Effect of organic acids-essential oils blend and oat fiber combination on broiler chicken growth performance, blood parameters, and intestinal health

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A B S T R A C T
This study evaluated the effect of organic acids-essential oils blend with or without oat hulls (OH) on growth performance, organ weights, blood parameters, gut morphology, microbiota, and short-chain fatty acids (SCFA) in broiler chickens. Day-old broiler chickens were randomly allocated to 4 dietary treatments consisting of 1) a corn-soybean meal-wheat based diet (BAS), 2) BAS + 0.05% bacitracin methylene disalicylate (BMD), 3) BAS + protected organic acids-essential oils at 300 g/1,000 kg of feed (OE), and 4) BAS + protected organic acids-essential oils at 300 g/1,000 kg of feed + 3% OH (OEOH), in 8 replicate groups. Feeding was in starter (d 0 to 14), grower (d 14 to 24), and finisher (d 24 to 36) phases.

Body weight (BW), feed intake (FI), feed conversion ratio (FCR), and mortality were determined weekly. On d 36, 8 chickens per treatment were sampled for blood biochemistry, organ weights, cecal SCFA production, and microbiota. Treatments had no effect on FI and FCR at all phases. Both OE and OEOH treatments reduced (P < 0.001) the body weight gain of birds at the starter phase. Birds fed the OEOH treatment had higher (P < 0.001) gizzard weight, while those offered the BMD diet showed a tendency (P = 0.08) to have higher cecal weight. Birds in the OEOH treatment recorded increased ileal villus height and villus height-to-crypt depth ratio, as well as reduced duodenal crypt depth, while birds in the OE treatment had increased jejunal villus height and villus height-to-crypt depth ratio. Both OEOH and OE treatments increased the number of goblet cells produced in the duodenum and jejunum. Treatments had no effect on FI and FCR at all phases. Both OE and OEOH treatments reduced (P < 0.001) the body weight gain of birds at the starter phase. Birds fed the OEOH treatment had higher (P < 0.001) gizzard weight, while those offered the BMD diet showed a tendency (P = 0.08) to have higher cecal weight. Birds in the OEOH treatment recorded increased ileal villus height and villus height-to-crypt depth ratio, as well as reduced duodenal crypt depth, while birds in the OE treatment had increased jejunal villus height and villus height-to-crypt depth ratio. Both OEOH and OE treatments increased the number of goblet cells produced in the duodenum and jejunum. Treatments had no effect on SCFA concentrations. Birds in the OE treatment recorded the lowest concentration of blood urea (P = 0.05) and cholesterol (P < 0.05). Both OE and OEOH treatments increased (P < 0.05) the relative abundance of potentially beneficial bacteria in the genus Firmicutes, unclassified, Ruminococcus, Turicibacter, and Erysipelotrichaceae, unclassified, while reducing (P < 0.001) the relative abundance of potentially harmful Coprobacillus. Conclusively, both protected organic acids-essential oils blend and its combination with oat fibers show potential as tools to achieve antibiotics reduction in broiler production.

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1. Introduction

To preserve the potency of antibiotics, there has been a drastic reduction in their use in food animal production. In the past, antibiotics have played a major role in promoting growth and maintaining gut functionality in broiler chickens (Mehdi et al., 2018). With the current reduction and anticipated elimination of the preventive use of medically important antibiotics in poultry production, there is a need to investigate potential alternatives so that farmers can maintain or improve productivity.

Organic acids, also known as acidifiers, occur naturally and have been used as feed preservatives to prevent contamination by...
bacteria and fungi (Theron and Lues, 2011). They have also been reported to suppress the growth of acid intolerant pathogenic bacteria (Dhawale, 2005) and reduce pH in the stomach, which enhances peptic activity and increase the digestibility of nitrogen, phosphorus, and minerals (Dibner and Buttin, 2002). Organic acids have been reported to reduce bacterial production of toxic components and change the morphology of poultry intestinal wall in a way that reduced colonization of pathogens, thus preventing epithelial cell damage (Langhout, 2000). Cherinton et al. (1991) and Hajati (2018) presented the mode of action of organic acids on bacteria as penetrating the cell wall of certain types of bacteria, including Escherichia coli, Salmonella spp., and Clostridium perfringens and some coliforms and disrupting their normal physiology, by inhibiting enzymatic reactions and denaturing proteins and DNA.

Essential oils are plant-derived mixtures of volatile compounds with less toxicity (Zhai et al., 2018) and have the ability to improve feed consumption and utilization in chickens (Krishan and Narang, 2014). Essential oils have been reported to possess antioxidant (Fernandez-Panchon et al., 2008), anti-inflammatory (Craig 2001), immunomodulatory (Szigi et al., 1998), and digestion enhancement properties (Jang et al., 2007), which result in enhanced production and health of chickens.

The use of organic acids and essential oils as possible alternatives for antibiotics and immune modulator is gaining tremendous interest in the poultry industry (Yang et al., 2018). However, a combination of these 2 compounds may be more effective in improving gut health and modulating the immune system of broiler chickens. Recent studies that investigated the beneficial effects of protected organic acids-essential oils blends in broiler chickens have reported increased growth performance and intestinal morphology (Abdelli et al., 2020; Gao et al., 2019; Yang et al., 2018), improved intestinal integrity and barrier function (Stefanello et al., 2020; Pham et al., 2020), and increased digestive enzyme activity (Yang et al., 2018, 2019). To our knowledge, only a few studies (Pham et al., 2020) have investigated the effect of the association of organic acids and essential oils on gut microbiota using next-generation DNA sequencing methods. Only a few studies (Yang et al., 2019) have also reported the effect of such blends on short-chain fatty acids (SCFA), and production and blood parameters in broiler chickens.

High fiber ingredients have proven prebiotic effects, and their use in monogastric animals to modulate gut microbiota, promote gut health and growth performance has been reported (Adewole, 2020; Ndou et al., 2018). Oat (Avena sativa) hulls (OH) have high contents of lignin, an insoluble fiber (Kim et al., 2008; Jimenez- Moreno et al., 2009) and has the potential to modulate gastrointestinal microbiota and increase SCFA production, thus enhancing gut barrier integrity (Ndou et al., 2018). Short-chain fatty acids are microbial metabolites, which have been shown to exert multiple beneficial effects on mammalian energy metabolism through the complex interplay between diet, gut microbiota, and intestinal barrier, and immunity (Den Besten et al., 2013). The potential of the combinational use of organic acids and essential oils blends (having an antibacterial effect) with OH (having prebiotic effects) to promote gut microbiota of poultry has not been explored. In reality, it is more likely that a combination of additives will achieve the needed breakthroughs to eliminate or reduce the use of antibiotics in poultry production. This could be observed in the limited successes that have been recorded in most additives that were singly used. It is anticipated that this combinational approach would provide new insight into the application of non-antibiotic compounds and enhance production performance and health of chickens. Moreover, OH are currently under-utilized products, hence contributing to environmental burden (Perruzza, 2010); their use in poultry production would not only reduce feed cost but also improve ecological sustainability. Therefore, the objective of this study was to determine the effect of a protected organic acids and essential oils blend with or without OH on growth performance, organ weights, blood parameters, and ceca SCFA and microbiota in broiler chickens.

