Dietary Oregano Essential Oil Supplementation Influences Production Performance and Gut Microbiota in Late-Phase Laying Hens Fed Wheat-Based Diets

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Simple Summary: Essential oils (EOs) are antibacterial and anti-inflammatory, and are called natural antibiotics. Within the great variety of EOs, oregano essential oil (OEO) is known for its antimicrobial activity. So, we focused on comparing the effect of flavomycin and OEO on the egg-laying performance and intestinal flora of laying hens. This study found that OEO improved the egg-production performance and altered microbial composition. The results revealed that OEO could be an effective alternative to flavomycin.

Abstract: This study aimed to investigate the potential effects of OEO on production performance, egg quality, fatty acid composition in yolk, and cecum microbiota of hens in the late phase of production. A total of 350 58-week-old Jing Tint Six laying hens were randomly divided into five groups: (1) fed a basal diet (control); (2) fed a basal diet + 5 mg/kg flavomycin (AGP); (3) fed a basal diet + 100 mg/kg oregano essential oil + 20 mg/kg cinnamaldehyde (EO1); (4) fed a basal diet + 200 mg/kg oregano essential oil + 20 mg/kg cinnamaldehyde (EO2); (5) fed a basal diet + 300 mg/kg oregano essential oil + 20 mg/kg cinnamaldehyde (EO3). Compared to the control group, group EO2 exhibited higher (p < 0.05) egg production during weeks 5–8 and 1–8. EO2 had a lower feed conversion ratio than the control group during weeks 1–8. The content of monounsaturated fatty acid (MUFA) in EO2 was higher (p < 0.05) than that of the control and AGP groups. EO2 increased (p < 0.05) the abundance of Actinobacteriota and decreased the abundance of Desulfovibrio in the cecum. The abundances of Anaerofilum, Fournierella, Fusobacterium, and Sutterella were positively correlated with egg production, feed conversion ratio, and average daily feed intake, while the abundances of Bacteroides, Desulfovibrio, Lactobacillus, Methanobrevibacter, and Rikenellaceae_RC9_gut_group were negatively correlated with egg production, feed conversion ratio, and average daily feed intake. Dietary supplementation with 200 mg/kg OEO and 20 mg/kg cinnamaldehyde could improve egg-production performance, decrease feed conversion ratio, and alter the fatty acid and microbial composition of eggs from late-phase laying hens.

Keywords: essential oils; production performance; fatty acid composition; bacterial community; laying hen

1. Introduction

Antibiotics have been widely used in the poultry industry as animal-growth promoters for decades. However, the long-term use of sub-therapeutic antibiotics caused a series of...
problems, such as making bacteria resistant to antibiotics, antibiotic residues, endogenous infections, and double infections, which pose serious threats to livestock and poultry production and human health [1,2]. Now, China has banned feed antibiotics, leading to increased demand for green feed additives.

Essential oils (EOs) are concentrated hydrophobic liquids containing volatile aromatic compounds obtained from plants [3]. Oregano essential oil (OEO) has strong antibacterial properties on various pathogenic bacteria, such as *Salmonella enteritidis*, *Escherichia coli*, *Staphylococcus aureus*, *Campylobacter*, etc. [4–6], and is considered to be a substitute for antibiotics. OEO, or its main components (thymol and carvacrol), can destroy the cell membrane structure to exert antibacterial activity [3,7]. Furthermore, EOs has been widely used in poultry and pigs for many years. A supplement of OEO (containing thymol and carvacrol) could alleviate intestinal injuries in broiler chickens under *Clostridium perfringens* challenge [8], enhance the growth performance and intestinal health of broilers [9–13], inhibit the growth of pathogenic bacteria, and modulate the intestinal microbial composition of broilers [14,15].

The efficacy of EOs (with thymol, carvacrol, or menthol as active components) on improving laying performance and egg quality has been reported [16–18]. Cinnamaldehyde is the major compound of cinnamon essential oil, which can alter microbial composition [19]. It has been reported that cinnamaldehyde could enhance growth performance by improving intestinal histomorphology in broilers under a necrotic enteritis challenge [20]. Dietary supplementation of cinnamaldehyde could improve nutrient utilization and decrease total nitrogen excretion in broilers [21]. However, there are few investigations on the effects of OEO and cinnamaldehyde on the production performance, egg quality, and intestinal microbial community of laying hens in the late phase of production.

In this study, we hypothesized that OEO may serve as an antibiotic alternative to positively alter microbial composition, subsequently leading to improvements in production performance, egg quality, and fatty acid composition of yolks. Therefore, the purpose of this study was to investigate the effects of dietary oregano essential oil supplementation on laying performance, egg quality, and microbial community in the cecum of laying hens in the late phase of production.

2. Material and Methods

2.1. Animals and Experimental Design

A total of 350 58-week-old Jing Tint Six laying hens were randomly divided into 5 groups with 5 replicates of 14 birds. The control group (CK) received a basal diet. The positive control group (AGP) received a basal diet supplemented with 5 mg/kg flavomycin. The three treatment groups (EO1, EO2, and EO3) received a basal diet and 20 mg/kg cinnamaldehyde supplemented with 100, 200, and 300 mg/kg oregano essential oil, respectively. The trial lasted 8 weeks and the birds were provided by Gu’an Songhe Poultry Breeding Co., Ltd. (Hebei China). An OEO product (containing 8% oregano essential oil) was obtained from a commercial supply (Ropapharm International B.V. Zaandam Netherlands) and its major active ingredients were carvacrol and thymol, with wheat flour and silicon dioxide as carriers. The actual concentrations of OEO in the EO1, EO2, and EO3 diets were 8, 16, and 24 mg/kg, respectively. The concentrations of carvacrol and thymol in the EO were ≥3.6% and ≥0.1%, respectively. The cinnamaldehyde was purchased from Shanghai Aladdin Biochemical Technology Co., Ltd. (Shanghai, China). All birds were housed in four-tier battery cages in an environmentally controlled house with the temperature maintained at approximately 23 °C and humidity maintained at 30–48%. All birds were provided ad libitum access to fresh water and mash feed. The basal diet (Table 1) was formulated according to NRC (1994) recommendations. All birds remained in good health and no medical intervention was applied to any birds during the feeding period.
Table 1. Ingredient and nutrient levels of the experimental diets (% as-fed basis).

| Item                  | Value     |
|-----------------------|-----------|
| Wheat                 | 72.00     |
| Soybean meal (44% CP) | 14.50     |
| Soybean oil           | 1.00      |
| Limestone             | 10.30     |
| Premix 1              | 2.20      |
| Total                 | 100.00    |

Calculated nutrient levels

- Crude protein: 16.06%
- Calcium: 3.86%
- Metabolizable energy, MJ/kg: 10.96
- Available phosphorus: 0.32
- SID lysine: 0.66
- SID methionine: 0.37
- SID threonine: 0.52
- SID tryptophan: 0.19

1 Premix provided the following per kg of the diet: vitamin A, 443,040 IU; vitamin D3, 99,968 IU; vitamin E, 113.6 IU; vitamin K3, 113.6 mg; vitamin B1, 113.6 mg; vitamin B2, 284.0 mg; vitamin B6, 170.4 mg; vitamin B12, 0.9 mg; D-biotin, 13.6 mg; D-pantothenic acid, 511.2 mg; niacinamide, 1818 mg; Choline chloride, 13,638 mg; Fe, 3636 mg; Cu, 681.0 mg; Mn, 5454 mg; Zn, 4090 mg; L-Lysine, 7000 mg; DL-Methionine, 60,000 mg; NaCl, 3000 mg.

