Efficacy of natural alternatives to antibiotic on the growth performance, gut microbial population, intestinal morphology, and serum biochemical metabolites of broiler chickens

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ABSTRACT
The present study investigated the effects of different feed additives on growth performance, carcass characteristics, gut microbial population, intestinal morphology, and blood metabolites of broiler chickens. A total of 540, day-old Ross 308 male chicks were randomly distributed into 6 dietary treatments with 6 replicate pens per treatment for 35 days. The birds fed on a basal diet without feed additive (CON) or the basal diet supplemented with 0.25 g/kg antibiotic as growth promoter (AGP), 1 g/kg essential oils (EO), 1.7 g/kg synbiotic (SYN), 3 g/kg medium-chain fatty acids (MCFA), and 1 mL/L essential oils in drinking water (EOW). The additives improved the body weight gain (p = .004) and feed conversion ratio (p = .02) compared to the CON group during the whole trial. The serum concentration of cholesterol was lower in the birds fed the MCFA diet and serum concentration of low-density lipoprotein cholesterol (LDL) decreased in MCFA and SYN groups. The relative weight of spleen was the greatest in MCFA group (p = .01). Feeding birds diets containing different additives decreased the population of coliform (p = .002) and Clostridium perfringens (p = .01) while Lactobacillus population was greater in broilers offered EOW, SYN, and MCFA supplements (p = .02). The jejunal villus height enhanced in the broiler chickens which received AGP and MCFA additives (p = .008). It is concluded that EOW, SYN, MCFA, and EO as alternatives for AGP improved the growth performance and intestinal morphometric indices and reduced the caecal pathogenic bacteria in broiler chickens.

HIGHLIGHTS
- Adding MCFA to diet and EO to the drinking water improved BWG and FCR in broiler chickens.
- Dietary supplementation of MCFA decreased serum total cholesterol, increased caecal LAB population, and improved intestinal villus height.
- All supplements as AGP alternatives had beneficial effects on suppressing the pathogenic bacteria.

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INTRODUCTION

Over the past few decades, in-feed antibiotics have been used at sub-therapeutic doses in poultry production to control pathogenic bacteria and improve the growth performance and quality of livestock products (Jazi et al. 2018). However, excessive antibiotic use has consequently led to the emergence of resistant bacteria and antibiotic residues in poultry meat products, which pose a serious risk to human health. This has led many countries to ban the use of antibiotics as growth promoters (AGP) in poultry nutrition (Shirani et al. 2019). Therefore, there is a global effort to identify and develop effective, economical, and safe alternatives to AGP for poultry production. Among the possible alternatives, phytogenic feed additives such as essential oils, organic acids, medium-chain fatty acids (MCFA), probiotics, prebiotics, and synbiotics have shown antibacterial activity to control dysbiosis and improve the growth performance of poultry (Gadde et al. 2017).

Medicinal plants and phytogenic products have been used for centuries to treat a variety of human diseases due to their excellent therapeutic effects (Park and Kim 2018). The therapeutic effects of...
medicinal plants and phytogenic feed additives have been attributed to combinations of active phytochemicals and secondary compounds such as organic acids, terpenoids, phenolic compounds, and aldehydes, which can exert antibacterial, anti-inflammatory, and antioxidant effects. Inclusion of phytogenic products in the poultry feed may have beneficial effects through balancing gut microbiota ecosystem, stimulating digestive enzymes secretion, maintaining gut integrity, regulating lipid metabolism, and modulating immune response (Hashemipour et al. 2013; Hajiaghapour and Rezaeipour 2018; Hazrati et al. 2019; Shirani et al. 2019; Kolbadinejad and Rezaeipour 2020). Ajowan (source of thymol and γ-terpinene), thyme (source of thymol and p-Cymene) (Gilani et al. 2005), are among the plants that have strong antioxidant properties due to their active ingredients and are important due to their antimicrobial and antifungal properties and have been considered by the poultry industry (Aeschbach et al. 1994). Today, the effect of garlic on the immune system and its antibiotic and antifungal properties has been considered by poultry farmers. Most of the antimicrobial properties of garlic are related to alicine, which is produced by the phospho-pyridoxal alliinase enzyme (Yoshida et al. 1987). The antioxidant properties of turmeric and its derivatives have been proven in various experiments. Curcumin is one of the main components of turmeric, which neutralises oxygen free radicals (Ruby et al. 1995). Lemon balm is a medicinal plant from the mint genus. The most common properties of Lemon balm are antispasmodic, antioxidant, antibacterial, antiviral, and anti-inflammatory effects (Yosofi et al. 2011).