2. Materials and methods

The experimental procedures for this study were approved by Dalhousie University Animal Use and Care Committee (Ethical code 2020-011), and chickens were cared for in accordance with the guidelines of the Canadian Council on Animal Care (CCAC, 2009).

2.1. Birds and housing

Day-old broiler chickens (Ross 308) were obtained from a commercial source. Upon arrival, chicks (mixed-sex) were weighed in groups of 26 birds and assigned to floor pens (0.93 m × 2.14 m), at a stocking density of 0.076 m²/bird. The temperature in the broiler room was monitored daily and was gradually reduced from 30 to 22.6 °C from d 0 to 36. The lighting program was set to produce 18 h of light and 6 h of darkness throughout the experimental period, and illumination was gradually reduced from 20 lx on d 0 to 5 lx on d 36.

2.2. Ingredients, diets, and chemical analysis

The OH used in this study was obtained from Grain Millers, Yorton, Saskatchewan, Canada. They were received as unground coarse particle-sized. The chemical composition of the coarse OH has been reported previously by Adewole (2020). The combination of organic acids and essential oils used was provided by JEFo Nutrition Incorporation, Saint-Hyacinthe, QC, Canada as a Protected Organic Acids and Essential Oils – P(OA + EO) formula containing organic acids (fumaric, sorbic, malic, and citric acids) and essential oils (thymol, vanillin, and eugenol). The active ingredients are embedded by Jefo matrix technology, made of an emulsion of hydrogenated vegetable oil according to U.S. Patent WO2018089516 (Ferket et al., 2017). Four dietary treatments were assigned to 8 replicate pens per treatment in a completely randomized design. The dietary treatments consisted of a corn-soybean meal-wheat basal diet (BAS), BAS + 0.05% bacitracin methylene disalicylate (BMD), BAS + P(OA + EO) at 300 g/1,000 kg of feed (OE) and BAS + P(OA + EO) + 3% OH (OEHO). The BAS, BMD, and OE diets were isoennergetic and iso-nitrogenous, but the OEHO diet had reduced energy due to the addition of OH (Table 1). The reduced energy as a result of OH addition is attributable to the low energy value of oat hulls compared to their cereal components (McNab and Boorman, 2002). Mossami (2011) reported that oats with higher hull content have less metabolizable energy compared to less-hulled oats. All diets were formulated on a digestible amino acid basis and analyzed according to AOAC (1990) nutrient requirements for broiler chickens. The phase-feeding program that consisted of starter phase (d 0 to 14), grower phase (d 14 to 24), and finisher phase (d 24 to 36) was utilized. Diets were fed in crumbled form during the starter phase and in pelleted form during the grower and finisher phases. The ingredient and nutritional compositions of the diets in phases 1 to 3 are presented in Table 1. The diet samples were finely ground and were all analyzed for dry matter, crude protein (CP), fat, and minerals. Dry matter was determined according to AOAC (1990) method (925.06) by oven drying a 5.0 g sample at 105 °C overnight. Nitrogen content was determined using the combustion method (990.03: AOAC, 1990) with an N analyzer (Model CNS-2000; LECO Corp., St. Joseph, MO).
determined after hexane extraction (Method 920.39; AOAC, 1990) and CP was calculated as N × 6.25. Ether extract in samples was determined after hexane extraction (Method 920.39; AOAC, 1990) in an Ankom extraction system (Macedon, NY). Samples were analyzed for minerals (Ca, Na, K, Mg, P, Mn, and Zn) after ashing at 500 °C in an Ankom furnace. Minerals were determined using inductively coupled plasma mass spectrometry (ICP-AES; Vista, Varian, Palo Alto, CA) according to the method of AOAC (2005, 250 g/kg). Lysine HCl 0.03 0.03 0.03 0.03
Available phosphorus 0.48 0.48 0.48 0.48 0.50 0.50 0.50 0.50 0.50 0.50 0.50 0.50
Available phosphorus 0.48 0.48 0.48 0.48 0.44 0.44 0.44 0.44 0.39 0.39 0.39 0.39
Dextrin solubilized 0.40 0.40 0.40 0.40 0.37 0.37 0.37 0.37 0.38 0.38 0.38 0.38
Pelle binding agent 0.50 0.50 0.50 0.50 0.50 0.50 0.50 0.50 0.50 0.50 0.50 0.50
BMD 110 G 0.05 0.05 0.05 0.05 0.05 0.05 0.05 0.05
Vitamin/Mineral Premix 0.50 0.50 0.50 0.50 0.50 0.50 0.50 0.50 0.50 0.50 0.50 0.50

Table 1: Ingredient and nutrient compositions of experimental diets (as-fed basis, %, unless otherwise stated)².

| Item | 1 to 14 | Production period (days of age) | 14 to 24 | 24 to 36 |
|------|---------|--------------------------------|---------|---------|
|      | BAS     | BMD | OE | OEOH | BAS | BMD | OE | OEOH | BAS | BMD | OE | OEOH |
| Ingredients | | | | | | | | | | | | |
| Corn | 41.3 | 41.2 | 41.3 | 37.9 | 44.3 | 44.2 | 44.3 | 40.9 | 48.5 | 48.4 | 48.4 | 45.1 |
| Soybean meal | 40.2 | 40.2 | 40.2 | 40.6 | 36.5 | 36.5 | 36.5 | 36.9 | 31.5 | 31.5 | 31.5 | 31.9 |
| Wheat | 10.0 | 10.0 | 10.0 | 10.0 | 10.0 | 10.0 | 10.0 | 10.0 | 10.0 | 10.0 | 10.0 | 10.0 |
| Oat hulls | – | – | 0.03 | 0.03 | 0.03 | 0.03 | 0.03 | 0.03 | – | – | – | – |
| Organic acids-essential oils | 3.44 | 3.47 | 3.46 | 3.46 | 4.59 | 4.63 | 4.62 | 4.62 | 5.67 | 5.70 | 5.69 | 5.69 |
| Animal/Vegetable fat | 1.80 | 1.80 | 1.80 | 1.79 | 1.65 | 1.65 | 1.65 | 1.64 | 1.52 | 1.52 | 1.52 | 1.51 |
| Limestone | 1.23 | 1.23 | 1.23 | 1.24 | 1.06 | 1.06 | 1.06 | 1.07 | 0.93 | 0.93 | 0.93 | 0.94 |
| DL-Methionine Premix | 0.61 | 0.61 | 0.61 | 0.62 | 0.53 | 0.53 | 0.53 | 0.54 | 0.49 | 0.49 | 0.49 | 0.50 |
| Lysine HCl | 0.03 | 0.03 | 0.03 | 0.02 | – | – | – | – | 0.01 | 0.009 | 0.01 | 0.003 |
| Iodized salt | 0.40 | 0.40 | 0.40 | 0.40 | 0.37 0.37 0.37 0.37 0.38 0.38 0.38 0.38
| Pellet binding agent | 0.50 | 0.50 | 0.50 | 0.50 | 0.50 | 0.50 | 0.50 | 0.50 | 0.50 | 0.50 | 0.50 | 0.50 |
| BMD 110 G | – | 0.05 | – | – | – | 0.05 | – | – | – | 0.05 | – | – |
| Vitamin/Mineral Premix | 0.50 | 0.50 | 0.50 | 0.50 | 0.50 | 0.50 | 0.50 | 0.50 | 0.50 | 0.50 | 0.50 | 0.50 |