2.2. Sample Collection

Three eggs per replicate were randomly collected for egg quality determination on days 28 and 56. Two eggs in each replicate were randomly sampled on day 56 to separate the egg yolk from egg whites. The egg yolk was freeze-dried for fatty acid determination. On day 56, 1 bird per replicate (5 per treatment) was slaughtered by cervical dislocation after fasting for 12 h. Cecal contents were collected and kept frozen at −80 °C for assessment of microbial composition.

2.3. Laying Performance and Egg Quality

During the experimental period, egg production, broken-egg production, and egg weight were recorded daily by replicate, and feed consumption for each replicate was weighed every week. The feed conversion ratio (FCR) was calculated as grams of feed consumed/egg weight for each replicate. Egg length (mm) and width (mm) were recorded for shape index calculation (shape index = length/width). The breaking strength and thickness of eggshells were measured by an Egg Force Reader (EFR-01, Israel Orka Food Technology Ltd., Bountiful, UT, USA) and Egg Shell Thickness Gauge (ESTG-1, Israel Orka Food Technology Ltd., Bountiful, UT, USA), respectively. Egg weights, Haugh unit values, and albumen heights were measured by an Egg Analyzer (EA-01, Israel Orka Food Technology Ltd., Bountiful, UT, USA).

2.4. Analysis of Ileal Microbiota

Total genome DNA from samples was extracted using the CTAB method. DNA concentration and purity was monitored on 1% agarose gels. According to the concentration, DNA was diluted to 1 ng/µL using sterile water. The V3-V4 region of the 16S rRNA gene was amplified using the primer pair 341F/806R (5′-CCTAYGGGRBGCASCAG-3′ and 5′-GGACTACHVGGGTWTCTAAT-3′). Sequencing libraries were generated using an Illumina TruSeq DNA PCR-Free Library Preparation Kit (Illumina, San Diego, CA, USA) following the manufacturer’s recommendations, and index codes were added. The library quality was assessed on the Qubit@ 2.0 Fluorometer (Thermo Scientific, Waltham, MA, USA) and Agilent Bioanalyzer 2100 system. The library was sequenced on an Illumina NovaSeq platform and 250 bp paired-end reads were generated. Sequencing and bioinformatics analysis were performed by Novogene Bioinformatics Technology Co. (Beijing, China).
2.5. Statistical Analysis

Data were analyzed by a one-way Analysis of Variance (ANOVA) procedure and differences were examined using LSD’s Multiple Range Test by IBM SPSS Statistics 23. The differences in the relative abundances of bacteria between groups were assessed using Wilcoxon rank tests. Data were presented as mean ± SEM. Significant differences were defined as \( p < 0.05 \), and tendency was defined as \( 0.05 \leq p < 0.10 \).

3. Results

3.1. Laying Performance and Egg Quality

Dietary OEO supplementation had no significant influences (\( p > 0.05 \)) on average daily feed intake (ADFI), or on broken-egg production of laying hens during weeks 1–8 of the experiment (Table 2). However, EO2 increased (\( p < 0.05 \)) egg production of laying hens in comparison with the control in weeks 5–8 and 1–8. Average egg weight in group EO2 was lower (\( p < 0.05 \)) than that in the AGP in weeks 1–4, but it was higher (\( p < 0.05 \)) than that in the control during weeks 5–8 and 1–8. The EO2 group decreased (\( p < 0.05 \)) the FCR when compared to groups EO1 and EO3. With respect to egg quality, there were no significant effects (\( p > 0.05 \)) of dietary OEO or cinnamaldehyde supplementation on eggshell strength, shape index, albumen height, or Haugh unit at the end of weeks 4 or 8 (Table 3). Eggshell thickness in EO2 and EO3 was lower (\( p < 0.05 \)) than that in the control and AGP in week 4.

### Table 2. Effects of dietary supplementation with oregano essential oil on laying performance of laying hens

| Items                              | Treatments                          | Control | AGP  | EO1  | EO2  | EO3  | SEM    | \( p \)-Value |
|------------------------------------|-------------------------------------|---------|------|------|------|------|--------|-------------|
| Egg production, %                  |                                     |         |      |      |      |      |        |             |
| Weeks 1–4                          |                                     | 90.13 a | 91.32 a | 85.36 b | 91.42 a | 86.82 b | 0.89 | 0.001 |
| Weeks 5–8                          |                                     | 87.46 b | 92.38 a | 85.71 a | 91.95 a | 86.50 b | 0.90 | 0.001 |
| Weeks 1–8                          |                                     | 88.77 b | 91.86 a | 85.48 c | 91.64 a | 86.67 c | 0.67 | 0.001 |
| Average egg weight, g              |                                     | 58.68 b | 59.46 a | 59.62 a | 58.97 b | 59.47 a | 0.19 | 0.001 |
| Weeks 1–4                          |                                     | 58.98 c | 59.65 ab | 59.96 a | 59.34 b | 59.93 a | 0.18 | 0.001 |
| Weeks 5–8                          |                                     | 58.81 c | 59.36 c | 59.795 a | 59.18 b | 59.70 a | 0.13 | 0.001 |
| Weeks 1–8                          |                                     | 115.34 | 120.34 | 115.96 | 116.93 | 118.99 | 3.70 | 0.649 |
| ADFI, g/hen per day                |                                     | 109.61 | 116.34 | 109.63 | 111.03 | 114.81 | 3.95 | 0.389 |
| Weeks 1–8                          |                                     | 112.47 | 118.32 | 112.79 | 113.98 | 116.85 | 2.87 | 0.193 |
| FCR, g/g                           |                                     | 2.18    | 2.22   | 2.28   | 2.16   | 2.30   | 0.06 | 0.117 |
| Weeks 1–4                          |                                     | 2.25    | 2.24   | 2.27   | 2.15   | 2.32   | 0.08 | 0.349 |
| Weeks 5–8                          |                                     | 2.22 ab | 2.23 ab | 2.28 a | 2.16 b | 2.31 a | 0.05 | 0.026 |
| Broken-egg production              |                                     | 1.57    | 3.20   | 3.61   | 1.78   | 2.21   | 0.78 | 0.076 |
| Weeks 1–4                          |                                     | 3.60    | 4.91   | 3.09   | 3.34   | 5.21   | 0.85 | 0.082 |
| Weeks 5–8                          |                                     | 2.58    | 4.05   | 3.35   | 2.60   | 3.72   | 0.75 | 0.203 |