Previous studies report MCFA antimicrobial activity against the most common poultry pathogenic bacteria such as Escherichia coli (Skrivanova et al. 2012) and Clostridium perfringens (Skrivanova et al. 2012). In addition, it is reported that MCFA can increase the release of secretin and free protons and decrease the digesta pH which promotes the secretion of digestive enzymes (Shabani et al. 2019). The aforementioned modes of action of MCFA also improves overall gut functionality and nutrients digestibility leading to a better growth performance of birds (Menconi et al. 2014).

Combination of probiotic and prebiotic, called symbiotic, are one of the most popular functional feed supplements in poultry nutrition (Hazrati et al. 2019; Dev et al. 2020). Probiotics are live microbial feed additives that beneficially alter the gut microbial balance and improve the host animal’s health and growth performance (Al-Khalaifah 2018). The prebiotics are non-digestible fibre compounds which are food for beneficial bacteria and hence, affect the composition or activity of the hindgut and caecal microbiota (Pourabedin and Zhao 2015). The inclusion of symbiotics in the diets of broiler chickens improves growth performance, utilisation of nutrients, and immunity (Dev et al. 2020). However, the beneficial effects of the aforementioned feed additives in poultry production have not always been consistent (Toghyani et al. 2010; Mookiah et al. 2014; Nosrati et al. 2017). Therefore, more in-depth research needs to be done to come along with satisfactory and consistent results. Hence, the aim of the present study was to evaluate and compare different feed additives including EO, MCFA, and symbiotic on growth performance, carcass characteristics, gut microbial population, intestinal morphology, and serum metabolites of broiler chickens.

Materials and methods

Experimental design and management

Day-old male Ross 308 broiler chickens (n = 540) were purchased from a commercial hatchery and transferred to the Research Centre at the Qaemshahr Branch, Islamic Azad University (Qaemshahr, Iran). On arrival, the broilers were assigned to 36 floor pens with bedding of softwood shavings in an environmentally controlled room. The dietary treatments consisted of a corn-soybean meal basal diet without feed additives as the control (CON); the basal diet supplemented with 0.25 g/kg antibiotic growth promoter (AGP; Kimilacin® 8.8, Kimia Faam Pharmaceutical Co., Iran); the basal diet supplemented with 1 g/kg essential oil (EO mix contains garlic, thyme, turmeric, lemon balm and few other medicinal herbs, Bioherbal®, Pars Imen Daru Co., Iran), the basal diet supplemented with 1.5 g/kg prebiotic plus 0.2 g probiotic (symbiotic, probiotic: Lacto feed, Tak Genezist, Iran; Prebiotic: Mito Healthy Friend (MHF-Y), MEITO Co., Japan); the basal diet supplemented with 3 g/kg MCFA (Selacid®, Selko B.V., Tilburg, Netherland contains a mixture of caproic, caprylic, capric, and lauric acids); and a water-supplemented treatment with 1 mL/L EO (EOW mix contains ferula gummosa, eucalyptus, clove, peppermint, thyme, fennel, ajwain and satureja, Arkia Z-9; Bein Daru, Iran). Broiler chickens were fed experimental diets (mash form) for the whole experimental period in three phases: starter (d 1–10), grower (d 11–24), and finisher (d 25–35). The basal diets were formulated to meet or exceed Aviagen (2018) recommendations (Table 1). Each diet was replicated 6 times.
Table 1. Ingredients and nutrient levels of the basal diets.