Table 1: Ingredient and nutrient compositions of experimental diets (as-fed basis, %, unless otherwise stated)².

| Item | 1 to 14 | Production period (days of age) | 14 to 24 | 24 to 36 |
|------|---------|--------------------------------|---------|---------|
|      | BAS     | BMD | OE | OEOH | BAS | BMD | OE | OEOH | BAS | BMD | OE | OEOH |
| Metabolizable energy, kcal/kg | 3.000 | 3.000 | 3.000 | 2.909 | 3.100 | 3.100 | 3.100 | 3.099 | 3.200 | 3.200 | 3.200 | 3.109 |
| Calcium | 0.96 | 0.96 | 0.96 | 0.96 | 0.87 | 0.87 | 0.87 | 0.87 | 0.78 | 0.78 | 0.78 | 0.78 |
| Available phosphorus | 0.48 | 0.48 | 0.48 | 0.48 | 0.44 | 0.44 | 0.44 | 0.44 | 0.39 | 0.39 | 0.39 | 0.39 |
| Digestible lysine | 1.28 | 1.28 | 1.28 | 1.28 | 1.16 | 1.16 | 1.16 | 1.17 | 1.02 | 1.02 | 1.03 | 1.03 |
| Digestible methionine + Cystine | 0.95 | 0.95 | 0.95 | 0.95 | 0.87 | 0.87 | 0.87 | 0.87 | 0.80 | 0.80 | 0.80 | 0.80 |
| Sodium | 0.19 | 0.19 | 0.19 | 0.19 | 0.18 | 0.18 | 0.18 | 0.18 | 0.18 | 0.18 | 0.18 | 0.18 |

2.3. Sampling and measurements

On d 8 and every week thereafter, body weight (BW) and feed intake (FI) were measured for each pen, and body weight gain (BWG) and feed conversion ratio (FCR) which was corrected for mortality were calculated. On d 36, one chicken was randomly selected from each pen, individually weighed, and euthanized by electrical stunning and exsanguination. Blood samples were collected from the euthanized bird into 5-mL heparinized tubes for the determination of blood biochemistry. The weights of the bursa of Fabricius and spleen and the empty weights of gizzard and ceca were determined by trained personnel. Digesta from the pair of ceca were mixed and divided into 2 subsamples; one part was stored in plastic RNase-, and DNase-free tubes, placed in liquid nitrogen and kept at −80 °C for analyses of the gut microbiota. The other part was placed in BioFreeze kits (Alimetrics Diagnostics, Espoo, Finland) for the determination of SCFA. For intestinal morphology determination, a 0.5 cm tissue section was collected from the middle of the duodenum, jejunum, and ileum and preserved in 10% formalin.

2.3.1. Blood biochemistry analysis

Samples for blood biochemical analysis were centrifuged at 5,000 × g at 4 °C for 10 min and shipped on ice to Atlantic Veterinary College, University of Prince Edward Island Pathology Laboratory, where samples were analyzed using cobas 6,000 analyzer series.

2.3.2. Short-chain fatty acids and bacterial density

Samples were immediately preserved using BioFreeze sampling kits (Alimetrics Diagnostics Ltd., Espoo, Finland) following the recommended protocol by the manufacturer. Bacterial density and SCFA profiles were analyzed by Alimetrics Diagnostics Ltd. as described previously by Adewole (2020) and Apajalahti et al. (2019).

2.3.3. Gut morphology

Gut morphology (duodenum, jejunum, and ileum) recovered from 8 birds per treatment were subjected to gut morphological processing using the “I See Inside” (ISI) histology methodology (Belote et al., 2018), adapted from Kraeski et al. (2017). The ISI methodology is based on a numeric score of histological alterations. For each macroscopic and microscopic alteration observed, an
2.3.5. Bioinformatics analysis

The analytical flowchart for bioinformatics analysis of cecal microbiota data is presented in Appendix Fig. 1. Amplicons were sequenced with an Illumina MiSeq using the 300-bp paired-end kit (v.3). Sequences were denoised, taxonomically classified using Greengenes (v. 13_8) as the reference database, and clustered into operational taxonomic units (OTU) with the mothur software package (v. 1.39.5) (Schloss et al., 2009), following the recommended procedure (https://www.mothur.org/wiki/MiSeq_SOP; accessed Nov 2017). The potential for contamination was addressed by co-sequencing DNA amplified from specimens and from 3 each of template-free controls and extraction kit reagents processed the same way as the specimens. Operational taxonomic units were considered putative contaminants (and were removed) if their mean abundance in controls reached or exceeded 25% of their mean abundance in specimens. Alpha diversity was estimated with the Shannon index on raw OTU abundance tables after filtering out contaminants. The significance of diversity differences was tested by analysis of variance (ANOVA). To estimate beta diversity across samples, we excluded OTU occurring with a count of less than 3 in at least 10% of the samples and then computed Bray–Curtis indices. We visualized beta diversity, emphasizing differences across samples, using principal coordinate analysis (PCoA) ordination. Variation in community structure was assessed with permutational multivariate analyses of variance (PERMANOVA) with treatment group as the main fixed factor and using 9,999 permutations for significance testing. Statistical analysis on cecal microbiota data was conducted in the R environment.

2.4. Statistical analysis

One-way ANOVA was carried out using the mixed procedure of SAS with treatments (BAS, BMD, OE, or OEHO) as factor and the following parameters as variables: feed intake, body weight, body weight gain, feed conversion ratio, concentrations of total SCFA, acetic acid, propionic acid, butyric acid, valeric acid, lactic acid, branched chained fatty acids (BCFA), volatile fatty acids (VFA), and blood chemistry parameters. Normal probability plot of residuals was used to ascertain the normality of all datasets in the same statistical package. Datasets found to be non-normal were log base 10 transformed. Lipase dataset was inverse transformed, whereas the total bilirubin dataset failed to achieve normality with transformation. Hence a non-parametric Kruskal–Wallis test was used to test the effect of treatment on levels of blood total bilirubin acid. Following ANOVA, differences between significant means were tested using Tukey’s honest significant difference (HSD) test in the same statistical package. Parametric gut morphology data were analyzed by one-way ANOVA, while non-parametric data were analyzed by Mood’s median test (Mangiafico, 2016), in the same statistical package. Analyzed data are presented as means, standard error of the mean (SEM), and probability values. Values were considered statistically different at $P < 0.05$.