1 \( n = 5 \) replicates per treatment. 2 Control, a basal diet; AGP, a basal diet + 5 mg/kg flavomycin; EO1, a basal diet + 100 mg/kg oregano essential oil + 20 mg/kg cinnamaldehyde; EO2, a basal diet + 200 mg/kg oregano essential oil + 20 mg/kg cinnamaldehyde; and EO3, a basal diet + 300 mg/kg oregano essential oil + 20 mg/kg cinnamaldehyde. ADFI, average daily feed intake; FCR, feed conversion ratio. a–c Values within a row with no common superscripts differ significantly (\( p < 0.05 \)).

3.2. Yolk Fatty Acids Composition

Fatty acid composition and content of yolks at week 8 are shown in Table 4. The concentration of butyric acid (C4:0) in EO3 was significantly increased compared to the control and AGP groups. The concentration of lauric acid (C12:0) in EO1, EO2, and EO3 was significantly increased compared to the control group. The concentration of pentadecanoic acid (C15:0) in EO2 and EO3 was significantly lower than that in the control.
and AGP groups. The concentration of stearic acid (C18:0) in EO1 and EO3 was significantly increased compared to the control group. Yolk exhibited greater concentrations of oleic acid (C18:1, cis(n-9)) in EO2 and EO3, and greater concentrations of eicosenoic (C20:1) and eicosapentaenoic (C20:5) acids in EO1, EO2, and EO3, as compared to the control group \((p < 0.05)\). Additionally, the concentration of MUFA in EO2 and EO3 was higher \((p < 0.05)\) than that in the control and AGP groups at week 8.

Table 3. Effects of dietary supplementation with oregano essential oil on egg quality of laying hens

| Items                        | Treatments 2 | SEM  | \( p \)-Value |
|------------------------------|--------------|------|---------------|
|                              | Control      | AGP  | EO1           | EO2           | EO3           |
| Eggshell thickness, \(10^{-2}\) mm |              |      |               |               |
| Week 4                       | 28.78        | 28.56 | 28.24         | 27.91         | 27.87         |
| Week 8                       | 28.73        | 28.89 | 29.02         | 29.42         | 29.20         |
| Eggshell strength, kg        | 3.55         | 3.45  | 3.45          | 3.46          | 3.57          |
| Week 4                       | 3.42         | 3.41  | 3.45          | 3.61          | 3.55          |
| Week 8                       | 3.35         | 3.34  | 3.14          | 3.16          | 3.13          |
| Shape index                  | 1.35         | 1.34  | 1.34          | 1.36          | 1.38          |
| Week 4                       | 1.34         | 1.35  | 1.34          | 1.34          | 1.37          |
| Week 8                       | 1.25         | 1.25  | 1.25          | 1.25          | 1.25          |
| Albumen height, mm           | 6.53         | 6.44  | 6.14          | 6.40          | 5.76          |
| Week 4                       | 6.99         | 6.10  | 5.98          | 5.96          | 5.90          |
| Week 8                       | 7.50         | 7.81  | 7.21          | 7.60          | 7.28          |
| Haugh unit                   | 79.53        | 78.96 | 77.83         | 78.77         | 73.65         |
| Week 4                       | 75.70        | 75.81 | 76.21         | 76.70         | 74.28         |
|                              | 3.51         | 3.94  | 3.66          | 3.66          | 0.97         |

\(^1\) \(n = 5\) replicates per treatment. \(^2\) Control, a basal diet; AGP, a basal diet + 5 mg/kg flavomycin; EO1, a basal diet + 100 mg/kg oregano essential oil + 20 mg/kg cinnamaldehyde; EO2, a basal diet + 200 mg/kg oregano essential oil + 20 mg/kg cinnamaldehyde; and EO3, a basal diet + 300 mg/kg oregano essential oil + 20 mg/kg cinnamaldehyde. \(^{a-c}\) Values within a row with no common superscripts differ significantly \((p < 0.05)\).

3.3. Cecal Microbial Profile

As shown in Table 5, there was a tendency for Chao1 and ACE to increase in the EO2 group. The Venn analysis of ASVs identified 571, 432, and 971 unique ASVs in the control, AGP, and EO2 groups, respectively. These three treatments shared 895 ASVs among their cecum microbiota (Figure 1A). The PCA results suggested that there was a clear difference between the bacterial communities of the control group and EO2, while the separation between the control group and AGP could hardly be detected (Figure 1B).

The dominant phyla in the three treatment groups were Bacteroidota and Firmicutes, together accounting for more than 75% of all phyla (Figure 2A). Laying hens from group EO2 had a higher abundance of Bacteroidota and a lower abundance of Firmicutes compared to the AGP group. At the class level, the dominant classes were Bacteroidia, Clostridia, and Fusobacteriia, which collectively accounted for more than 73% of the total sequences (Figure 2B). At the family level (Figure 2C), the cecal microbiota were dominated by Bacteroidaceae in all groups.