| Ingredients, % | Stater period (d 1–10) | Grower period (d 11–24) | Finisher period (d 25–35) |
|----------------|------------------------|-------------------------|---------------------------|
| Corn           | 53.88                  | 57.33                   | 62.20                     |
| Soybean meal   | 40.78                  | 36.98                   | 31.82                     |
| Soybean oil    | 1.10                   | 1.84                    | 2.41                      |
| Limestone      | 0.87                   | 0.81                    | 0.75                      |
| Dicalcium phosphate | 1.84       | 1.64                    | 1.47                      |
| Salt           | 0.38                   | 0.38                    | 0.38                      |
| Vitamin premixa | 0.25                 | 0.25                    | 0.25                      |
| Mineral premixb | 0.25                 | 0.25                    | 0.25                      |
| L-lysine HCL   | 0.22                   | 0.16                    | 0.16                      |
| L-threonine    | 0.08                   | 0.05                    | 0.03                      |
| Total          | 100                    | 100                     | 100                       |
| Calculated composition |          |                         |                           |
| AME, kcal/kg   | 2800                   | 2900                    | 3000                      |
| Crude protein, % | 21.47                 | 20.11                   | 18.28                     |
| Lys, %         | 1.36                   | 1.22                    | 1.10                      |
| Met, %         | 0.66                   | 0.60                    | 0.55                      |
| Met+Cys, %     | 1.00                   | 0.93                    | 0.86                      |
| Thr, %         | 0.90                   | 0.82                    | 0.73                      |
| Ca, %          | 0.89                   | 0.82                    | 0.75                      |
| Available P, % | 0.40                   | 0.36                    | 0.33                      |
| Na, %          | 0.16                   | 0.15                    | 0.15                      |
| Cl, %          | 0.29                   | 0.29                    | 0.29                      |

Vitamin premix provided the following per kilogram of diet: vitamin A, 11,500 U; cholecalciferol, 2,100 U; vitamin E, 22 U; vitamin K3, 1.50 mg; thiamine, 3 mg; riboflavin, 4.4 mg; pantothenic acid, 25 mg; niacin, 40 mg; choline chloride, 560 mg; biotin, 0.1 mg; folic acid, 0.8 mg; pyridoxine 10 mg; vitamin B12, 0.060 mg.

Trace mineral premix provided the following in milligrams per kilogram of diet: iron, 50 mg; zinc, 55 mg; manganese, 75 mg; iodine, 1.8 mg; copper, 8 mg; selenium, 0.3 mg; cobalt, 0.2 mg.

and each replicate pen housed 15 broiler chickens. During the experiment, the birds had ad libitum access to feed and water and were provided with 23 h of light and 1 h of dark. The room temperature was set at 33–34 °C for the first 5 d, then gradually reduced to 21 °C, and kept stable until the end of the study.

Measurements and sampling

Feed intake (FI) and body weight (BW) per pen were recorded on days 0, 10, 24, and 35, and the feed conversion ratio (FCR) was calculated as the FI divided by body weight gain (BWG).

At the end of the trial (d 35), one bird from each replicate was randomly selected and blood samples were obtained from the brachial vein of each chick and centrifuged (2000 × g for 10 min) at 4 °C. Then, the concentrations of triglyceride, cholesterol, high-density lipoproteins (HDL), total protein, and albumin in the serum were analysed by an automatic biochemical analyser and using the corresponding commercial kits from Pars Azmoon Company (Tehran, Iran), according to the manufacturer’s instructions. Very low-density lipoprotein cholesterol (VLDL-C) values were calculated by dividing triglyceride values to unit 5 and low-density lipoprotein cholesterol (LDL-C) values subtracting total values of HDL-C and VLDL-C from total cholesterol (Jazi et al. 2018).

Following blood sample collection, the birds were individually weighed and euthanized by CO2 asphyxiation, and dissected to collect samples for carcass composition, internal organs, caecal microbial evaluation, gut morphology, and meat quality studies. The weight of the carcass, breast, thighs, liver, spleen, and bursa of Fabricius were recorded and the relative weight of the organs was expressed as a percentage of BW.

For caecal microbial evaluation, one gram of caecal digesta content was diluted serially in 0.9% sterile saline solution. Then, 0.1 mL of each dilution was plated onto MRS agar, tryptose sulphite cycloserine agar, MacConkey agar, and plate count agar to count the lactic acid bacteria (LAB), clostridium perfringens, coliforms, and total anaerobic bacteria (TAB), respectively. Tryptose sulphite cycloserine agar and MRS agar plates were incubated anaerobically for 24–48 hours at 37 °C. The MacConkey agar and plate count agar plates were incubated aerobically for 24 hours at 37 °C. The bacterial colonies in each plate were counted using a colony counter, and the results expressed as colony-forming units per gram of sample (log10 CFU/g).