3. Results

3.1. Growth performance

The effect of all dietary treatments on the growth performance of birds is presented in Table 2. No effect of dietary treatment on FI was recorded in this study, across all phases. In the starter phase, the BWG of birds fed the BAS and BMD were not different, but they each recorded higher ($P < 0.001$) BWG than birds fed the OE and OEHO diets. In the grower phase, birds fed the BMD diet had the highest BWG and was significantly ($P < 0.005$) different from birds fed the OEHO diet; birds fed the BAS and OE diets had intermediate BWG. In the finisher phase, BWG was not affected by dietary treatments. Over the entire trial period, birds offered the BMD diet recorded the highest BWG, which was significantly higher ($P = 0.01$) than birds in the OEHO treatment. Birds offered the BAS, and OE treatments retained intermediate BWG values at the end of the trial. Dietary treatments recorded no significant effect on FCR in this study throughout all phases. Nonetheless, at the end of the trial period, dietary treatment OE had numerically better FCR than the other treatments.

Table 2

| Item                          | Treatments1          | SEM2   | P-value   |
|-------------------------------|----------------------|--------|-----------|
| Feed intake, g/bird           | BAS                  | BMD    | OE        | OEHO     |
| Starter (d 1 to 14)           | 542                  | 548    | 522       | 519      | 10.64  | 0.1725 |
| Grower (d 14 to 28)           | 1,890                | 1,857  | 1,762     | 1,768    | 40.89  | 0.0037 |
| Finisher (d 28 to 36)         | 1,165                | 1,153  | 1,148     | 1,156    | 31.44  | 0.0739 |
| Overall (d 1 to 36)           | 3,585                | 3,587  | 3,438     | 3,441    | 88.73  | 0.3577 |
| Body weight gain, g/bird      |                      |        |           |          |        |        |
| Starter (d 1 to 14)           | 447a                 | 455a   | 413b      | 413b     | 7.206  | 0.0004 |
| Grower (d 14 to 28)           | 1,168ab              | 1,177ab| 1,118ab   | 1,088ab  | 21.92  | 0.0001 |
| Finisher (d 28 to 36)         | 817                  | 828    | 836       | 802      | 27.59  | 0.0000 |
| Overall (d 1 to 36)           | 2,449ab              | 2,460ab| 2,371ab   | 2,303ab  | 35.14  | 0.0134 |
| Feed conversion ratio         |                      |        |           |          |        |        |
| Starter (d 1 to 14)           | 1.21                 | 1.21   | 1.23      | 1.24     | 0.017  | 0.3737 |
| Grower (d 14 to 28)           | 1.62                 | 1.58   | 1.58      | 1.63     | 0.030  | 0.5159 |
| Finisher (d 28 to 36)         | 1.44                 | 1.40   | 1.40      | 1.45     | 0.064  | 0.8830 |
| Overall (d 1 to 36)           | 1.50                 | 1.46   | 1.45      | 1.46     | 0.028  | 0.5855 |

a,b In a row, means assigned different lowercase letters are significantly different, $P < 0.05$ (Tukey’s procedure).

1 BAS, basal diet; BMD, antibiotic diet; OE, diet containing 300 g of protected organic acids-essential oils blend/1,000 kg of feed; OEHO, diet containing 300 g of protected organic acids-essential oils blend/1,000 kg of feed + 3% coarse oat hulls.

2 Standard error of means.
3.2. Organ weights

Of all relative organ (bursa, spleen, gizzard, and ceca) weights and bird slaughter weight examined in this study, only the relative empty weight of gizzard was affected by dietary treatments (Table 3). Birds fed the OEOH treatment had significantly higher ($P < 0.001$) gizzard weight compared to other dietary treatments. Birds fed the BMD diet showed a tendency ($P = 0.08$) to have higher cecal weight compared to other treatments.

3.3. Ceca short-chain fatty acid concentration

Dietary treatments had no significant effect on SCFA concentrations in this study (Table 4). Nonetheless, birds of the OEOH treatment recorded the highest concentrations of acetic acid, butyric acid, lactic acid, total SCFA, and volatile fatty acids (VFA). Concentrations of propionic and valeric acid were numerically higher in the BMD treatment, compared to the other groups, and BCFA concentrations were found highest in the BAS treatment. No effect of dietary treatment was also recorded for Total Eubacteria (Table 4).

3.4. Blood parameters

The effect of dietary treatments on blood biochemical components is presented in Table 5. Birds in the OE treatment recorded the lowest levels of urea ($P = 0.05$) and cholesterol ($P < 0.05$), relative to other treatments. The levels of blood enzymes and proteins were not significantly affected by dietary treatments in this study. However, blood amylose levels in the OE treatment was at least 10% higher ($P > 0.05$) than the other treatments.

3.5. Gut morphology

Gut morphometric properties and intestinal health indicators in the three segments of the small intestine (duodenum, jejunum, and ileum) are presented in Table 6. In the duodenum, the antibiotic treatment recorded the lowest ($P < 0.001$) duodenal villus length, with other treatments being statistically similar. Duodenal crypt depth of birds in the OEOH treatment was the most shallow ($P < 0.05$) and was 18% shallower than those of the OE treatment. Similarly, the duodenal villus height-to-crypt depth ratio in the OEOH treatment was 23% higher ($P < 0.05$) than that of the antibiotic treatment, whereas other treatments expressed statistical similarity. In terms of duodenum health index, ISI total scores were not significantly different between treatments, even though OEOH treatment recorded a higher ($P < 0.05$) increase in goblet cells relative to other treatments. Birds in the OE treatment had the highest ($P < 0.001$) villus length and villus height-to-crypt depth ratio in the jejunum, compared to other treatments with respectively 44% and 46% improvement by comparison to the BAS treatment. Although crypt depth in the jejunum was not significant between treatments, crypt depth in the OEOH treatment was the most shallow ($P > 0.05$). Compared to other treatments, antibiotic treatment recorded numerically higher ISI total scores, attributable to significantly increased ($P < 0.05$) goblet cells; nonetheless, increased goblet cells were statistically similar across treatments. In the ileum, OEOH treatment recorded the highest ($P < 0.001$) villus height and villus height-to-crypt depth ratio, while crypt depth was found deepest ($P < 0.05$) in the BAS treatment, relative to the BMD and OE treatments. Birds in the OEOH treatment had intermediate crypt depth. Additionally, ileum ISI total scores were significantly higher ($P < 0.001$) in the OE treatment, relative to the BMD and the OEOH treatment; the BAS treatment had intermediate ISI total scores.