At the phylum level, the abundance of Desulfobacterota in the control group was higher \((p < 0.05)\) than that in AGP and EO2, and EO2 had a higher \((p < 0.05)\) abundance of Actinobacteriota than the control and AGP groups (Table 6). At the class level, the control had a higher \((p < 0.05)\) abundance of Desulfovibrionia than AGP and EO2. At the family level, AGP had a higher \((p < 0.05)\) abundance of Lachnospiraceae than the control and EO2, and the control had a higher \((p < 0.05)\) abundance of Desulfovibrionaceae than AGP and EO2. At the genus level, the control had a higher \((p < 0.05)\) abundance of *Desulfovibrio* than AGP and EO2.
Table 4. Effects of dietary supplementation with oregano essential oil on fatty acid composition of egg yolk (g/100 g) 1.

| Fatty Acid | Treatments 2 | SEM | p-Value |
|------------|--------------|-----|---------|
|            | Control      | AGP | EO1      | EO2      | EO3      |       |
| C4:0       | 0.002 b      | 0.003 b | 0.005 b | 0.005 b | 0.008 a | 0.001 | 0.005 |
| C12:0      | 0.005 b      | 0.006 a | 0.006 a | 0.006 a | 0.006 a | 0.000 | 0.003 |
| C14:0      | 0.277        | 0.273 | 0.269    | 0.267    | 0.271    | 0.009 | 0.814 |
| C15:0      | 0.044 a      | 0.043 a | 0.041 ab | 0.037 bc | 0.034 c | 0.003 | 0.006 |
| C16:0      | 18.656       | 18.581 | 19.411   | 19.050   | 19.220   | 0.554 | 0.520 |
| C18:0      | 6.136 b      | 6.565 a | 6.748 a | 6.499 b | 6.624 a | 0.175 | 0.026 |
| C21:0      | 0.047        | 0.057 | 0.060    | 0.059    | 0.057    | 0.004 | 0.056 |
| C24:0      | 0.097        | 0.092 | 0.091    | 0.090    | 0.098    | 0.007 | 0.716 |
| C16:1      | 2.660        | 2.818 | 2.664    | 2.799    | 2.753    | 0.186 | 0.866 |
| C18:1, cis(n-9) | 15.191 c | 15.245 bc | 15.490 bc | 16.069 ab | 16.452 ab | 0.379 | 0.012 |
| C20:1 n9   | 0.131 b      | 0.146 ab | 0.156 a | 0.158 a | 0.153 a | 0.008 | 0.034 |
| C18:2, cis(n-6) | 9.518 | 9.505 | 9.336 | 9.157 | 9.573 | 0.472 | 0.899 |
| C18:3 n-3  | 0.360        | 0.368 | 0.361    | 0.333    | 0.349    | 0.025 | 0.675 |
| C20:2      | 0.138        | 0.153 | 0.159    | 0.148    | 0.142    | 0.007 | 0.059 |
| C20:3 n-6  | 0.149        | 0.154 | 0.155    | 0.143    | 0.139    | 0.006 | 0.057 |
| C20:4 n-6  | 1.397        | 1.430 | 1.420    | 1.382    | 1.460    | 0.050 | 0.578 |
| C20:5      | 0.010 b      | 0.013 a | 0.014 a | 0.013 a | 0.014 a | 0.001 | 0.001 |
| C22:6      | 0.701        | 0.731 | 0.713    | 0.715    | 0.780    | 0.037 | 0.292 |
| SFA        | 25.392       | 26.309 | 26.778   | 26.013   | 26.319   | 0.599 | 0.204 |
| UFA        | 30.260       | 30.565 | 30.468   | 30.917   | 31.816   | 0.529 | 0.061 |
| MUFA       | 17.983 c     | 18.212 c | 18.311 bc | 19.025 ab | 19.358 a | 0.349 | 0.003 |
| n-3 PUFA   | 12.276       | 12.355 | 12.158   | 11.891   | 12.457   | 0.552 | 0.867 |
| n-6 PUFA   | 11.064       | 11.089 | 10.911   | 10.682   | 11.172   | 0.056 | 0.627 |
| n-3/n-6    | 0.096        | 0.099 | 0.099    | 0.098    | 0.101    | 0.003 | 0.605 |
| UFA/SFA    | 1.197        | 1.196 | 1.144    | 1.189    | 1.210    | 0.028 | 0.221 |
| EFA        | 11.276       | 11.303 | 11.117   | 10.872   | 11.383   | 0.522 | 0.874 |

1 n = 5 replicates per treatment. 2 Control, a basal diet; AGP, a basal diet + 5 mg/kg flavomycin; EO1, a basal diet + 100 mg/kg oregano essential oil + 20 mg/kg cinnamaldehyde; EO2, a basal diet + 200 mg/kg oregano essential oil + 20 mg/kg cinnamaldehyde; and EO3, a basal diet + 300 mg/kg oregano essential oil + 20 mg/kg cinnamaldehyde. SFA, saturated fatty acid; UFA, unsaturated fatty acid; MUFA, monounsaturated fatty acid; PUFA, polyunsaturated fatty acid; EFA, essential fatty acids; n-3 PUFA = C18:3 n-3 + C20:5 n-6 + C20:4 n-6; n-6 PUFA = C18:2, cis(n-6) + C20:3 n-6 + C20:4 n-6. a–c Values within a row with no common superscripts differ significantly (p < 0.05).

Table 5. Effect of dietary supplementation with oregano essential oil on alpha diversity in cecum 1.

| Items      | Treatments 2 | SEM | p-Value |
|------------|--------------|-----|---------|
|            | Control      | AGP | EO2      |       |
| Shannon    | 7.94         | 7.84 | 8.18     | 0.32   | 0.579 |
| Simpson    | 0.99         | 0.98 | 0.99     | 0.01   | 0.627 |
| Chao1      | 855.41       | 790.28 | 1006.06 | 79.45  | 0.050 |
| ACE        | 850.20       | 786.00 | 996.60  | 78.94  | 0.055 |

1 n = 5 replicates per treatment. 2 Control, a basal diet; AGP, a basal diet + 5 mg/kg flavomycin; EO2, a basal diet + 200 mg/kg oregano essential oil + 20 mg/kg cinnamaldehyde.

3.4. Correlation between Cecal Microbiota and Laying Performance

As shown in Figure 3, the egg production was positively correlated with the abundances of Sutterella and Anaerofilum, but it was negative correlated with Lactobacillus, Methanobrevibacter, Desulfovibrio, and Rikenellaceae_RC9_gut_group abundances. FCR was positively correlated with Fusobacterium, Fournierella, Sutterella, and Anaerofilum, while showing a negative correlation with Bacteroides. There was a positive correlation between ADFI and Anaerofilum, Sutterella, and Fournierella, and a negative correlation between ADFI and Lactobacillus, Methanobrevibacter, Rikenellaceae_RC9_gut_group, Desulfovibrio, and Bacteroides.
and EO2. At the genus level, the control had a higher \((p < 0.05)\) abundance of *Desulfovibrio* than AGP and EO2.

**Figure 1.** Venn diagram of ASV (A) and PCA (B) of cecum microbiota in the three groups. Control, hens received a basal diet; AGP, hens received a basal diet supplemented with 5 mg/kg flavomycin; EO2, hens received a basal diet supplemented with 200 mg/kg oregano essential oil and 20 mg/kg cinnamaldehyde.