For gut histomorphology study, a 1-cm segment from the midpoint of the duodenum, jejunum, and ileum of sampled birds was collected and flushed with distilled water and then fixed in 10% buffered formal-dehyde solution. Each of the collected segments was embedded in paraffin, and a 5-μm section of each sample was cut (Semi-automated Microtome, Model Leica RM 2145) and mounted on a glass slide and subsequently stained with haematoxylin and eosin. A light microscope (Model U- TV0.5 XC-2, Olympus corporation, BX41) was used scan the slides and study the gut morphology parameters. Villus height (from the tip of the villus to the crypt junction), crypt depth (from the base of the villus to the sub mucosa), and villus width (VW: at the middle point of the villus) were measured with the ImageJ software package. A total of 10 intact, well-oriented crypt-villus units were selected in duplicate from each tissue sample, and the averages of 20 values were obtained for each replicate (Jazi et al. 2018).

Statistical analysis

Data were analysed by one-way analysis of variance using the GLM procedure of SAS software (SAS Institute, Inc 2003) as a completely randomised design.
Results

Growth performance and carcass characteristics

The effect of different feed additives on growth performance of broilers is summarised in Table 2. During the starter period (day 1–10), BWG (p = .12) and FCR (p = .14) tended to improve in all supplemented groups compared to the CON group, however, FI was not affected by experimental treatments. During the grower period (day 11–24), birds fed AGP had a tendency for a higher BWG compared to the treatments other (p = .10). The feed intake was significantly higher in the AGP, EO, and CON groups compared to the MCFA, SYN, and EOW (p = .04). The FCR, BWG and FI were not affected by experimental treatments for the finisher phase. For the overall grow-out period, broiler chickens fed the AGP diet had highest BWG (p = .004) and lowest FCR than others (p = .02). Birds fed MCFA and EOW had a higher BWG compared to the CON, but not different from the EO and SYN (p = .004). Birds fed CON, MCFA and EO diets had the second lowest FCR (p = .02). However, no significant difference was recorded for FI among the experimental treatments for the overall experiment.

The effect of experimental diets on the carcass yield and organ weights is presented in Table 3. There were no significant differences in relative weights of carcass, breast, thigh, liver, and bursa of Fabricius among treatment groups. However, broiler chickens fed diets containing MCFA showed an increase in the weight of spleen compared to the other experimental treatment (p = .01).

Caecal microbiota

The effect of different feed additives on caecal microbial count of broilers is presented in Table 4. The greatest decrease in the populations of TAB (p = .02), LAB (p = .02), coliform (p = .002), and Clostridium perfringens (p = .01) were observed in the AGP group. However, MCFA showed a similar effect as AGP on TAB, coliforms, and Clostridium perfringens, and had a significantly higher LA than AGP (p = .02), although not different to the CON, EO, and SYN. Likewise, other feed additives SYN, MCFA, EO, and EOW decreased the numbers of coliform and Clostridium perfringens in

Table 2. Effect of the experimental diets on growth performance of broiler chickens

