Dietary supplementation with ferric tyrosine improves zootchnical performance and reduces caecal Campylobacter spp. load in broilers

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ABSTRACT
1. The objective of this study was to evaluate the effect of ferric tyrosine on the reduction of Campylobacter spp. and zootchnical performance in broilers exposed to Campylobacter spp. using a natural challenge model to simulate commercial conditions. Additionally, the minimum inhibitory concentrations (MICs) of ferric tyrosine against common enteropathogens were evaluated.
2. At the start of the trial, 840 healthy male 1-d-old birds (Ross 308) were randomly allocated to 6 replicate pens of 35 birds each and fed diets containing different concentrations of ferric tyrosine (0, 0.02, 0.05 and 0.2 g/kg) in mash form for 42 d.
3. Broilers fed diets containing ferric tyrosine showed significantly higher body weight at d 42 and weight gain compared to the control group. However, birds fed ferric tyrosine ate significantly more than the control birds so significant improvements in feed conversion rate were not observed.
4. Microbiological analyses of caecal samples collected on d 42 of the study showed, per gram of sample, 2–3 log10 reduction in Campylobacter spp. and 1 log10 reduction in Escherichia coli in the groups fed diets containing ferric tyrosine compared to the control.
5. The MICs of ferric tyrosine was >400 mg/l for C. jejuni and >200 mg/l for E. coli and Salmonella enterica, indicating that ferric tyrosine did not exert antimicrobial activity.
6. The results showed that birds fed ferric tyrosine grew faster and consumed more feed compared to the control group, indicating potential benefits of faster time to reach slaughter weight with no significant reduction on feed efficiency. Moreover, ferric tyrosine significantly reduced caecal Campylobacter spp. and E. coli indicating potential as a non-antibiotic feed additive to lower the risk of infections transmitted through the food chain.

Introduction
Campylobacteriosis is the most common human food-borne illness in the European Union (EU) (EFSA 2017a) and, along with other enteropathogenic bacteria such as Salmonella spp. and Escherichia coli (Chaveerach et al. 2004b; Santini et al. 2010; Hermans et al. 2011), Campylobacter spp. pose a serious public health risk. Contaminated chicken meat is a major source of human infection (Friedman et al. 2004; Adak et al. 2005; Bull et al. 2008), with ca. 200,000 reported cases of campylobacteriosis per year (EFSA 2016). It has been estimated that 75% of EU broiler meat samples are contaminated with Campylobacter spp. (EFSA 2010). The prevalence of this pathogen can be very high in poultry flocks and can be transmitted through the food chain (EFSA 2010, 2011). Reducing the number of contaminated carcasses entering the food chain can reduce the incidence of human cases of campylobacteriosis, hence control measures must be implemented on poultry farms to reduce human exposure (European Food Safety Authority (EFSA) 2011). It has been estimated that reducing caecal Campylobacter spp. numbers by 3 log10 CFU/g (colony forming units per gram) can reduce public health risk by 90% (Romero-Barios et al. 2013). However, controlling contamination on farms poses several serious challenges. A single bird infected with low numbers of Campylobacter spp. can infect a whole flock (Stern et al. 2001), and these chickens appear asymptomatic, meaning that infection can go undetected (European Commission (EC) 2017). Strict biosecurity measures have proven to be effective in excluding Campylobacter from housed flocks in northern Europe and the United Kingdom but are difficult to maintain in the long term under commercial farming conditions (ACMSF 2004; Bull et al. 2008). Antibiotics are no longer a viable option for control and are subject to global pressure to reduce use drastically, due to growing concerns about antimicrobial resistance (AMR). EFSA has recently reported that Campylobacter spp. strains isolated from humans and pigs are resistant to ciprofloxacin and tetracyclines, which are critically important antibiotics for human use (EFSA 2017b). Similar data have been reported for Salmonella spp. and E. coli isolates from fattening pigs, highlighting the growing problem of AMR. The EU banned the use of antibiotics as growth promoters in animal feeds in 2006 (EMA and EFSA 2017), hence, there is an urgent need for alternatives that can protect farm animals and limit the establishment and growth of bacterial pathogens, in particular zoonotic microorganisms.

Various feed additives have been proposed to reduce Campylobacter colonisation in chickens, including probiotics, prebiotics, organic acids, bacteriophages, bacteriocins and plant-derivatives, some of which have shown promising results (Hermans et al. 2011; Guyard-Nicodème et al. 2015).
Recently, in-feed chelated iron (III) complexes have shown to be effective against *Campylobacter* and other pathogenic bacteria in broilers (Khattak et al. 2018). However, in the study performed by Khattak et al., birds were artificially challenged, so the purpose of the present study was to investigate whether iron chelates have comparable effects under more natural infection conditions. The aim of the present study was to evaluate the effect of ferric tyrosine (TYPLEX®, Akeso Biomedical Inc., USA) on broiler zootechnical performance and reduction of caecal *Campylobacter* spp. using naturally infected birds to simulate farm conditions. Additionally, the minimum inhibitory concentrations (MICs) of ferric tyrosine against common enteropathogens were evaluated to ascertain whether ferric tyrosine exerts antimicrobial activity.