**Figure 2.** Microbial composition in the cecum of laying hens at phylum level (A), class level (B), family level (C), and genus level (D). Control, hens received a basal diet; AGP, hens received a basal diet supplemented with 5 mg/kg flavomycin; EO2, hens received a basal diet supplemented with 200 mg/kg oregano essential oil and 20 mg/kg cinnamaldehyde.
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Differences of bacterial distribution in cecal digesta between the control, AGP, and EO2 groups.

| Items, % | Treatments | SEM | p-Value |
|----------|------------|-----|---------|
| Phylum   |            |     |         |
| Desulfo bacterota | 3.72 a | 2.04 b | 2.66 b | 0.45 | 0.010 |
| Actinobacterota | 1.34 b | 1.51 b | 3.88 a | 0.91 | 0.016 |
| Class     |            |     |         |
| Desulfovibrionia | 3.69 a | 2.02 b | 2.61 b | 0.44 | 0.008 |
| Family    |            |     |         |
| Lachnospiraceae | 6.79 b | 10.16 a | 6.69 b | 0.85 | 0.002 |
| Desulfovibrionaceae | 3.69 a | 2.02 b | 2.61 b | 0.44 | 0.008 |
| Genus     |            |     |         |
| Desulfovibrio | 3.63 a | 2.00 b | 2.56 b | 0.44 | 0.010 |

n = 5 replicates per treatment. Control, a basal diet; AGP, a basal diet + 5 mg/kg flavomycin; EO2, a basal diet + 200 mg/kg oregano essential oil + 20 mg/kg cinnamaldehyde. a,b Values within a row with no common superscripts differ significantly (p < 0.05).

Figure 3. Pearson’s correlation analysis between the abundances of cecal microbiota (at genus level) and egg production. FCR, feed conversion ratio; ADFI, average daily feed intake.

4. Discussion

Essential oils and their active ingredients have been extensively studied for their growth-promoting properties, which make them a potential alternative to AGPs [22]. OEO can increase the villus height and decrease the crypt depth, promoting the absorption of nutrients in the intestine [23]. In the present study, egg production was improved in the EO2 treatments, but there was no significant effect on FCR in groups EO1, EO2, and EO3 when compared to the control and AGP groups. In line with our study, several previous studies had reported that OEO improved egg production in laying hens [24,25]. This beneficial effect could be attributed to the active ingredients (i.e., thymol and carvacrol) in OEO and cinnamaldehyde, which have been proven to have antibacterial and anti-inflammatory properties, and to improve intestinal health and nutrient utilization [21,26]. In contrast, other studies had shown that OEO (or its main compounds) did not significantly improve laying performance of laying hens [27,28]. The inconsistent effects of OEO on production...
performance may be caused by the composition and supplemental levels of OEO, the type of laying hen, the feeding phase, and the environmental conditions. It is noteworthy that the FCR of the EO2 treatment group was lower \((p < 0.05)\) than that of the EO3 group, which may be due to the negative effects of high concentrations of essential oils on intestinal epithelial cells and intestinal probiotics [29,30].

Eggshell thickness is an important indicator of egg quality and is critical for egg transportation and storage [31]. During eggshell calcification, the availability of intestinal calcium is vital as it plays a key role in providing sufficient amounts of calcium to satisfy shell quality requirements [32]. It has been reported that dietary essential oils could significantly increase eggshell thickness [24,33]. But in our study, the eggshell thickness of EO1 and EO2 was lower \((p < 0.05)\) than that of the control and AGP at week 4, although the difference disappeared at week 8, which may be due to altered flora structure [34].

The lipid composition of eggs has received attention due to the relationship between dietary lipids and the development of coronary heart disease [35]. MUFAs have a significant impact on improving cardiovascular and cerebrovascular health and reducing oxidative stress damage in the body [36]. A MUFA-rich diet can improve insulin sensitivity, and positively affects blood lipids, systemic inflammatory response, and endothelial dysfunction [37]. Some countries have even advocated that MUFAs should become healthy substitutes in the daily diet [38]. Previous research has reported that dietary EOs and rosemary extract supplementation in the diet of laying hens did not significantly influence the fatty acid composition of their egg yolks [24,39]. Another study found that dietary bergamot oils could significantly increase the proportion of DHA and the n-3 PUFA proportion of egg yolk [40]. In the present study, the content of MUFAs in groups EO2 and EO3 was higher \((p < 0.05)\) than that of the control and AGP treatments, which may be due to OEO and cinnamaldehyde affecting lipid metabolism in the serum and liver of laying hens [40,41].

Intestinal flora is involved in food digestion and metabolism, body growth and development, and immune suppression and activation. As one of the three major components of the intestinal barrier, intestinal flora is a direct factor in whether pathogenic bacteria can invade [42–44]. OEO can cause irreversible damage to bacteria cell membranes and can cause leakage of biological macromolecules. [45] The present study was performed to better understand the effect of OEO and cinnamaldehyde on microbiota. Since the beneficial effects of OEO on production performance were mainly observed in the EO2 group, the modulatory roles of OEO on cecal microbial composition were assessed in the control, AGP, and EO2 groups. ACE and Chao1 indices were used to estimate species richness, while Shannon and Simpson indices were used to estimate species diversity [46]. The Chao1 of EO2 was higher \((p < 0.05)\) than that of the AGP group, indicating that the combination of OEO and cinnamaldehyde increased the abundance of cecal flora. According to the beta-diversity results of the three treatments, significant clustering was observed, indicating that the addition of OEO and cinnamaldehyde altered the cecal microbial community structure. Further analysis of the changes in microbiota composition and the specific taxa present upon addition of OEO and cinnamaldehyde was then performed. At the phylum level, Bacteroidota and Firmicutes, as dominant flora, accounted for more than 75% of the total microbial community. Firmicutes and Bacteroidetes are involved in the metabolism of nutrients in the body (such as enzymes encoding polysaccharide decomposition, involved in sugar metabolism), and are cooperatively involved in amino acid metabolism [47,48]. Actinobacteriota is reported to improve feed utilization by producing extracellular enzymes [49], and to decompose undigested components in feed through secreting endogenous enzymes [50]. In addition, Actinobacteriota is helpful for the maintenance of overall microbial structure. Due to the production of bacteriocins and the ability to convert feed into fermentable microbial biomass [51], Actinobacteriota is considered to be a key group that regulates the function of gut microbiota. In the present study, the abundance of Actinobacteriota in group EO2 was higher \((p < 0.05)\) than that of the control and AGP, indicating that dietary supplementation OEO and cinnamaldehyde may increase
the abundance of beneficial bacteria to promote the production performance and feed utilization of the late-stage laying hens. Short chain fatty acids (SCFAs) play important roles in the gut, such as inflammation reduction, cancer prevention, clearance of drug-resistant pathogenic bacteria, and regulation of gene expression [52–54]. Studies have shown that Lachnospiraceae can promote SCFAs production [55]. At the family level, the abundance of Lachnospiraceae in AGP was higher \((p < 0.05)\) than that of the control and EO2, suggesting that AGP might promote production performance by increasing SCFA-producing bacteria, but the specific mechanism of action needs to be studied in depth. At the genus level, the abundance of Desulfovibrio in AGP and EO2 was lower \((p < 0.05)\) than that of the control. Desulfovibrio are considered to be harmful bacteria, because Desulfovibrio acts as a hydrogen sink in the cecal ecosystem of birds, and hydrogen inhibits the production of short-chain fatty acids [56,57]. Therefore, Desulfovibrio was highly negatively correlated with laying hens' egg production and ADFI in our study. Lactobacilli is considered a beneficial bacteria due to its tendency to reduce colonization by enteric pathogens through competitive exclusion, antagonistic activity, and production of bacteriocins [58]. Interestingly, the results of this study have shown that Lactobacillus was negatively correlated with laying hens' egg production and ADFI, which may be related to Lactobacillus deconjugating bile acids, affecting lipid metabolism and energy utilization [59,60].