| Item                  | Treatmente | CON     | AGP     | EO      | MCFA    | SYN     | EOW     | SEM     | p-Value |
|-----------------------|------------|---------|---------|---------|---------|---------|---------|---------|---------|
| Body weight gain, g   | 209.16     | 260.10  | 253.13  | 247.40  | 255.23  | 247.33  | 5.01    | .12     |
| Feed intake, g        | 232.67     | 270.00  | 250.00  | 259.00  | 272.00  | 260.00  | 5.06    | .30     |
| Feed conversion ratio | 1.11       | 1.04    | 0.99    | 1.04    | 1.06    | 1.05    | 0.01    | .14     |
| Body weight gain, g   | 926.17     | 956.67  | 928.67  | 918.20  | 832.10  | 843.13  | 14.12   | .10     |
| Feed intake, g        | 1330.00    | 1325.00 | 1333.33 | 1273.33 | 1182.00 | 1190.00 | 16.00   | .04     |
| Feed conversion ratio | 1.43       | 1.38    | 1.43    | 1.38    | 1.44    | 1.41    | 0.01    | .59     |
| Body weight gain, g   | 841.33     | 946.33  | 823.67  | 905.67  | 931.67  | 977.00  | 19.42   | .23     |
| Feed intake, g        | 1623.70    | 1773.30 | 1686.70 | 1739.70 | 1800.00 | 1813.33 | 32.32   | .54     |
| Feed conversion ratio | 1.93       | 1.88    | 2.05    | 1.92    | 1.93    | 1.86    | 0.02    | .22     |
| Body weight gain, g   | 1976.67bc  | 2163.10c | 2005.47bc | 2071.27b | 2010.00bc | 2067.47bc | 10.84   | .004    |
| Feed intake, g        | 3186.33    | 3368.33 | 3272.00 | 3270.00 | 3254.00 | 3263.33 | 25.86   | .54     |
| Feed conversion ratio | 1.61ab     | 1.55c   | 1.63a   | 1.58bc  | 1.62ab  | 1.58bc  | 0.005   | .02     |

a,b Means with different superscripts in each row are significantly different (p < .05).

Table 3. Effect of the experimental diets on carcass characteristics of broiler chickens

| Item                  | Treatment | CON     | AGP     | EO      | MCFA    | SYN     | EOW     | SEM     | p-Value |
|-----------------------|-----------|---------|---------|---------|---------|---------|---------|---------|---------|
| Carcass               | 64.95     | 64.07   | 65.82   | 64.14   | 67.38   | 65.26   | 0.42    | .25     |
| Breast                | 23.50     | 24.63   | 22.42   | 24.51   | 25.07   | 24.05   | 0.27    | .69     |
| Thigh                 | 19.75     | 18.89   | 19.58   | 19.05   | 20.22   | 19.62   | 0.17    | .29     |
| Liver                 | 2.15      | 2.17    | 2.02    | 2.18    | 2.28    | 2.23    | 0.057   | .87     |
| Spleen                | 0.09b     | 0.08b   | 0.09b   | 0.13a   | 0.09b   | 0.08b   | 0.01    | .01     |
| Bursa of fabricius    | 0.10      | 0.08    | 0.09    | 0.10    | 0.11    | 0.07    | 0.01    | .43     |

a,b Means with different superscripts in each row are significantly different (p < .05).
caecum \( (p < .05) \). Coliforms were reduced by all additives including AGP, EO, MCFA, SYN, and EO compared to the CON \( (p = .002) \). *Clostridium perfringens* Was reduced in AGP, MCFA, and SYN compared to the CON \( (p = .01) \).

### Intestinal morphology

The effects of experimental diets on intestinal morphology of broiler chicks are listed in Table 5. The results show that broiler chickens fed MCFA and AGP diets had higher villus height in jejunum than others \( (p = .008) \). The rest of the measurements did not indicate a considerable impact on the other morphometric values in the duodenum, jejunum, and ileum of birds fed experimental diets.

### Serum biochemical parameters

The effects of experimental diets on blood serum biochemistry of broiler chicks are shown in Table 6. Serum concentrations of albumin, total protein, triglycerides, HDL, and VLDL were not affected by dietary treatments. However, feeding birds with diets supplemented with MCFA decreased serum cholesterol compared to the other treatments \( (p = .001) \). Furthermore, the serum LDL in birds fed with SYN and MCFA diets was lower than others \( (p = .02) \).

**Discussion**

In the past two decades, interest in the use of natural feed additives as alternatives to in-feed antibiotics in poultry nutrition has increased dramatically, particularly after European Union ban on AGP in poultry production in 2006 (Shirani et al. 2019). The aim of the present study was to test whether different feed additives including EO, MCFA, SYN, and EOW, could be considered as a promising alternative to AGP and study their effect on production performance, gut health, blood metabolites, and meat quality of broiler chickens. Based on the findings obtained in this study, the use of feed additives of AGP, MCFA, and EOW in broilers nutrition increased BWG and decreased FCR. Previous studies have shown that the addition of phytogenic products such as EO to the drinking water of broilers improved BWG and FCR compared to the control group (Adaszyńska-Skwirzyńska and Szczepanska 2019). This improvement in growth performance is due to the bioactive ingredients in phytogenic products’ ability in stimulating the secretion of pancreatic digestive enzymes such as amylase, maltase, and trypsin, reducing pathogenic bacteria, as well as improving...

