen Lynch: writing, review and editing. Aidan Coffey: Data Curation, writing, review and editing, project administration, funding acquisition. John Egan: Data Curation, writing, review and editing, project administration, funding acquisition. Lisa O’Connor: Writing, review and editing, project administration, funding acquisition. Declan Bolton: Conceptualization, methodology, data curation, formal analysis, visualization, writing, original draft preparation, review and editing, supervision, project administration, funding acquisition.

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