**Material and methods**

**Experimental birds and diets**

The study protocol was approved by the Roslin Nutrition Ltd. Ethical Review Committee and the UK Food Standards Agency. The study birds were managed and handled in compliance with local animal welfare standards and Directive 2010/63/EU.

A total of 1100 pre-sexed, male 1d-old broilers (Ross 308) were purchased from a local commercial hatchery and delivered to the trial site (56.0092° N, 2.8594° W) in Aberlady, Scotland. The trial site was an experimental research facility with animal housing set-up to simulate commercial conditions. Any chicks showing signs of ill-health, injury or in poor condition were excluded from the selection process. The poultry house was lit by programmable artificial light. The standard lighting program was 23 h of light per day, followed by 1 h of darkness. Environmental conditions (temperature, humidity and ventilation rate) were automatically controlled and appropriate for the age of the broilers.

Ferric tyrosine (TYPLEX®, Akeso Biomedical, Inc., USA) is an organo-iron complex of iron (III) coordinated with L-tyrosine (4-hydroxyphenylalanine). The dietary treatments are summarised in Table 1. The control group (T1) was fed the basal diet (starter and grower), and the treatment groups received the basal diets supplemented with ferric tyrosine at 0.02 g/kg (T2), 0.05 g/kg (T3) or 0.20 g/kg feed (T4). Birds were fed a starter diet from 0 to 21 d and a grower diet from 21 to 42 d.

All diets were formulated according to recommended specifications (NRC 1996) and analysed (AOAC 2007) for crude protein, ether extract, dry matter, iron and ash (Tables 2 and 3). Feed and water were offered *ad libitum*. Coloured tracers (Micro-Tracers Inc., San Francisco, CA, USA) were added to ferric tyrosine at 10% w/w, to enable visual confirmation of its content in feeds. Proximate analyses of feed samples confirmed that feed nutrients were within expected ranges. The diets were in a mash form and did not contain any other added iron compounds, coccidiostats or veterinary antibiotics.

**Study design**

On d 0, out of a pool of 1100 birds, 840 healthy birds were randomly allocated to the 4 treatment groups to give 6 replicate pens per group, each pen containing 35 birds, according to a randomised complete block design. The birds were weighed per pen on arrival and then at 21 and 42 d of the trial. Average bird body weight (BW) was calculated by dividing the average weight of the pen by the number of birds. Feed consumption and feed refusals were recorded by pen on d 21 and 42, and mortality and/or culls were recorded daily. Average pen weight gain (AWG), feed intake (AFI) and feed conversion rate (FCR, feed/gain) were calculated for periods 0–21, 22–42 and 0–42 d on trial. At the end of the trial (42 d), 5 birds/pen were humanely euthanised, and caecal samples were collected and sent for microbiology. The trial terminated after 42 d and all birds were humanely euthanised by cervical dislocation and the carcasses destroyed.

| Treatment | Ferric tyrosine (g/kg feed) | Microtracer (g/kg feed) |
|-----------|----------------------------|--------------------------|
| T1 | Control – 0 g/kg | 0 |
| T2 | T1 + 0.02 g/kg feed | 0.022² |
| T3 | T1 + 0.50 g/kg feed | 0.055² |
| T4 | T1 + 0.20 g/kg feed | 0.220² |

Table 1. Experimental diets.

1One gram of microtracer contains 60,000 violet graphite particles.

2Microtracers at 10% in test products.

**Campylobacter spp. challenge**

A natural *Campylobacter* spp. challenge model was used, whereby study birds were bedded on fresh wood shavings, which was overlaid with litter from the previous batch of broilers. Litter samples from previous batches of birds taken from the barn used for this study had tested positive for

**Table 2. Feed composition and calculated analyses.**

| Ingredients (%) | Starter mash 1–21 d of age | Grower mash 22–42 d of age |
|-----------------|----------------------------|---------------------------|
| Wheat           | 69.862                     | 67.354                    |
| Barley          | –                          | 1.3                       |
| Soya bean meal, 48% CP | 23.4                     | 21.4                      |
| Sodium bicarbonate | 0.13                    | 0.22                      |
| Fishmeal 66%    | 2.5                        | 1.4                       |
| L-Tyrosine      | 0.022                      | 0.022                     |
| O-Methionine    | 0.123                      | 0.164                     |
| Choline chloride | 0.067                     | 0.067                     |
| Dicalcium phosphate | 0.13                    | 0.32                      |
| Calcium carbonate | 1.74                     | 0.74                      |
| Sodium chloride | 0.12                       | 0.16                      |
| Minerals and vitamins | 0.5                    | 0.5                       |
| Total           | 100                        | 100                       |