### Table 3

Effect of a protected organic acids-essential oils blend with or without oat hulls on relative organ weights of broiler chickens (% BW, unless otherwise stated).

| Item          | BAS     | BMD     | OE      | OEOH    | SEM² | $P$-value |
|---------------|---------|---------|---------|---------|------|-----------|
| Slaughter weight, kg | 2.80    | 2.82    | 2.75    | 2.63    | 0.080| 0.3559    |
| Bursa          | 0.18    | 0.17    | 0.18    | 0.20    | 0.006| 0.6352    |
| Spleen         | 0.10    | 0.09    | 0.10    | 0.08    | 0.008| 0.2431    |
| Gizzard        | 1.17*   | 1.17*   | 1.19*   | 1.67*   | 0.073| <0.0001   |
| Ceca¹          | 0.36    | 0.42    | 0.39    | 0.34    | 0.024| 0.0765    |

a In a row, means assigned different lowercase letters are significantly different. $P < 0.05$ (Tukey’s procedure).

### Table 4

Effect of a protected organic acids-essential oils blend with or without oat hulls on cecal short chain fatty acids (mmol/kg) and total eubacteria (16S rRNA copies/gram of sample) of broiler chickens.

| Item                  | BAS | BMD | OE | OEOH | SEM² | $P$-value |
|-----------------------|-----|-----|----|------|------|-----------|
| Acetic acid           | 51.5| 51.1| 54.9| 59.5  | 5.622| 0.6946    |
| Propionic acid        | 4.29| 5.26| 4.24| 3.87  | 0.459| 0.2071    |
| Butyric acid          | 12.2| 12.3| 12.1| 12.8  | 1.466| 0.9880    |
| Valeric acid          | 0.94| 1.06| 0.98| 0.831 | 0.087| 0.2589    |
| Lactic acid           | 3.00| 1.58| 2.98| 3.05  | 0.914| 0.3812    |
| Total SCFA            | 74.2| 71.3| 77.2| 80.3  | 7.299| 0.8395    |
| BCFA                  | 2.23| 1.78| 1.78| 1.85  | 0.262| 0.5731    |
| VFA                   | 71.2| 69.8| 74.2| 77.2  | 7.325| 0.8905    |
| Total eubacteria      | 12.0| 11.9| 11.8| 12.0  | 0.019| 0.6832    |

SCFA = short chain fatty acids; BCFA = branched chain fatty acids; VFA = volatile fatty acids.

¹ BAS, basal diet; BMD, antibiotic diet; OE, diet containing 300 g of protected organic acids-essential oils blend/1,000 kg of feed; OEOH, diet containing 300 g of protected organic acids-essential oils blend/1,000 kg of feed + 3% coarse oat hulls.
² Standard error of means.

Datasets from bioinformatic analysis of cecal samples revealed 53,470 OTU at an average of 14, 285 quality-filtered reads per sample at the 97% similarity level. More information on sequence quality is presented in Appendix Fig. 2A and B. The relative abundance of the most abundant taxa are presented in Figs. 1 and 2 and Appendix Figs. 3–6. Firmicutes was the most abundant (>85%) phylum across all treatment groups, and Lachnospiraceae was the most abundant family (>65%) across treatment groups. Phylum level analysis showed that treatment affected ($P < 0.05$) the relative abundance of Tenericutes but not the abundances of other bacterial phyla in d 36 (Fig. 1B). At the genus level, the relative abundance of Firmicutes_unclassified, RF39_unclassified, Rumino-coccus, Turicibacter, Erysipelotrichaceae_unclassified, and g__SM53 general were all enhanced ($P < 0.05$) by OE and OEOH treatments (Fig. 2). This relative abundance was statistically similar to those of the BMD treatment, in most cases. The OE treatment increased ($P < 0.05$) the relative abundance of Streptococcus; however, the relative abundance of Coprobacillus was reduced ($P < 0.001$) in both OE and OEOH treatments (Fig. 2E and H).

Alpha diversity, a measure of species richness and evenness expressed as the number of observed OTU by Shannon diversity index, showed no significant difference between treatments.
Effect of a protected organic acids-essential oils blend with or without oat hulls on blood biochemical components of broiler chickens.

| Item                      | BAS      | BMD     | OE      | OEOH     | SEM² | P-value |
|---------------------------|----------|---------|---------|----------|------|---------|
| **Electrolytes and minerals, mmol/L** |          |         |         |          |      |         |
| Sodium                    | 152      | 154     | 162     | 169      | 9.97 | 0.5941 |
| Potassium                 | 5.76     | 5.09    | 5.61    | 5.95     | 0.31 | 0.2572 |
| Chloride                  | 111      | 112     | 119     | 124      | 8.47 | 0.6493 |
| Calcium                   | 2.68     | 2.72    | 2.75    | 2.94     | 0.14 | 0.6026 |
| Phosphorus                | 1.77     | 1.59    | 1.74    | 1.77     | 0.13 | 0.7706 |
| Magnesium                 | 0.83     | 0.82    | 0.73    | 0.82     | 0.06 | 0.6080 |
| **Metabolites, mmol/L**   |          |         |         |          |      |         |
| Urea                      | 0.34ᵃ     | 0.26ᵇ    | 0.24ᵇ   | 0.29ᵃ    | 0.03 | 0.0473 |
| Creatinine                | 2.00     |         | 1.13    | 3.13     | 1.14 | 0.6198 |
| Glucose                   | 14.7ᵃ     |         | 17.8    | 17.3     | 1.15 | 0.1572 |
| Cholesterol               | 2.94ᵇᵃ    | 3.41ᵇ   | 2.46ᵇ   | 3.29ᵃ    | 0.25 | 0.0205 |
| Iron                      | 17.9     | 23.0    | 21.9    | 16.4     | 2.35 | 0.1685 |
| Bile acids                | 17.0     | 14.0    | 18.3    | 18.3     | 3.29 | 0.7734 |
| Uric acid                 | 401      | 347     | 347     | 372      | 49.6 | 0.9367 |
| Total Bilirubin           | 0.63     | 0.38    | 0.25    | 0.37     | 0.18 | 0.5163 |
| **Enzymes, U/L**          |          |         |         |          |      |         |
| Amylase                   | 410      | 483     | 654     | 586      | 144  | 0.4234 |
| Alkaline phosphatase      | 3243     | 2722    | 2972    | 3303     | 634  | 0.7807 |
| Creatine kinase           | 19,820   | 19,784  | 19,670  | 19,011   | 4011 | 0.3819 |
| Acid phosphatase          | 324      | 308     | 225     | 320      | 36.6 | 0.1255 |
| Gamma-glutamyl transferase| 10.0     | 11.9    | 10.5    | 14.0     | 1.57 | 0.2933 |
| Lipase                    | 22.0     | 17.8    | 20.0    | 20.3     | 4.15 | 0.9113 |
| **Proteins, g/L**         |          |         |         |          |      |         |
| Total protein             | 28.0     | 28.4    | 24.3    | 31.5     | 2.30 | 0.1981 |
| Albumin                   | 12.0     | 12.1    | 10.6    | 13.4     | 1.02 | 0.3180 |
| Globulin                  | 16.0     | 16.3    | 13.6    | 18.1     | 1.38 | 0.1704 |

¹ In a row, means assigned different lowercase letters are significantly different, P < 0.05 (Tukey’s procedure).
² SEM – standard error of the mean; ISI – intestinal score.