### Table 4. Effect of the experimental diets on cecalmicroflora population (log10cfu/g) of broiler chickens.

| Item                    | CON  | AGP  | EO   | MCFA | SYN  | EOW  | SEM | p-Value |
|-------------------------|------|------|------|------|------|------|-----|---------|
| Total anaerobic bacteria| 9.23 | 9.10 | 9.23 | 9.14 | 9.23 | 9.19 | 0.01| .02     |
| Lactic acid bacteria    | 7.94 | 7.84 | 7.96 | 7.99 | 7.96 | 7.86 | 0.01| .02     |
| Coliforms               | 8.32 | 8.15 | 8.23 | 8.18 | 8.25 | 8.25 | 0.01| .002    |
| Clostridium perfringens | 6.00 | 5.78 | 5.88 | 5.72 | 5.84 | 5.89 | 0.02| .01     |

*a–c* Means with different superscripts in each row are significantly different \( (p < .05) \).

**Table 5. Effect of the experimental diets on intestinal morphology (mm) of broiler chickens.**

| Item                    | CON  | AGP  | EO   | MCFA | SYN  | EOW  | SEM | p-Value |
|-------------------------|------|------|------|------|------|------|-----|---------|
| Duodenum                |      |      |      |      |      |      |     |         |
| Villus height           | 1370.17 | 1304.67 | 1339.67 | 1277.67 | 1333.33 | 1276.50 | 13.11   | .27     |
| Villus width            | 184.83 | 186.00 | 175.00 | 189.50 | 192.50 | 175.00 | 13.11   | .27     |
| Jejunum                 |      |      |      |      |      |      |     |         |
| Villus height           | 1204.17 | 1364.50 | 1279.83 | 1461.00 | 1247.33 | 1264.33 | 19.50 | .008    |
| Villus width            | 208.33 | 186.00 | 192.00 | 219.17 | 200.33 | 206.33 | 8.46    | .97     |
| Ileum                   |      |      |      |      |      |      |     |         |
| Villus height           | 1016.33 | 993.17 | 1015.83 | 1054.00 | 918.83 | 1062.33 | 26.88 | .69     |
| Villus width            | 208.83 | 213.00 | 203.17 | 171.33 | 169.00 | 208.50 | 7.80    | .38     |

*a–c* Means with different superscripts in each row are significantly different \( (p < .05) \).

**Legend**

| Treatment            | Description                                      |
|----------------------|--------------------------------------------------|
| CON                  | Basal diet without feed additive                 |
| AGP                  | Basal diet + antibiotic growth promoter          |
| EO                   | Basal diet + essential oils                      |
| MCFA                 | Basal diet + medium-chain fatty acids            |
| SYN                  | Basal diet + symbiotic                           |
| EOW                  | Drinking water + essential oils                  |
Table 6. Effect of the experimental diets on serum biochemical parameters of broiler chickens.

| Item                  | CON  | AGP  | EO    | MCFA | SYN  | EOW  | SEM  | p-value |
|-----------------------|------|------|-------|------|------|------|------|---------|
| Cholesterol, mg/dL    | 146.16<sup>a</sup> | 142.33<sup>a</sup> | 142.66<sup>a</sup> | 109.00<sup>b</sup> | 134.33<sup>a</sup> | 142.32<sup>a</sup> | 2.06  | .001    |
| Triglycerides, mg/dL  | 105.50 | 102.83 | 98.66  | 103.33 | 97.83 | 103.33 | 1.84  | .81     |
| VLDL-C<sub>f</sub>, mg/dL | 71.83 | 71.66 | 73.83  | 73.66 | 78.00 | 66.33 | 1.57  | .42     |
| LDL-C<sub>g</sub>, mg/dL | 21.10 | 20.56 | 19.73  | 20.66 | 19.56 | 20.66 | 0.37  | .81     |
| HDL-C<sub>e</sub>, mg/dL | 53.23 | 50.10 | 49.43<sup>abc</sup> | 39.70<sup>bc</sup> | 36.76<sup>c</sup> | 55.66<sup>a</sup> | 1.72  | .02     |
| Total protein, g/dL   | 4.67  | 4.28  | 4.87   | 4.53  | 4.30  | 4.58  | 0.76  | .24     |
| Albumin, mg/dL        | 1.41  | 1.38  | 1.45   | 1.34  | 1.17  | 1.25  | 0.04  | .26     |