2Supplies per kg feed: Vit A: 0.010 MIU; Vit D3: 0.005MIU; Vit E: 50mg; Vit K3: 3 mg; Vit B1: 2.0 mg; Vit B2: 7 mg; Vit B6: 5 mg; Vit B12: 15 μg; Folic acid: 1.0 mg; Biotin: 0.2 mg; Panthothenic acid: 15 mg; xA315 niacinamide: 50 mg; Mo 0.5 mg; Mn: 100 mg; Zn: 80 mg; I: 1.0 mg; Cu: 10 mg; Se: 0.20 mg; Fe: 267 mg.
Campylobacter spp. Furthermore, birds previously housed in this barn had tested positive for Campylobacter spp. on several occasions. This natural challenge model was developed at Roslin Nutrition and was selected to replicate as far as possible, infection under commercial conditions.

### Microbiology

On d 42, 5 birds/pen were humanely euthanised by cervical dislocation. The caeca from each individual bird were removed and tied off to preserve the contents, placed in a pre-labelled ziplock bag and immediately placed on dry ice. The birds were processed in descending order of ferric tyrosine concentration, with the control birds processed last to reduce the likelihood of cross-contamination. Sterile equipment was used and changed between each treatment group. Latex gloves were worn by study staff responsible for the removal of the caeca and were changed between treatment groups. The samples were sent via courier to the microbiology laboratory for Campylobacter spp. and *E. coli* enumeration by conventional culture. Caeca were stored frozen (−80°C) until analysis. Prior to analysis, the caecal samples were removed from the freezer and allowed to defrost, and a sterile scalpel was used to cut off the blind end of both caecal sacks. From each caecum, 0.5 g of contents, in total 1 g, was weighed into sterile Universal bottles, diluted with 2 ml sterile Maximum Recovery Diluent (MRD, Oxoid, Basingstoke, UK), and mixed thoroughly to give a 1:2 dilution (w/v). Further serial dilutions were made using MRD and 10 µl of each dilution were inoculated on charcoal cefoperazone deoxycholate agar (CCDA) and Brilliance CampyCount Agar plates (Oxoid), incubated microaerophilically at 42°C for 24–48 h and then assessed for the presence or absence of thermotolerant Campylobacter spp. The individual caeca from 5 birds/pen were analysed in duplicate. Plates of an appropriate dilution were selected and putative colonies enumerated. For confirmation, two colonies from each presumptively positive plate were selected and subcultured onto paired blood agar plates (Oxoid). These plates were incubated at 37°C for 48 h, one plate aerobically, one plate microaerophilically. The presence of Campylobacter spp. was indicated by a lack of aerobic growth and the formation of colonies with Campylobacter morphology that grow microaerophilically. In addition, Gram stains were carried out on all presumptively positive samples. As a further step, oxidase strips (Oxoid) were used to confirm that samples were oxidase positive (Cowan and Steel 1965; Corry et al. 1995). The same series of samples were tested for the presence and absence of *E. coli* using chromogenic plates (Oxoid) and incubated for 20 h at 37°C, using the same procedure as reported for Campylobacter spp. enumeration. All results were expressed as CFU/g of caecal contents.

In addition, polymerase chain reaction (PCR) was conducted on 5 representative colonies isolated from CCDA plates from each treatment group to confirm the presence of *C. jejuni* vs. *C. coli*. The primer sets in the multiplex PCR target the identification of *C. jejuni* and *C. coli* based on the amplification of the two genes, mapA (589 bp) *C. jejuni* and *ceuE* *C. coli* (462 bp). In addition, a 16S primer (800 bp) set was included as quality assurance of the DNA-preparation and analysis (internal control). Between 3 and 4 colony morphotypes from each treatment group were examined. To avoid false negatives, three different concentrations of each isolate’s template were used for PCR amplification.