5. Conclusions

Based on the results above, we conclude that dietary supplementation with OEO and cinnamaldehyde improved performance and decreased the feed conversion ratio of late-phase laying hens by the selective modulations of cecum microbial communities. These findings may provide useful information for developing an effective and safe alternative to AGP in the poultry industry.

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References
1. Barton, M.D. Antibiotic use in animal feed and its impact on human health. *Nutr. Res. Rev.* 2000, 13, 279–299. [CrossRef]
2. Phillips, I. Does the use of antibiotics in food animals pose a risk to human health? A critical review of published data. *J. Antimicrob. Chemother.* 2003, 53, 28–52. [CrossRef]
3. Zeng, Z.; Zhang, S.; Wang, H.; Piao, X. Essential oil and aromatic plants as feed additives in non-ruminant nutrition: A review. *J. Anim. Sci. Biotechnol.* 2015, 6, 7. [CrossRef]
4. Calo, J.R.; Crandall, P.G.; O’Bryan, C.A.; Ricke, S.C. Essential oils as antimicrobials in food systems–A review. *Food Control* 2015, 54, 111–119. [CrossRef]
5. Hao, Y.; Li, J.; Zhang, W.; Sun, M.; Li, H.; Xia, F.; Cui, H.; Bai, H.; Shi, L. Analysis of the chemical profiles and anti-*S. aureus* activities of essential oils extracted from different parts of three oregano cultivars. *Foods* 2021, 10, 2328. [CrossRef]
6. Hao, Y.; Kang, J.; Yang, R.; Li, H.; Cui, H.; Bai, H.; Andrey, T.; Li, J.; Shi, L. Multidimensional exploration of essential oils generated via eight oregano cultivars: Compositions, chemodiversities, and antibacterial capacities. *Food Chem.* 2022, 374, 131629. [CrossRef]
7. Zhu, S.Y.; Yang, Y.; Yu, H.D.; Ying, Y.; Zou, G.L. Chemical composition and antimicrobial activity of the essential oils of *Chrysanthemum indicum*. *Ind. Ethnopharmacol.* 2005, 96, 151–158.

8. Du, E.; Wang, W.; Gan, L.; Li, Z.; Guo, S.; Guo, Y. Effects of thymol and carvacrol supplementation on intestinal integrity and immune responses of broiler chickens challenged with *Clostridium perfringens*. *J. Anim. Sci. Biotechnol.* 2016, 7, 19. [CrossRef]

9. Hernández-Coronado, A.C.; Silva-Vázquez, R.; Rangel-Nava, Z.E.; Hernández-Martínez, C.A.; Kawas-Garza, J.R.; Hume, M.E.; Méndez-Zamora, G. Mexican oregano essential oil fluids given in drinking water on performance, carcass traits, and meat quality of broilers. *Poult. Sci.* 2019, 98, 3050–3058. [CrossRef]

10. Xue, F.; Shi, L.; Li, Y.; Ma, H.; Sun, Y.; Chen, J. Effects of replacing dietary Aureomycin with a combination of plant essential oils on production performance and gastrointestinal health of broilers. *Poult. Sci.* 2020, 99, 4521–4529. [CrossRef]

11. Peng, Q.Y.; Li, J.D.; Li, Z.; Duan, Z.Y.; Wu, Y.P. Effects of dietary supplementation with oregano essential oil on growth performance, carcass traits and jejunal morphology in broiler chickens. *Anim. Feed Sci. Technol.* 2016, 214, 148–153. [CrossRef]

12. Abdul, H.; Zia, U.; Rifat, U.K.; Qudrat, U.; Shabana, N. Effect of diet supplemented with coconut essential oil on performance and villus histomorphology in broiler exposed to avian coccidiosis. *Trop. Anim. Health Prod.* 2020, 52, 2499–2504. [CrossRef]

13. Cheng, H.; Chen, J.F.; Tang, S.G.; Guo, S.C.; He, C.Q.; Qu, X.Y. Effects of essential oil/palygorskite composite on performance, egg quality, plasma biochemistry, antioxidant status, immune response and intestinal morphology of laying hens. *Poult. Sci.* 2022, 101, 101632. [CrossRef]

14. Yang, C.; Kennes, Y.M.; Lepp, D.; Yin, X.; Wang, Q.; Yu, H.; Yang, C.; Gong, J.; Diarra, M.S. Effects of encapsulated cinnamonaldehyde and citral on the performance and cecal microbiota of broilers vaccinated or not vaccinated against coccidiosis. *Poult. Sci.* 2020, 99, 936–948. [CrossRef]

15. Yin, D.; Du, D.; Yuan, J.E.; Gao, J.; Wang, Y.; Aggrey, S.E.; Guo, Y. Supplemental thymol and carvacrol increases ileum Lactobacillus population and reduces effect of necrotic enteritis caused by Clostridium perfringens in chickens. *Sci. Rep.* 2017, 7, 7334. [CrossRef]

16. Abdel-Wareth, A.A.A.; Lohakare, J.D. Productive performance, egg quality, nutrients digestibility, and physiological response of bovans brown hens fed various dietary inclusion levels of peppermint oil. *Anim. Feed Sci. Technol.* 2020, 267, 114554. [CrossRef]

17. Migliorini, M.J.; Boiago, M.M.; Stefani, L.M.; Zampar, A.; Roza, L.F.; Barreta, M.; Arno, A.; Robazza, W.S.; Giuratti, J.; Galvão, A.C.; et al. Oregano essential oil in the diet of laying hens in winter reduces lipid peroxidation in yolks and increases shelf life in eggs. *J. Therm. Biol.* 2019, 85, 102409. [CrossRef]