<sup>a</sup>Means with different superscripts in each row are significantly different (p < .05).
<sup>b</sup>CON: basal diet without feed additive; AGP: basal diet + antibiotic growth promoter; EO: basal diet + essential oils; MCFA: basal diet + medium-chain fatty acids; SYN: basal diet + symbiotic; EOW: drinking water + essential oils.
<sup>c</sup>HDL-C: high density lipoprotein cholesterol.
<sup>d</sup>LDL-C: low density lipoprotein cholesterol.
<sup>e</sup>VLDL-C: very low density lipoprotein cholesterol.

The MCFA have two main resources: milk, and coconut oil. It has been reported that the MCFA supplementation in diets exhibit positive effects on growth performance in broiler chickens (Nguyen et al. 2018) which relates to the antimicrobial activity of organic acid, helps the reduction of the pathogenic microbial load, and leads to decreased metabolic demands of microbes, and hence enhanced availability of dietary nutrients to the host animals (Lee et al. 2015). In addition, organic acids can stimulate the release of secretin and free protons and reduce the digesta pH which increases the secretion of digestive enzymes (Shabani et al. 2019). Therefore, such changes can effectively increase nutrient digestibility and thus improve growth performance. In the present study, the use of the SYN supplement in the broiler diets had no significant effect on the performance traits. Similarly, there are several studies indicating no positive effect of SYN supplementation on the growth performance of broiler chickens (Erdoğan et al. 2010; Mookiah et al. 2014). However, some reports have shown that SYN supplementation could improve the growth performance of broiler chickens (Dev et al. 2020). The variations in the findings pertaining to the performance of broilers in response to dietary SYN may be related to differences in the composition and inclusion level of the supplements used, diet composition, the administration route, environmental factors, and animal factors such as strain and age.

In the current study, dietary supplementation of MCFA increased the development of the spleen. Spleen is an immune organ and its weight measurement is a common method for assessing the immune status of chickens (Nguyen et al. 2018). The immune organs make up the body's immune system together with lymphatic organs and immune cells. Proliferation, differentiation, and maturation of immune cells usually occur in the thymus, spleen, and bursa (Brekelmans and Van Ewijk 1990). Therefore, the weight of the lymphatic organs indicates the body's ability to provide lymph cells during the immune response.

Pathogenic microorganisms reduce the growth rate and health status of broilers by producing toxins, utilising nutrients, and suppressing beneficial microbes that synthesise vitamins or other growth factors (Falaki et al. 2010). Therefore, the stabilisation of intestinal microflora is critical to intestinal health and function (Mohebodini et al. 2019). In the present study, all feed additives reduced the numbers of coliform and Clostridium perfringens in caecal content. In addition, feeding diets MCFA increased the population of LAB in caecal digesta. In vitro studies have shown that the EO bioactive compounds have strong antimicrobial activity against pathogenic bacteria such as Salmonella, E. coli, and Clostridium perfringens (Du et al. 2015). Other in vivo studies indicated that feeding EO in broiler chickens decreases populations of coliform and Clostridium perfringens in caecal (Park and Kim 2018; Barbarestani et al. 2020). The decrease of pathogenic bacteria is due to the ability of bioactive ingredients of EO to stimulate the secretion of mucus in the intestine and disrupt the bacterial cell membrane by breakdown of the outer membrane and leakage of ions and other components (Zeng et al. 2015). Similar to the present results, Mohammed et al. (2019) reported that the supplementation of SYN (LAB and fructo-oligosaccharides) increased populations of coliform and E. coli and increased lactobacillus spp. and Bifidobacterium spp. counts in the caecal content. Symbiotic (probiotic and prebiotic) play an important role in improving the gut microflora balance by supply food for beneficial bacteria. The proliferation of the beneficial bacteria, improves gut ecosystem by producing antimicrobial compounds such as bacteriocins, stimulating the immune system, and competitive exclusion of pathogenic microbes (Kridtayopas et al.
The antibacterial mode of action of MCFA includes the entry of organic acids into the gram-negative bacteria cell (such as coliform and E.coli), leading to bacterial membrane disruption and prevention of bacterial enzyme’s activities. Eventually, with blocking the bacterial enzymatic processes, the proton motive force falls, leading to cellular death (Suiryanrayna and Ramana 2015). On the other hand, organic acids provide suitable conditions for the growth and multiplication of beneficial bacteria such as LAB by acidifying the intestinal environment (Shabani et al. 2019). The results of this study confirm the above reports by showing a significant increase in the number of LAB and a decrease in the number of coliform and Clostridium perfringens in diets supplemented with MCFA, which is consistent with other reported studies (Nguyen et al. 2018).