### Minimum inhibitory concentration assays (growth inhibition studies with Campylobacter jejuni, Escherichia coli and Salmonella enterica)

Ferric tyrosine was subjected to two digestive phases to mimic digestion in the avian gut. The product is poorly soluble and the digestive steps were included to enhance its solubility and bioavailability. A pepsin digestion phase was performed to mimic conditions in the acidic proventriculus and a pancreatin digestion phase mimicked conditions in the neutral duodenum. In brief, 240 mg ferric tyrosine was suspended in 5 ml of 50 mM Na-phosphate buffer (pH 6.5). Following this step, 2.25 ml of 150 mM HCl and 0.75 ml of activated pepsin (1 mg/ml) in 10 mM HCl was added; and the pH adjusted to pH 2.1. The resulting suspension was digested for 1 h at 37°C. Following pepsin digestion, 4 ml of 150 mM NaHCO₃, 2 ml of bovine bile (125 mg/ml in 150 mM NaHCO₃) and 2 ml of porcine pancreatin (12.5 mg/ml in 150 mM NaHCO₃) were added to the digested suspension and the pH was adjusted to 6.5 with NaOH. The suspension was then left to digest for 3 h at 37°C after which the total volume was adjusted to 20 ml. A positive control (PC) was prepared by following the steps described above, with no added ferric tyrosine. The two digests (PC digest and 20 mM ferric tyrosine digest) were sterilised by UV light before use in the MIC studies.

For the MIC dilution study, *C. jejuni* strain DSM4688 grown in Müller–Hinton growth medium, and *E. coli* strain 156/97 F4+ and *S. enterica* serovar Typhimurium strain IR715 both grown in Luria broth were added to 96-well microtiter plates (Merck, Germany) containing the ferric tyrosine digest at concentrations ranging from 25.5 to 408 mg/l for *C. jejuni* and 0.39 to 200 mg/l for *E. coli* and *S. enterica*, and the PC digest in dilutions corresponding to

| Sample | Diet | Ferric tyrosine content (g/kg) | Moisture (%) | Crude protein (%) | Ether extract (%) | Ash (%) | Fe (mg/kg) | Ferric tyrosine-microtracer (%) recovery | Calculated ferric tyrosine content (g/kg) |
|--------|------|-------------------------------|--------------|------------------|-----------------|--------|-----------|---------------------------------------|--------------------------------------|
| T1     | Starter | 0 | 11.6 | 20.4 | 3.1 | 5.5 | 125 | NA | NA |
| T2     | 0.02 | 11.5 | 20.3 | 2.9 | 5.4 | 189 | 131 | 0.026 |
| T3     | 0.05 | 11.3 | 20.6 | 2.9 | 5.5 | 198 | 118 | 0.059 |
| T4     | 0.20 | 11.3 | 20.5 | 2.9 | 5.3 | 196 | 107 | 0.214 |
| T1     | Grower | 0 | 11.9 | 19.3 | 3.2 | 4.2 | 171 | NA | NA |
| T2     | 0.02 | 11.9 | 18.7 | 3.0 | 4.2 | 159 | 98 | 0.020 |
| T3     | 0.05 | 11.9 | 18.7 | 3.0 | 4.7 | 185 | 85 | 0.043 |
| T4     | 0.20 | 11.5 | 19.1 | 3.0 | 4.4 | 166 | 81 | 0.162 |

NA: Not applicable.

1Calculated ferric tyrosine = % recovery of microtracer × ferric tyrosine dose.
the amounts of digest added with the ferric tyrosine. The range of concentrations selected was chosen to meet or exceed the practical doses used in feed. All plates were incubated at 38°C. Plates containing C. jejuni were read after 24 h by measuring fluorescence with a Perkin Elmer multimode plate reader after rendering bacterial cells fluorescent with SYBR Green dye (Sigma-Aldrich, Darmstadt, Germany). Plates containing E. coli and S. enterica were read at 4 and 20 h. Turbidity was measured using a spectrophotometer at a wavelength 600 nm. The MIC value was defined as the lowest product concentration that yielded a near-significant trend (0.05 < P ≤ 0.10). Text in bold – significant result (P < 0.05); text in italics – near-significant trend (0.05 < P ≤ 0.10).

Results show least square mean of 6 replicate pens. No. of replicates/treatment = 6 pens of 35 male birds/treatment; Means separated by Tukey’s test.

Values in same column with no common abc superscript are significantly different (P ≤ 0.05); Values in same column with no common xy superscript exhibit a near-significant trend (0.05 < P ≤ 0.10). Text in bold – significant result (P < 0.05); text in italics – near-significant trend (0.05 < P ≤ 0.10). SE: standard error; BW: mean bird body weight; AWG: mean pen weight gain; AFI: mean pen feed intake; FCR: feed/gain; NS: not significant.

Results

The effect of ferric tyrosine on broiler performance during each study period is summarised in Table 4. The mortality rate (including culled birds) was low and there were no significant differences in mortality between treatment groups (T1, 6/210 (2.9%); T2, 4/210 (1.9%); T3, 4/210 (1.9%); T4, 6/210 (2.9%)). The majority (13/20) of birds were culled early in the study as poor or non-starters or small birds. During the first period (0–21 d on trial), broilers fed diets supplemented with ferric tyrosine (T2, T3 and T4) weighed significantly more at 21 d (+117 g; 1385, 1385, 1323 vs. 1268 g; P = 0.003, respectively), and gained significantly more weight (+110, +130, +63 g; 588, 608, 541 vs. 478 g; P = 0.008; P = 0.009, respectively) compared to broilers fed the T1 control diet. No significant differences were seen for FCR.