18. Wang, H.; Liang, S.; Li, X.; Yang, X.; Long, F.; Yang, X. Effects of encapsulated essential oils and organic acids on laying performance, egg quality, intestinal morphology, barrier function, and microflora count of hens during the early laying period. *Poult. Sci.* 2019, 98, 6751–6760. [CrossRef]

19. Chen, Y.; Wang, J.; Yu, L.; Xu, T.; Zhu, N. Microbiota and metabolome responses in the cecum and serum of broiler chickens fed with plant essential oils or virginiamycin. *Sci. Rep.* 2020, 10, 5382. [CrossRef]

20. Abdelli, N.; Pérez, J.F.; Vilarrasa, E.; Cabeza Luna, I.; Melo-Duran, D.M.; Angelo, D.; Solá-Oriol, D. Targeted-release organic acids and essential oils improve performance and digestive function in broilers under a necrotic enteritis challenge. *Animals* 2020, 10, 259. [CrossRef]

21. Chowdhury, S.; Mandal, G.P.; Patra, A.K.; Kumar, P.; Samanta, I.; Pradhan, S.; Samanta, A.K. Different essential oils in diets of broiler chickens: 2. Gut microbes and morphology, immune response, and some blood profile and antioxidant enzymes. *Anim. Feed Sci. Technol.* 2018, 236, 39–47. [CrossRef]

22. Windisch, W.; Schedle, K.; Plitzner, C.; Kroismayr, A. Use of phytogenic products as feed additives for swine and poultry. *J. Anim. Sci.* 2008, 86, E140–E148. [CrossRef] [PubMed]

23. Shima, A.A.; Samar, A.T.; Dina, M.M.A.; Doaa, M.A.F.; Aziza, M.H.; Abdallah, E.M. Effect of supplemental glycerol monolaurate and oregano essential oil blend on the growth performance, intestinal morphology, and amino acid digestibility of broiler chickens. *Vet. Res.* 2021, 17, 312.

24. Ding, X.; Yu, Y.; Su, Z.; Zhang, K. Effects of essential oils on performance, egg quality, nutrient digestibility and yolk fatty acid profile in laying hens. *Anim. Nutr.* 2017, 3, 127–131. [CrossRef] [PubMed]

25. Nadia, R.; Hassan, R.A.; Qota, E.M.; Fayek, H.M. Effect of natural antioxidant on oxidative stability of eggs and productive and reproductive performance of laying hens. *Int. J. Poult. Sci.* 2008, 7, 134–150. [CrossRef]

26. Yu, C.; Wei, J.; Yang, C.; Yang, Z.; Yang, W.; Jiang, S. Effects of star anise (*Illicium verum* Hook. f.) essential oil on laying performance and antioxidant status of laying hens. *Poult. Sci.* 2018, 97, 3957–3966. [CrossRef]

27. Botsoglou, N.; Florou-Paneri, P.; Botsoglou, E.; Dotas, V.; Giannenas, I.; Koidis, A.; Mitrakos, P. The effect of feeding rosemary, oregano, saffron and alpha-tocopheryl acetate on hen performance and oxidative stability of eggs. *S. Afr. J. Anim. Sci.* 2005, 35, 143–151.

28. Bozkurt, M.; Kucukyilmaz, K.; Catti, A.U.; Cinar, M.; Bintas, E.; Coven, F. Performance, egg quality, and immune response of laying hens fed diets supplemented with mannan-oligosaccharide or an essential oil mixture under moderate and hot environmental conditions. *Poult. Sci.* 2012, 91, 1379–1386. [CrossRef]

29. Bimczok, D.; Rau, H.; Sewekow, E.; Janczyk, P.; Souffrant, W.B.; Rothkotter, H.J. Influence of carvacrol on proliferation and survival of porcine lymphocytes and intestinal epithelial cells in vitro. *Toxicol. In Vitro* 2008, 22, 652–658. [CrossRef]

30. Thapa, D.; Losa, R.; Zweifel, B.; Wallace, R.J. Sensitivity of pathogenic and commensal bacteria from the human colon to essential oils. *Microbiology* 2012, 158, 2870–2877. [CrossRef]
31. Grobas, S.; Mendez, J.; De Blas, C.; Mateos, G.G. Influence of dietary energy, supplemental fat and linoleic acid concentration on performance of laying hens at two ages. *Br. Poult. Sci.* 1999, 40, 681–687. [CrossRef] [PubMed]

32. Skrivan, M.; Marounek, M.; Babuncova, I.; Podosednizek, M. Influence of limestone particle size on performance and egg quality in laying hens aged 24-36 weeks and 56-68 weeks. *Anim. Feed Sci. Technol.* 2010, 156, 110–114. [CrossRef]

33. Feng, J.; Lu, M.; Wang, J.; Zhang, H.; Qiu, K.; Qi, G.; Wu, S. Dietary oregano essential oil supplementation improves intestinal functions and alters gut microbiota in late-phase laying hens. *J. Anim. Sci. Biotechnol.* 2021, 12, 72. [CrossRef] [PubMed]

34. Li, C.; Pi, G.; Li, F. The role of intestinal flora in the regulation of bone homeostasis. *Front. Cell. Infect. Microbiol.* 2021, 11, 579323. [CrossRef]

35. Simopoulos, A.P.; Salem, N.J. Egg yolk as a source of long-chain polyunsaturated fatty acids in infant feeding. *Am. J. Clin. Nutr.* 1992, 55, 411–414. [CrossRef]

36. Plotz, T.; Krummel, B.; Laporte, A.; Pingitore, A.; Persaud, S.J.; Jorns, A.; Elsner, M.; Mehmeti, I.; Lenzen, S. The monounsaturated fatty acid oleate is the major physiological toxic free fatty acid for human beta cells. *Nutr. Diabetes* 2017, 7, 305. [CrossRef]

37. Gillingham, L.G.; Harris-Janzen, S.; Jones, P.J. Dietary monounsaturated fatty acids are protective against metabolic syndrome and cardiovascular disease risk factors. *Lipids* 2011, 46, 209–228. [CrossRef]

38. Willett, W.C. The Mediterranean diet: Science and practice. *Public Health Nutr.* 2006, 9, 105–110. [CrossRef]

39. Galobart, J.; Barroeta, A.C.; Baucells, M.D.; Codony, R.; Ternes, W. Effect of dietary supplementation with rosemary extract and alpha-tocopheryl acetate on lipid oxidation in eggs enriched with omega 3-fatty acids. *Poult. Sci.* 2001, 80, 460–467. [CrossRef]

40. Bolukbasi, S.C.; Urusan, H.; Erhan, M.K.; Kiziltunc, A. Effect of dietary supplementation with bergamot oil (*Citrus bergamia*) on performance and serum metabolic profile of hens, egg quality and yolk fatty acid composition during the late laying period. *Archiv. Fur. Geflugelkunde* 2010, 74, 172–177.