Intestinal morphology traits including villus height, crypt depth, and villus height to crypt depth ratio, are indicative of gut health and nutrient digestion and absorption capacity in birds. Increased villus height and villus height to crypt depth ratio are directly associated with greater nutrient digestion (Soumeh et al. 2019). Whilst reduced crypt depth results in slower tissue turnover and can lead to lower gut secretion and higher performance. In the present study, the results indicated that broiler chickens fed MCFA and AGP diets had higher villus height in the jejunum. Similarly, previous studies have also reported the positive effects of organic acids on morphological parameters of small intestine (Panda et al. 2009). This improvement which plays an important role in the intestinal morphology may be associated with a more balanced microflora such as increase in LAB population caused by MCFA in this experiment. The increased population of LAB in the gastrointestinal tract could inhibit the colonisation of pathogens such as coliforms by producing specific compounds such as bacteriocins and organic acids, and reduce the incidence of their adverse effects on the intestinal epithelium (Jazi et al. 2017). Therefore, this leads to lower inflammatory damage to the intestinal structure and tissue, which increases villus height (Cook and Bird 1973) thereby increasing the digestion and absorption of nutrients.

The serum biochemical parameters can indicate nutrient metabolism and physiological status of the body. The results of the present study indicated that feeding birds with diets supplemented with MCFA decreased serum cholesterol. In addition, a recent study indicated that MCFA supplementation could lower serum total cholesterol and LDL in broilers (Shokrollahi et al. 2014). The lower cholesterol levels observed in MCFA supplemented groups in the present study could be attributed to the increase in LAB population in the intestinal. Suggested modes of action for LAB to reduce and/or eliminate cholesterol are: enzymatic deconjugation of bile acids due to bile-salt hydrolysis activity; attachment of cholesterol into the LAB cell membrane, conversion of cholesterol to coprostanol; preventing the synthesis of hepatic cholesterol by metabolites of LAB such as propionate; inhibition of Acetyl-CoA carboxylase enzyme activity as a key enzyme for fatty acids synthesis in the liver; and reducing HMG-CoA reductase expression (Jazi et al. 2018).

Conclusions

In summary, the results obtained in the present study indicate that adding MCFA to diet and EO to the drinking water of broilers improved BWG and FCR compared to the CON, however, none could outperform the birds fed with AGP. The inclusion of SYN and EO in feed had less-pronounced growth-promoting effects. In addition, dietary supplementation of MCFA decreased serum total cholesterol, increased caecal LAB population, and improved villus height in the jejunum. Our results suggest that all the supplements had beneficial effects on suppressing the pathogenic bacteria load. Therefore, based on the results of the current study, it can be concluded that supplements, especially MCFA, could be used as a suitable alternative to AGP in broiler chickens, however, a slightly lower growth performance should be compromised to practice an antibiotic-free production.

Ethical approval

All animal protocols for this study were approved by the Animal Care and Use Committee at the Qaemshahr Branch, Islamic Azad University (Qaemshahr, Iran).

Disclosure statement

No potential conflict of interest was reported by the author(s).

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Data availability statement

All data generated and analysed during this study are included in this published article.

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