During the second growth period (22–42 d on trial), broilers fed T2, T3 and T4 diets weighed significantly more at the end of the trial (42 d) (+263, +219 g; 2751, 2707 vs. 2488 g; P = 0.002; P = 0.008; P = 0.009, respectively). Broilers fed T2 and T3 diets consumed significantly more feed (+263, +219 g; 2751, 2707 vs. 2488 g; P = 0.005; P = 0.021, respectively) compared to broilers fed the T1 control diet. Broilers fed the T3 diet presented a significantly higher FCR (1.934 vs. 1.845 vs. 1.827 g; P = 0.014; P = 0.003; respectively) compared to broilers fed the T1 control diet and the T4 diet. In addition, broilers receiving the T4 diet tended to gain more weight (+117 g; 1465 vs. 1348 g; P = 0.062) and to eat more (+187 g; 2675 vs. 2488 g; P = 0.057) compared to broilers fed the T1 control diet. Broilers fed the diets containing ferric tyrosine (T2, T3 and T4) gained significantly more weight (+212, +182, +179 g; 2039, 2009, 2006 vs. 1827 g; P = 0.002; P = 0.008; P = 0.009, respectively) and ate significantly more feed (+385, +385, +258 g; 3609, 3609, 3482 vs. 3224 g; P < 0.001; P < 0.001; P = 0.027, respectively) compared to broilers fed the T1 control diet. No significant differences in FCR were noted between the groups supplemented with ferric tyrosine and the T1 control group.

Microbiological counts from the caecal samples collected on d 42 are summarised in Table 5. All individual birds in T1 tested positive for Campylobacter spp. and E. coli. Figure 1 shows the distribution of the counts for each

### Table 4. Effect of dietary addition of ferric tyrosine on broiler zootechnical performance parameters for each study period.

| Parameter                        | Treatment          | T1 0 g/kg ferric tyrosine | T2 0.02 g/kg ferric tyrosine | T3 0.05 g/kg ferric tyrosine | T4 0.20 g/kg ferric tyrosine | SE  | Treatment P-value (ANOVA) |
|----------------------------------|--------------------|---------------------------|-----------------------------|-----------------------------|-----------------------------|-----|--------------------------|
| BW 1 d (g)                       | 41.81              | 42.00                     | 42.48                       | 42.19                       | 0.135                       | 0.3645 |
| BW 21 d (g)                      | 520b               | 630c                      | 650d                        | 583b                        | 11.6                        | 0.001 |
| BW 42 d (g)                      | 1868b              | 2081b                     | 2052b                       | 2048b                       | 24.0                        | 0.001 |
| AWG 1–21 d (g)                   | 478e               | 588e                      | 608e                        | 541b                        | 11.5                        | <0.001 |
| AWG 22–42 d (g)                  | 1348f              | 1451f                     | 1410f                       | 1403f                       | 17.2                        | 0.055 |
| AWG 1–42 d (g)                   | 1827f              | 2039f                     | 2009f                       | 2009f                       | 24.0                        | 0.002 |
| AF1 1–21 d (g)                   | 737f               | 859f                      | 901f                        | 807f                        | 16.5                        | <0.001 |
| AF1 22–42 (g)                    | 2488f,x            | 2751f,b                   | 2707f,b                    | 2675f,b,y                  | 30.8                        | 0.005 |
| AF1 1–42 d (g)                   | 3224f              | 3609f                     | 3609f                       | 3482f                       | 42.8                        | <0.001 |
| FCR 1–21 d (g)                   | 1.539              | 1.462                     | 1.484                       | 1.488                       | 0.0138                      | 0.2405 |
| FCR 22–42 (g)                    | 1.845f             | 1.898f,xy                 | 1.934f                      | 1.827f,y                   | 0.0124                      | 0.002 |
| FCR 1–42 d (g)                   | 1.765f,x           | 1.771f,y                  | 1.798f                      | 1.726f                      | 0.0087                      | 0.083 |
Table 5. Caecal Campylobacter spp. and E. coli counts at 42 d of age (log$_{10}$ CFU/g).

| Treatment       | Dose (g/kg) | Campylobacter spp. | E. coli |
|-----------------|-------------|--------------------|---------|
|                 |             | Caeca$^1$ | Caeca$^2$ | Caeca |
| T1 Control      | 0           | 5.879$^b$ | 4.799$^b$ | 6.438$^b$ |
| T2 Ferric tyrosine | 0.02 | 4.986$^{bc}$ | 3.621$^{b}$ | 5.449$^{bc}$ |
| T3 Ferric tyrosine | 0.05 | 4.104$^{ab}$ | 2.399$^*$ | 5.736$^*$ |
| T4 Ferric tyrosine | 0.20 | 3.366$^*$ | 1.681$^*$ | 5.118$^*$ |
| SEM             |             | 0.1301   | 0.1448   | 0.1843  |
| Treatment P-value (ANOVA) |       | <0.001   | <0.001   | 0.104   |