Nonetheless, BAS had the highest Shannon index, 5.889 ± 0.37 (Shannon mean ± Shannon standard deviation), while the BMD treatment recorded the lowest Shannon index, 5.453 ± 0.84. The similarity between the cecal microbiota composition, based on the Bray–Curtis dissimilarities index, is graphically presented in a PCoA ordination plot (Fig. 4). Permutational analysis of variance determined significant differences (P < 0.05) in beta-diversity among treatments. Post-hoc pairwise contrast among treatments revealed significant differences and tendencies between treatments: BAS vs. OE (P = 0.05), BAS vs. OE vs. OEOH (P = 0.06), and OE vs. OEOH (P = 0.06). Differential abundance testing revealed 13 differentially abundant OTUs at the species level among treatments (Table 7). In the BAS treatment, species belonging to unclassified Tenericutes RF39 and unclassified Firmicutes were differentially abundant relative to other treatments. Unclassified Ruminococcaceae and Turicibacter species were differentially abundant in the BMD treatment. The OE treatments were differentially comprised of unclassified Clostridiales and Firmicutes species. A host of unclassified Firmicutes and other unclassified bacteria species were also found differentially abundant in the OEOH treatment group.

4. Discussion
Several independent studies have reported the beneficial effects of organic acids (Paul et al., 2007; Adil et al., 2010; Kamal and Ragaa, 2014), essential oils (Brenes and Roura, 2010; Franz et al., 2010), and dietary fibers (specifically moderate levels of OH) (Hetland and Svihus, 2001; Mateos et al., 2012; Scholey et al., 2020) in poultry nutrition. Here, we present for the first time the synergistic effect of a protected organic acids and essential oils formula (containing organic acids: fumaric, sorbic, malic, citric acids; and essential oils:...
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Liu et al., 2017; Stefanello et al., 2020), turkey poults (Giannenas et al., 2014), and weaned piglets (Yang et al., 2019). Conversely, Wang et al. (2019) observed no effect of encapsulated organic acids and essential oils blend on body weight, mortality, average daily egg weight, and feed-to-egg ratio in laying hens, but observed a significant increase in resistance to mechanical stress for eggs as well as improvements in intestinal tract morphology and counts of bifidobacteria in cecal digesta. With respect to OH inclusion, Jiménez-Moreno et al. (2016) reported improved average daily gain and feed-to-gain ratio in broilers offered either of oat hulls, rice hulls, or sunflower hulls at 2.5% or 5% inclusion levels. García et al. (2019) recently observed no effect of 3% OH feeding on growth performance in broiler chickens. The varying performance effects observed from blends of organic acids and essential oils and OH supplementation across studies can be attributable to several factors, including organic acids and essential oils mix types and doses (e.g., free or protected blends, protection type, nature of essential oils and acids, the formula of the mix, etc.), the dietary composition of basal diets, age of the bird, management and environmental conditions (Zeng et al., 2015), as well as the type and level of dietary fiber and the genetic potential of the bird (Jiménez-Moreno et al., 2016).

With regards to the relative weight of assessed organs in this study, birds in the OEOH treatment recorded the highest gizzard weight, while birds of the BMD treatment tended to have higher ceca weight. No effect of dietary treatments was found for the relative weight of the bursa and spleen. This result reaffirms a recent report from our laboratory, which revealed that the delivery of free choice OH to broiler chickens significantly improved the relative weight of the gizzard (Adewole et al., 2020). Consistent with results from the current study, several studies with similar or higher OH inclusion levels have reported an increase in the relative weight of gizzard in broilers and pullets (Hetland et al., 2003; González-Alvarado et al., 2008, 2010; García et al., 2019). An enlarged gizzard positively influences nutrient digestibility by increasing feed retention time, feed grinding rigor, digestive enzyme secretion, and feed-enzyme contact time (Amerah et al., 2007; González-Alvarado et al., 2008; Svilus, 2011; Scholey et al., 2020). It is not entirely clear why the potentially enhanced nutrient digestibility associated with the OEOH treatment did not translate into a significant growth performance effect in this study. However, it is most likely that birds in this treatment did not consume enough feed to meet their nutritional requirement, which could have resulted in a lower BWG. The propensity of antibiotic treatment to increase ceca weight is not surprising, as intestinal and ceca enlargement are often consequences of selective antimicrobial inhibition (Franti et al., 1972; Tayeri et al., 2018). Increased ceca weight is associated with increased ceca fermentation activity and microbial activity (Oyarzabal and Conner, 1996; Khaksar et al., 2008). The lack of dietary treatment effect on the relative weight of lymphoid organs (bursa and spleen) observed in this study could be attributed to the controlled experimental conditions in which birds were raised. Hence, there was no strain on their immune system. Dietary organic acids and essentials oils blends delivered in challenge trials have yielded better immune responses (Emami et al., 2017).

Asides bird management and pathological condition, diet has been shown to influence gut morphometric properties in poultry birds (Cowieson et al., 2017). In the current study, both OE and OEOH treatments exerted beneficial effects on the gut morphology of assessed birds. Birds in the OEOH treatment had significantly reduced duodenal crypt depth and significantly increased villus height and villus height-to-crypt depth ratio in the ileum. Birds in the OE treatment had significantly increased villus height and villus height-to-crypt depth ratio in the jejunum. Although the jejunum

Fig. 1. Relative abundance of ceca microbial composition. (A) of the main bacteria phyla, and (B) phylum Tenericutes across the different treatments. Treatments include - BAS, basal diet; BMD, antibiotic diet; OE, diet containing 300 g of protected organic acids-essential oils blend/1,000 kg of feed; OEOH, diet containing 300 g of protected organic acids-essential oils blend/1,000 kg of feed + 3% coarse oat hulls. The top of each graph assigned different lowercase letters (a,b) are significantly different. Treatment comparisons reported with (*) indicate significance at the level P < 0.05.

thymol, vanillin, and eugenol) with or without OH in broiler chickens.