41. Abdel-Wareth, A. Effect of dietary supplementation of thymol, syntibiotic and their combination on performance, egg quality and serum metabolic profile of Hy-Line Brown hens. *Br. Poult. Sci.* 2016, 57, 114–122. [CrossRef] [PubMed]

42. Cukrowska, B.; Sowinska, A.; Bierla, J.B.; Czarnowska, E.; Rybak, A.; Grzybowska-Chlebowczyk, U. Intestinal epithelium, intraepithelial lymphocytes and the gut microbiota—Key players in the pathogenesis of celiac disease. *World J. Gastroenterol.* 2017, 23, 7505–7518. [CrossRef]

43. Xiao, S.; Li, Q.P.; Hu, K.; He, Y.; Ai, Q.; Hu, L.H.; Yu, J.L. Vitamin A and retinoic acid exhibit protective effects on Necrotizing enterocolitis by regulating intestinal flora and enhancing the intestinal epithelial barrier. *Arch. Med. Res.* 2018, 49, 1–9. [CrossRef] [PubMed]

44. Xue, M.L.; Ji, X.Q.; Liang, H.; Guo, Y.; Zhang, H.; Cheng, J.; Zhou, H. Inulin improves the egg production performance and affects short-chain fatty acids in laying hens. *J. Funct. Foods* 2018, 25, 511–522. [CrossRef]

45. Cui, H.; Zhang, C.; Li, C.; Lin, L. Antibacterial mechanism of oregano essential oil. *Microbiome* 2019, 7, 100809. [CrossRef] [PubMed]

46. Shang, H.; Zhao, J.; Dong, X.; Guo, Y.; Zhang, H.; Cheng, J.; Zhou, H. Inulin improves the egg production performance and intestinal microbiota of broilers. *Anim. Feed Sci. Technol.* 2018, 23, 7505–7518. [CrossRef]

47. Li, I.; Wu, S.; Ronan, P.; Hausner, M.; Neufeld, J.D. Recovering glycoside hydrolase genes from active tundra cellulolytic bacteria. *FEMS Microb. Lett.* 2014, 60, 209–228. [CrossRef]

48. Feng, J.; Lu, M.; Wang, J.; Zhang, H.; Qiu, K.; Qi, G.; Wu, S. Dietary oregano essential oil supplementation improves intestinal morphology, cecal short-chain fatty acid concentration, and cecal microbiota in broiler chickens. *Anim. Feed Sci. Technol.* 2010, 158, 115. [CrossRef]

49. Li, I.; Wu, S.; Liou, J.; Liu, H.; Chen, J.; Chen, C. Effects of *Deinococcus* spp. supplement on egg quality traits in laying hens. *Poult. Sci.* 2018, 97, 319–327. [CrossRef] [PubMed]

50. Oladokun, S.; Koehler, A.; Maclsaac, J.; Ibeagha-Awemu, E.M.; Adewole, D.I. *Bacillus subtilis* delivery route: Effect on growth performance, intestinal morphology, cecal short-chain fatty acid concentration, and cecal microbiota in broiler chickens. *Poult. Sci.* 2020, 100, 100809. [CrossRef] [PubMed]

51. Smith, P.M.; Howitt, M.R.; Panikov, N.; Michaud, M.; Gallini, C.A.; Bohlooly-y, M.; Glickman, J.N.; Garrett, W.S. The microbial metabolites, short-chain fatty acids, regulate colonic t-reg cell homeostasis. *Science* 2013, 341, 569–573. [CrossRef] [PubMed]

52. Fernandez, J.; Redondo-Blanco, S.; Gutierrez-del-Rio, I.; Miguelez, E.M.; Villar, C.J.; Lombo, F. Colon microbiota fermentation of dietary prebiotics towards short-chain fatty acids and their roles as anti-inflammatory and anti tumour agents: A review. *J. Funct. Foods* 2016, 25, 511–522. [CrossRef]

53. Smith, P.M.; Howitt, M.R.; Panikov, N.; Michaud, M.; Gallini, C.A.; Bohlooly-y, M.; Glickman, J.N.; Garrett, W.S. The microbial metabolites, short-chain fatty acids, regulate colonic t-reg cell homeostasis. *Science* 2013, 341, 569–573. [CrossRef] [PubMed]

54. Fernandez, J.; Redondo-Blanco, S.; Gutierrez-del-Rio, I.; Miguelez, E.M.; Villar, C.J.; Lombo, F. Colon microbiota fermentation of dietary prebiotics towards short-chain fatty acids and their roles as anti-inflammatory and anti tumour agents: A review. *J. Funct. Foods* 2016, 25, 511–522. [CrossRef]

55. Zhao, L.; Zhang, F.; Ding, X.; Wu, G.; Lam, Y.Y.; Wang, X.; Fu, H.; Xue, X.; Lu, C.; Ma, J.; et al. Gut bacteria selectively promoted the growth and intestinal microbiota of broilers. *J. Appl. Microbiol.* 2016, 120, 195–204. [CrossRef]

56. Serino, M. SCFAs—The thin microbial metabolic line between good and bad. *Nat. Rev. Endocrinol.* 2019, 15, 318–319. [CrossRef] [PubMed]

57. Smith, P.M.; Howitt, M.R.; Panikov, N.; Michaud, M.; Gallini, C.A.; Bohlooly-y, M.; Glickman, J.N.; Garrett, W.S. The microbial metabolites, short-chain fatty acids, regulate colonic t-reg cell homeostasis. *Science* 2013, 341, 569–573. [CrossRef] [PubMed]
58. Lan, Y.; Verstegen, M.; Tamminga, S.; Williams, B.A. The role of the commensal gut microbial community in broiler chickens. *World Poult. Sci. J.* 2005, 61, 95–104. [CrossRef]

59. Lin, J.; Hunkapiller, A.A.; Layton, A.C.; Chang, Y.; Robbins, K.R. Response of intestinal microbiota to antibiotic growth promoters in chickens. *Foodborne Pathog. Dis.* 2013, 10, 331–337. [CrossRef]

60. Ranjitkar, S.; Lawley, B.; Tannock, G.; Engberg, R.M. Bacterial succession in the broiler gastrointestinal tract. *Appl. Environ. Microbiol.* 2016, 82, 2399–2410. [CrossRef]