$^a$Caecal samples cultured on CCDA medium.
$^b$Caecal samples cultured on Brilliance medium.
No. of replicates = 6 replicate pens per treatment. Results show group least square mean of 6 replicate pens.
Values in same column with no common abc superscript are significantly different (P < 0.05).
Values in same column with no common xy superscript exhibit a near-significant trend (0.05 < P ≤ 0.10).
Results in bold text indicate a significant result (P < 0.05).
SEM: standard error of the mean.

treatment groups which demonstrated that all pens in T1 were positive for Campylobacter spp. and E. coli. The results showed a significant reduction in Campylobacter spp. in birds fed T3 and T4 diets compared to the birds fed the T1 control diet (1.8 log$_{10}$ reduction, P < 0.001 and 2.5 log$_{10}$ reduction, P < 0.001, respectively, Table 5 and Figure 1(a)) when samples were grown on CCDA medium. Moreover, when samples were grown on the Brilliance medium, Campylobacter spp. counts were significantly reduced in birds fed T2, T3 and T4 diets compared to the birds that were fed the T1 control diet (1.2 log$_{10}$ reduction, P = 0.043; 2.4 log$_{10}$ reduction, P = 0.001 and 3.1 log$_{10}$ reduction, P < 0.001, respectively, Table 5 and Figure 1(b)). There was a trend for reduced E. coli counts in broilers fed the T4 diet compared to broilers fed the T1 control diet (1.3 log$_{10}$ reduction, P = 0.083, Table 5 and Figure 1(c)). All birds from T2 and T3 had positive Campylobacter counts and only two birds from T4, each from different pens (pen 4 and pen 13), had a negative Campylobacter count. However, the other birds tested from pen 4 and 13 were positive. Analysis of the distribution of the counts in T1 birds and T1 pens showed that the distribution conformed to a Poisson distribution, where the mean and variance are equal, indicating that the counts were homogenous among control birds and pens and there was no significant over-dispersion of counts. In comparison, significant over-dispersion was observed for the Campylobacter spp. counts from Brilliance media for T3 and T4 (P = 0.03 and P < 0.001, respectively).

The MIC value for C. jejuni was >400 (Table 6) and >200 mg/l for E. coli and S. enterica, respectively (Table 7). After 24-h incubation, C. jejuni fluorescence increased by 29% when exposed to the PC digest at a dilution corresponding to 408 mg/l ferric tyrosine which increased by 13% when exposed to ferric tyrosine digest at 408 mg/l (Table 6).

After 20-h incubation, the turbidity of E. coli decreased by 61% with PC digest dilution corresponding to 49.9 mg/l ferric tyrosine digest and decreased by 14% with 200 mg/l ferric tyrosine digest (Table 7). Similarly, S. enterica turbidity decreased by 37% after 20-h incubation when exposed to the PC digest at 200 mg/l, and turbidity increased by 5% after 20 h when exposed to 200 mg/l ferric tyrosine (Table 7).

Discussion
The results from the present study showed that ferric tyrosine, when administered in the feed of broilers, significantly reduced caecal Campylobacter spp. (T3 and T4), reduced E. coli counts (T4), and significantly improved weight gain at d 42 but did not affect FCR. Under the conditions of this study, ferric tyrosine added to diets at 0.02, 0.05 and 0.20 g/kg which led to a 1.2 log$_{10}$, 2.4 log$_{10}$ and 3.1 log$_{10}$ CFU/g reduction in caecal Campylobacter spp. counts, respectively, when samples were grown on Brilliance media. These results agree with a recent study that evaluated ferric tyrosine in broiler diets (Khattak et al. 2018) where the authors reported caecal Campylobacter reductions of 0.8 log$_{10}$, 1.9 log$_{10}$ and 2.0 log$_{10}$ CFU/g in birds fed ferric tyrosine at 0.02, 0.05 and 0.20 g/kg, respectively. A

![Figure 1](https://example.com/figure1.png)

Figure 1. Boxplots showing the distribution of caecal Campylobacter spp. and E. coli counts at d 42. (a) Caecal Campylobacter spp. counts (log$_{10}$ CFU/g) grown on CCDA media, (b) caecal Campylobacter spp. counts (log$_{10}$ CFU/g) grown on Brilliance media, (c) caecal E. coli counts (log$_{10}$ CFU/g) grown on chromogenic media.