At the end of this trial (d 36), BWG was observed to be similar between the BAS, BMD, and the OE treatment, despite the reported growth-enhancing effect of antibiotics (Butaye et al., 2003; Castanon, 2007; Gadde et al., 2017). However, birds offered the BMD treatment had 6.4% higher BWG compared to those in the OEOH treatment in the same time period. The reduction in BWG in the OEOH treatment was unsurprising, as the OE treatment had a low metabolizable energy value (almost 100 kcal lower than the other treatments). Additionally, no effect of dietary treatment on feed intake was recorded in this study at all stages of the trial. Conversely, García et al. (2018) showed that chickens would normally increase feed intake to compensate for the low dietary energy provided by high fiber diets. Nonetheless, Adewole et al. (2020), as in this study, has shown that a 3% OH inclusion level may not be sufficient enough to elicit a significant effect on feed intake. Regardless of the low metabolizable energy values, the OEOH treatment had no negative effect on FCR, throughout the study. Varying effects of OE and OH supplementation on growth performance are reported in the literature. Organic acids and essential oils blends have been reported to elicit superior growth performance similar to antibiotics in broiler chickens (Spais et al., 2002;
has been described as the main site of intestinal absorption (Zeinali et al., 2017), the ileum has also been reported to play a substantial role in starch digestion and absorption, especially in fast-growing broiler chickens (Svihus, 2014). A deeper crypt is often indicative of a higher rate of enterocyte cell renewal (Zabek et al., 2020) and faster tissue turnover (Berrocoso et al., 2017), which results in an increased nutrient requirement for intestinal maintenance and, ultimately, poor bird performance. Also, increased villus height and villus height-to-crypt depth ratio is indicative of increased epithelial cell turnover and a well-differentiated intestinal mucosa, suggesting increased digestive and absorptive capacity (Fan et al., 1997; Jeuissen et al., 2002). In agreement with our results, other studies have reported that both essential oil and dietary fibers reduced duodenal crypt depth and increased villus height and villus height-to-crypt depth ratio in the ileum (Sarikhan et al., 2010; Basmacioglu-Malayoglu et al., 2016; He et al., 2017; Masouri et al.,...

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**Fig. 2.** Relative abundance of ceca microbial composition. (A) At the genus level, (B) comparison of the relative abundance of *Firmicutes* unclassified, (C) comparison of the relative abundance of *Turicibacter*, (D) comparison of the relative abundance of *g_SMB52*, (E) comparison of the relative abundance of *Coprobacillus*, (F) comparison of the relative abundance of *RF39_unclassified*, (G) comparison of the relative abundance of *Erysipelotrichaceae*, and (H) comparison of the relative abundance of *Streptococcus* across the different treatments. Treatments include: BAS, basal diet; BMD, antibiotic diet; OE, diet containing 300 g of protected organic acids-essential oils blend/1,000 kg of feed; OEOH, diet containing 300 g of protected organic acids-essential oils blend/1,000 kg of feed +3% coarse oat hulls. The top of each graph assigned different lowercase letters (a, b) are significantly different. Treatment comparisons reported with (*) and (****) indicate significance at the level P < 0.05 and P < 0.001, respectively. Treatment comparisons reported with $P = 0.07$ indicate a statistical trend.
coarse oat hulls.

300 g of protected organic acids-essential oils blend/1,000 kg of feed; OEOH, diet containing 300 g of protected organic acids-essential oils blend/1,000 kg of feed; BMD, antibiotic diet; OE, diet containing 300 g of protected organic acids-essential oils blend/1,000 kg of feed +3% coarse oat hulls.

Fig. 3. ANOVA determined no significant differences in the Shannon diversity index (P > 0.05; F value = 0.723). Cecal content was collected from 36-day-old broiler chickens. Treatments include: BAS, basal diet; BMD, antibiotic diet; OE, diet containing 300 g of protected organic acids-essential oils blend/1,000 kg of feed; OEOH, diet containing 300 g of protected organic acids-essential oils blend/1,000 kg of feed +3% coarse oat hulls.

Fig. 4. Permutational analysis of variance (Permanova) determined significant differences (P < 0.05; R-squared = 0.1463) in beta-diversity among treatments. BAS, basal diet; BMD, antibiotic diet; OE, diet containing 300 g of protected organic acids-essential oils blend/1,000 kg of feed; OEOH, diet containing 300 g of protected organic acids-essential oils blend/1,000 kg of feed +3% coarse oat hulls. PCoA = principal coordinate analysis.

2017). Additionally, Giannenas et al. (2018) have shown that feeding broilers herbal additives could increase villus height, especially in the jejunum. The active ingredients of most essential oils are reported to stimulate the secretion of endogenous digestive enzymes and balance the gut microbial ecosystem by pathogen exclusion through competition for epithelial binding sites (Chiang et al., 2010; Ghazanfari et al., 2014), hence contributing to the observed positive effect on gut morphology. With regards to dietary fibers, their type, inclusion levels, and physiochemical properties dictate their influence on gut morphology (Montagne et al., 2003). Insoluble fibers, as in this study, often results in enhanced gut morphometric properties (Rezaei et al., 2011; Rahmatnejad and Saki, 2016). Furthermore, in this study, both OEOH and OE significantly increased the number of goblet cells produced in the duodenum and jejunum, respectively. This is consistent with the results of Jamroz et al. (2006), Reisinger et al. (2011), and Rezaei et al. (2011), who all observed an increased number of goblet cells when birds were offered different essential oil blends and dietary insoluble fibers, respectively. Goblet cells are major secretory cells responsible for mucin production (Mccauley and Guasch, 2015). Mucin produced by the goblet cells serves several essential functions in the gut, including lubricating intestinal surfaces, trapping and neutralizing bacteria, detoxifying heavy metal binding, interacting with the intestinal immune system, and acting as a diffusion barrier for nutrients and macromolecules (Forstner and Forstner, 1994). Additionally, glycoproteins secreted by goblet cells can also prevent pathogens and other harmful substances from contacting the intestinal epithelial cells (Round et al., 2012). Despite these benefits, the proliferation of goblet cells has been associated with inflammation (Sanches, 2019). Birds with significantly increased goblet cell numbers may have increased capacity to provide more mucin layer protective functions in the gut. Furthermore, according to the ISI histology methodology utilized in this study, the OE and the BAS (negative control) treatments had statistical scores in the ileum, relative to other treatments. The ISI methodology is expected to improve on the deficiencies associated with current linear measures of gut villi and crypts dimensions, hence providing an adequate measure of gut mucosa status. Although lower ISI total scores have been described as indicators of better intestinal health (Cardinal et al., 2019), it is possible that the higher ISI total scores observed in the BAS and OE treatments are indicative of constant basal intestinal inflammatory and proliferative responses. This is normal even in control birds, as in this study, raised under clean experimental conditions (Sanches, 2019). This basal enteritis effect is further reaffirmed by the uncompromised growth performance results obtained in these treatments relative to other treatments.

Bacterial fermentation of dietary fibers in the ceca often results in SCFA production. These SCFA play essential roles in maintaining intestinal functionality and integrity (Meimandipour et al., 2010). In this study, dietary treatments had no significant effect on SCFA concentrations. Nonetheless, OEOH treatment numerically increased concentrations of total SCFA, VFA, acetic acid, butyric acid, and lactic acid. This is consistent with our previous study (Adewole et al., 2020), which reported no significant effect of 3% OH on SCFA concentration in broiler chickens. Contrarily; Leung et al. (2018) recorded higher acetic acid and total SCFA concentration in broiler breeder hens fed OH (33% of diets), relative to the control diet. SCFA concentrations in birds are affected by several factors including, fermentation site (which changes with age), dietary fiber type and amount, microbiota composition, and the age of the bird (Meimandipour et al., 2011; Walugembe et al., 2015; Sun et al., 2020).