![Table 5](https://example.com/table5.png)

| Treatment | Dose (g/kg) | Campylobacter spp. | E. coli |
|-----------|-------------|--------------------|---------|
| T1 Control | 0           | 5.879$^b$ | 4.799$^b$ | 6.438$^b$ |
| T2 Ferric tyrosine | 0.02 | 4.986$^{bc}$ | 3.621$^{b}$ | 5.449$^{bc}$ |
| T3 Ferric tyrosine | 0.05 | 4.104$^{ab}$ | 2.399$^*$ | 5.736$^*$ |
| T4 Ferric tyrosine | 0.20 | 3.366$^*$ | 1.681$^*$ | 5.118$^*$ |
| SEM |             | 0.1301   | 0.1448   | 0.1843  |
| Treatment P-value (ANOVA) |       | <0.001   | <0.001   | 0.104   |

$^a$Caecal samples cultured on CCDA medium.
$^b$Caecal samples cultured on Brilliance medium.
No. of replicates = 6 replicate pens per treatment. Results show group least square mean of 6 replicate pens.
Values in same column with no common abc superscript are significantly different (P < 0.05).
Values in same column with no common xy superscript exhibit a near-significant trend (0.05 < P ≤ 0.10).
Results in bold text indicate a significant result (P < 0.05).
SEM: standard error of the mean.
In recent quantitative microbial risk assessment (QMRA) estimated that reducing caecal colonisation of birds at flock level by 2 log,0 or 3 log,10 CFU/g could reduce the incidence of human campylobacteriosis attributed to contaminated broiler meat by 76% and 90%, respectively (Romero-Barios et al. 2013). Another earlier QMRA estimated that the incidence of disease in humans could be reduced by 48% if carcass contamination with E. coli and S. enterica can be reduced by 1, 2 or 3 log,10, respectively (Bull et al. 2008) as confirmed by an epidemiological study that reported increased E. coli in chicken carcasses infected with Campylobacter spp. (Duffy et al. 2014). Moreover, translocation of E. coli to the liver, spleen and caecum increases in birds infected with C. jejuni (Awad et al. 2016). This evidence would suggest that Campylobacter spp. infection may increase the establishment of other pathogenic microbial populations, which could have serious implications for public health. In addition, the emergence of antibiotic resistance to Campylobacter spp. in humans and animals underlines the need for non-antibiotic alternatives to aid pathogen control on farms.

In this study, a natural challenge model was used, whereby study birds were housed in a barn that had housed broilers that previously tested positive for Campylobacter spp. on several occasions and were placed in pens containing dirty litter from an earlier study, in which birds had tested positive. This study design did not quantify the level of infection before or during the study. However, on d 42, all caecal samples collected from control birds tested positive for Campylobacter spp., and the counts followed a Poisson distribution indicating that the infection was homogeneous among individual birds and pens. As the layout of pens followed a randomised block design, it is assumed that all pens were exposed to a similar level of Campylobacter spp. challenge. It has been shown that a single bird harbouring low numbers of Campylobacter spp. can infect a whole flock, (Stern et al. 2001) and once a flock becomes Campylobacter positive, the surrounding environment becomes widely contaminated (Herman et al. 2003) which can persist for several weeks (Johnsen et al. 2006).

The MIC results showed that ferric tyrosine did not exert antimicrobial activity against the strains of C. jejuni, E. coli and S. enterica tested. The MICs of >400 and >200 mg/l were reported for C. jejuni and E. coli and S. enterica, respectively, which are much higher than MIC thresholds used to monitor antimicrobial susceptibility and resistance. According to recent guidelines, cut-off values for erythromycin, tetracycline and ciprofloxacin against C. jejuni are ≤4, ≤2 and ≤0.5 mg/l, respectively, while the cut-off values for ampicillin, ciprofloxacin and colistin when tested against Salmonella spp. and E. coli are ≤8, ≤0.06 and ≤2 mg/l, respectively (European Center for Disease Control (ECDC) 2016).

Significant improvements in final BW and weight gain were observed in the birds fed ferric tyrosine in comparison to the birds fed the control diet. Similar results were observed in the study conducted by Khattak et al. (2018). C. jejuni infection can significantly impair the growth performance of poultry (Awad et al. 2014a), and a highly significant negative association between infection and FCR has been reported (Sparks 2016). Campylobacter spp.

Table 6. Effect of ferric tyrosine on the growth of Campylobacter jejuni DSM4688 and minimum inhibitory concentrations (MICs).

| Bacterium       | Ferric tyrosine (mg/l) | Fluorescence after 24-h incubation (×10⁴) | Positive control digest¹ | Ferric tyrosine digest | MIC (mg/l) |
|-----------------|-----------------------|------------------------------------------|--------------------------|-----------------------|------------|
| C. jejuni       | 25.5                  | 2.58                                     | 2.38                     | >400                  |            |
| 51              | 2.90                  | 3.75                                     |                          |                       |            |
| 102             | 2.82                  | 2.83                                     |                          |                       |            |
| 204             | 3.27                  | 3.41                                     |                          |                       |            |
| 408             | 3.34                  | 2.68                                     |                          |                       |            |

¹ No product was added to the positive control digest. The concentration indicated that dilution of the digest was the same as that used for the corresponding ferric tyrosine digest.

Table 7. Effect of ferric tyrosine on the growth of Escherichia coli 156/97 F4 + and Salmonella enterica serovar Typhimurium strain IR715 and the minimum inhibitory concentrations (MICs).

| Bacterium       | Ferric tyrosine (mg/l) | Turbidity at 600 nm (≤) | Positive control digest¹ | Ferric tyrosine digest | MIC (mg/l) |
|-----------------|-----------------------|-------------------------|--------------------------|-----------------------|------------|
| E. coli         | 0.39                  | 0.35                    | 0.19                     | 0.36                  | >200       |
| 0.78            | 0.17                  | 0.38                     | 0.18                     | 0.34                  |            |
| 1.56            | 0.16                  | 0.35                     | 0.18                     | 0.36                  |            |
| 3.12            | 0.15                  | 0.32                     | 0.19                     | 0.36                  |            |
| 6.24            | 0.15                  | 0.29                     | 0.20                     | 0.37                  |            |
| 12.5            | 0.15                  | 0.23                     | 0.20                     | 0.37                  |            |
| 24.9            | 0.16                  | 0.20                     | 0.20                     | 0.37                  |            |
| 49.9            | 0.14                  | 0.15                     | 0.20                     | 0.36                  |            |
| 99.8            | 0.15                  | 0.15                     | 0.19                     | 0.33                  |            |
| 200             | 0.17                  | 0.15                     | 0.18                     | 0.31                  |            |
| S. enterica     | 0.39                  | 0.25                     | 0.65                     | 0.28                   | >200       |
| 0.78            | 0.26                  | 0.64                     | 0.27                     | 0.61                  |            |
| 1.56            | 0.25                  | 0.65                     | 0.28                     | 0.61                  |            |
| 3.12            | 0.22                  | 0.62                     | 0.28                     | 0.62                  |            |
| 6.24            | 0.24                  | 0.63                     | 0.28                     | 0.62                  |            |
| 12.5            | 0.25                  | 0.61                     | 0.28                     | 0.62                  |            |
| 24.9            | 0.25                  | 0.61                     | 0.27                     | 0.63                  |            |
| 49.9            | 0.26                  | 0.60                     | 0.27                     | 0.62                  |            |
| 99.8            | 0.26                  | 0.49                     | 0.24                     | 0.61                  |            |
| 200             | 0.25                  | 0.41                     | 0.23                     | 0.64                  |            |

¹ No product was added with the positive control digest. The concentration shown indicates that dilution of the digest was the same as that used for the corresponding ferric tyrosine digest.
infection downregulates the gene expression of various carrier proteins responsible for the absorption of nutrients (Awad et al. 2014b), leading to decreased nutrient adsorption and reduced performance.

Aspects of Campylobacter spp. pathogenesis remain poorly understood, particularly molecular host–pathogen interactions. Human histoblood group antigens (BgAgs) are often targeted by mucosal organisms to aid adhesion prior to invasion. The BgAgs-binding adhesins of C. jejuni have been identified as the major subunit protein of the flagella (FlaA) and the major outer membrane protein (MOMP) (Mahdavi et al. 2014). MOMP is a member of the trimeric bacterial porin family that assists the mucosal adhesion and invasion of C. jejuni (Mahdavi et al. 2014). Porins are involved in the uptake of nutrients through the outer membrane by passive diffusion along concentration gradients (Ferrara et al. 2016). MOMP is able to bind to multiple host cell membranes by promoting biofilm formation and auto-aggregation. The actual mode of action of ferric tyrosine is unknown, but it is thought that ferric tyrosine may be able to bind to MOMP and block the interaction of MOMP on the surface of Campylobacter spp. with the BgAgs of the gastrointestinal epithelial cells. As a result, it prevents colonisation of the avian gut by reducing biofilm formation. A recent study has demonstrated that ferric tyrosine inhibited biofilm formation in vitro (Khattak et al. 2018), which supports the assumed mode of action.

In conclusion, the results from the present study illustrate that ferric tyrosine can significantly reduce caecal Campylobacter spp. and E. coli and improve bird weight gain, indicating that this feed additive may contribute to control of Campylobacter spp. under commercial poultry production conditions.

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No potential conflict of interest was reported by the authors.

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