The OE treatment exerted a hypocholesterolemic effect in broiler chickens in this study. The cholesterol-lowering effect of essential oils and dietary acidifiers are well-substantiated in the literature (Elson et al., 1989; Craig, 1999; Abdó, 2004; Essa Al-Mashhodani et al., 2011; Özek et al., 2011; Kamal and Ragaa, 2014; Ali, 2016; Matty and Hassan, 2020). This hypocholesterolemic effect is associated with the inhibition of the hepatic 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase activity, a key regulatory enzyme involved in cholesterol synthesis (Qureshi et al., 1988; Lee et al., 2004). Additionally, the OE diet significantly reduced plasma urea levels in the current study. Urea is a protein metabolite, and its level in the blood indicates the immune status of the bird. Increased blood urea levels are often correlated with toxic or disease stress conditions in birds (Tekce
and Gül, 2017). Despite the limited report on urea levels in broiler chickens in the literature, both essential oil and organic acids have been reported to reduce serum levels of biogenic urea (Abdel-Fattah et al., 2008; Windisch et al., 2008; Tekce and Gül, 2017), which are in agreement with the results of the current study. This result could imply enhanced protein, and amino acid utilization in birds fed the OE diet, as urea are products of protein metabolism (Kamal and Ragaa, 2014). Furthermore, blood electrolytes and mineral levels were within the normal physiological range for broiler chickens (Clinical Diagnostic Division, 1990) and were not affected by dietary treatments. Similarly, levels of evaluated blood enzymes in this study were also not affected by dietary treatments. These enzymes are indicative of the bird’s liver function (Sun et al., 2019). Our results on evaluated blood enzymes and blood proteins are indicative of the healthy physiological state of all birds, as values were in the range of published values for healthy broiler chickens (Ilo et al., 2019).

It is becoming increasingly evident that gut microbiota is an important determinant of host physiology and health status (Sekirov et al., 2010; Nielsen et al., 2013). Cecal microbiota profile was majorly (>85%) dominated by Firmicutes, Proteobacteria, Tenericutes, and Actinobacteria at the phylum level, in this study (Fig. 1 A). Similar to antibiotic treatment, OEOH treatment reduced the relative abundance of Tenericutes compared to the OE treatment (Fig. 1 B). In agreement with our results, Das et al. (2020) have recently reported the tendency of a fiber-containing feed treatment using 2% blueberry pomace supplemented diet to reduce the relative abundance of Tenericutes in the cecum of broiler chickens. Tenericutes were negatively correlated with cumulative feed efficiency in their study. Tenericutes are also implicated in mycoplasma pathology in chickens. At the genus level, the relative abundances of Firmicutes_unclassified, Ruminococcus, Turicibacter, and Erysipelotrichaceae_unclassified were increased by both OE and OEOH treatments (Fig. 2). Firmicutes are associated with polysaccharide degradation, butyrate production, and enhanced nutrient absorption capacity (Louis et al., 2014; Meehan and Beiko, 2014). Ruminococcus is also correlated with polysaccharide degradation and butyrate production (Gong et al., 2007; Xiao et al., 2017). Turicibacter contributes to host metabolic and homeostatic mechanisms via cholesterol metabolism (Li et al., 2017; Hoffman and Margolis, 2020). Abdelli et al. (2020) recently showed that the targeted release of microencapsulated organic acids and essential oils blend increases the abundance of Erysipelotrichaceae in NE challenged broilers. Erysipelotrichaceae are also linked to butyrate production (De Maesschalck et al., 2014). The OE treatment increased the relative abundance of Streptococcus compared to OEOH treatment in this study (Fig. 2H). Although Streptococcus might be associated with poultry infection (Sekizaki et al., 2008), depending on the specific strain, they are also capable of reducing gut pathogen load (Roto et al., 2015). Coprobacillus have been referred to as “intestinal dysbiosis associated genera” (Janssens et al., 2018; Adhikari, 2019). Both OE and OEOH treatments in our study reduced the relative abundance of Coprobacillus. Similar to our results (Fig. 2C), Adhikari (2019) reported an increase in the genus Coprobacillus in non-supplemented control birds in their study.

Also, no difference in α-diversity was found between treatments in this study (Fig. 3). Both Pham et al. (2020), who researched a dietary encapsulated essential oil and organic acids mixture (though formulation differs from this study), and Bortoluzzi et al. (2017) who researched a sodium butyrate additive, have equally reported no difference in α-diversity. Conversely, permutational analysis of variance indicated that OE diet modified cecal microbiota diversity, with OEOH diet showing a tendency to elicit a similar response (Fig. 4). Pham et al. (2020) recently reported the potential of the organic acids and essentials oils blend they tested to modify the intestinal bacterial community profiles of broiler chickens. Furthermore, Firmicutes unclassified species were found differentially abundant in birds belonging to both the OE and OEOH treatments in this study. Birds of the OE treatment recorded differentially abundant Clostridiales_unclassified species. A positive correlation between body weight and Clostridia has been reported in broiler chickens offered essential oil supplemented diet (Betancourt et al., 2019). Overall, both OE and OEOH supplemented diet seemed to stimulate the growth of potentially beneficial intestinal microorganisms, whilst also inhibiting the proliferation of pathogenic bacteria.

5. Conclusion

The OE treatment reduced plasma cholesterol and urea levels in broiler chickens. Both OE and OEOH treatment improved gut morphology and positively influenced birds’ gut health by shifting gut microbiota diversity and stimulating the growth of potentially beneficial intestinal microorganisms. An additional beneficial effect on gut health was observed with the use of OEOH treatment, as
evidenced by the increased relative weight of the gizzard. However, this benefit did not translate into increased growth performance effect in birds offered the OEOH treatment. More studies are needed to determine the optimum dietary fiber inclusion level (beyond the 3% OH utilized in this study) that could elicit a superior growth performance effect. Conclusively, this study shows that both protected organic acids–essential oils blend and its combination with oat fibers show potential as an important strategy to achieve antibiotics reduction in broiler production.

Author contributions
Deborah I. Adewole: Conceptualization, Methodology, Software, Writing – original draft preparation, writing - reviewing & editing. Funding acquisition, Supervision. Samson Oladokun: Investigation, Data curation, Writing – original draft preparation, writing - reviewing & editing. Elizabeth Santin: Resources, Investigation, writing - reviewing & editing.

Conflict of interest
The authors declare the following competing interest(s): Elizabeth Santin is a co-author in this manuscript. She is an employee at Jefo Nutrition Inc. that provided the organic acids-essential oils blend for this work.

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Appendix A. Supplementary data
Supplementary data to this article can be found online at https://doi.org/10.1016/j.aninu.2021.02.001.

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