Impact of essential oils and organic acids on the growth performance, digestive functions and immunity of broiler chickens

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ABSTRACT

The aim of the experiment was to study the effects of feeding blends of sorbic acid, fumaric acid, and thymol (EOA) on growth performance, digestive functions, and immunity of broiler chickens. A total of 640 one-day-old male Cobb 500 chicks with similar BW (41.8 ± 0.6 g) were randomly divided into 4 dietary treatment groups consisting of 10 replicates with 16 birds per replicate and fed a basal diet until d 42 (CON) or diets with 0.15 g/kg enramycin during the grower period (AG), 0.30 g/kg EOA during the grower period (EG), or 0.30 g/kg EOA during the finisher period (EF). At d 42, the feed conversion ratio was reduced (P < 0.05) for birds in EG group compared with other groups. Birds in EG group showed a higher villus height of the duodenum and jejunum and muscular layers of the duodenum and ileum than birds in CON group (P < 0.05). Compared with other groups, crypt depth of the jejunum and ileum was markedly increased (P < 0.05) by EOA supplementation during the finisher period at d 42. The EOA supplementation during grower period increased significantly lipase, trypsin and chymotrypsin activities of the duodenum at d 21 and 42, as well as lipase and trypsin at d 21, and trypsin and chymotrypsin at d 42 in the jejunum, and trypsin and chymotrypsin activities of the ileum at d 21 compared to the control diet (P < 0.05). Birds of EG and EF groups showed a higher spleen index than birds of CON group. The level of secretory immunoglobulin A in duodenal and ileal mucosa was increased (P < 0.05) in EF group at d 42 compared with other groups. In conclusion, the results indicate that EOA can be effectively applied in broiler diets, especially during the grower phase by improving intestinal morphology and increasing digestive enzyme activity.

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1. Introduction

The avian gut plays an important role in the digestion and absorption of nutrients, while exerting an innate barrier function. Poor gut health has been led the broiler industry to suffer more challenges (i.e., diseases). Nowadays, modern countries have restricted or even banned the use of antibiotics in feed, due to the growing concerns about drug residues and resistant bacteria (Diarra and Malouin, 2014). Withdrawal of antibiotics from the poultry diets has triggered a search for suitable alternatives such as pre- and pro-biotics, organic acids, and essential oils.

Organic acids, for instance, propionate and medium-chain fatty acids, which have growth promoting properties and antimicrobial activities, have shown a greater capacity as an alternative to antibiotics (Paul et al., 2007; Rasschaert et al., 2016). Organic acids have been shown to decrease mortality in experimentally infected chickens through reducing the concentrations of Escherichia coli, Salmonella spp. and coliform in the small intestine (Jang et al., 2007; Amerah et al., 2012; Cerisuelo et al., 2014). In recent years, plant extracts, especially essential oils, have attracted attention from poultry industries. Essential oils contain many different compounds with antimicrobial activity, such as hydrocarbons, phenols, ketones, esters, and ethers (Solórzano-Santos and Miranda-Novales, 2012; Marchese et al., 2017).
There are complex and diverse effects due to different chemical constituents and processing methods. Therefore, in recent years, there is a widespread trend to study the complementary effects on growth performance and gut health of substituting antibiotics in the poultry industry (Placha et al., 2014; Pirgozliev et al., 2015; Sun et al., 2015). To the best of author’s knowledge, combining the effects of organic acids and essential oils as an alternatives option to the use of antibiotics in broiler diets, with possible complementary effect, has not yet been investigated. Therefore, the purpose of this experiment was to investigate the effects of feeding blends of sorbic acid, fumaric acid, and thymol (EOA) on the performance, immunity and the digestive tract functions in broiler chickens.

2. Materials and methods

2.1. Animals and experimental design

All experiment protocols were approved by the Animal Care and Use Committee of Northwest A & F University (protocol number NWAFAC1008). A total of 640 one-day-old male chicks (Cobb 500) with similar body weight (41.8 ± 0.6 g) were allotted into 4 treatment groups consisting of 10 replicates with 16 birds per replicate. Mash feed and fresh water were provided ad libitum. The birds were assigned to 4 groups, including a control group (basal diet [CON]) and 3 treatment groups (basal diet + 0.15 g/kg enramycin during the grower period [AG], basal diet + 0.30 g/kg EOA during the grower period [EF], basal diet + 0.30 g/kg EOA during the finisher period [EFf]). The birds were reared up to 42 days of age. The ingredient composition and chemical analysis of the basal diets formulated to meet nutrient requirements (NRC, 1994) are shown in Table 1. The crude protein was analysed by using official method 990.03 (nitrogen × 6.25), calcium (official method 968.08D) and total phosphorus (official method 965.17) contents of the basal diets were determined (AOAC, 2005). The amino acids were determined according to Palliyeguru et al. (2010) using a Hitachi L-8900 amino acid analyzer (Hitachi, Tokyo, Japan). The EOA ([containing a minimum of 200 g/kg of sorbic acid, a minimum of 200 g/kg fumaric acid, a minimum of 100 g/kg thymol, 350 g/kg of silicon dioxide, and 150 g/kg of glycides] was provided by Jefo Nutrition Inc., St-Hyacinthe, Quebec, Canada.

Feed intake and BW were recorded on d 21 and 42 for each replicate. The average daily gain (ADG), average daily feed intake (ADFI) and feed conversion ratios (FCR) were calculated. Mortality was recorded daily, and ADG, ADFI and FCR were corrected by mortality.

2.2. Immune organ index

After fasting for 12 h, 2 birds from each replicate group were randomly weighed at d 21 and 42. The body weights were recorded, and the birds were euthanised by an intraperitoneal injection of sodium pentobarbitone (28 mg/kg BW; Sinopharm Chemical Reagent Beijing Co., Ltd., Beijing, China). The thymus, spleen, and bursa of Fabricius were dissected and removed. Weights of the immune organs were individually recorded and expressed as relative to BW (g of organ/kg of BW).

2.3. Intestinal morphology

For intestinal morphological examination, gut samples (approximately 2 cm in length) from the middle of the duodenum, jejunum and ileum were collected. The samples were fixed according to the procedure of Xu et al. (2003). Histological sections were examined with a Nikon phase-contrast microscope coupled with a MicroComp integrated digital imaging analysis system (Nikon Eclipse 80i, Nikon, Tokyo, Japan). The variables measured included the villus height, crypt depth, and thickness of the muscular layers (Fan et al., 1997). Villus height to crypt depth ratio was also calculated. Five villi per section and 2 sections per sample were measured, and the average value was used for the statistical analysis.

2.4. Digestive enzyme activity

The enzymatic activities of amylase, lipase, trypsin, and chymotrypsin were examined according to the commercial kit instructions (Nanjing Jiancheng Bioengineering Institute, Nanjing, China).

2.5. Secretory immunoglobulin A (SIgA)

Intestinal mucosa was scraped at d 21 and 42. Ten centimeters mid-sections of duodenum, jejunum and ileum were obtained and rinsed using saline to remove their content. Then intestinal segments were opened longitudinally with small sharp scissors. Mucosa were scraped, placed in centrifuge tubes and stored at −80 °C. The SIgA levels in the duodenum, jejunum, and the ileum were determined using a commercial Radio Immune Assay kit (China Institute of Atomic Energy, Beijing, China). The protein contents were measured by Bradford method (Carlsson et al., 2011).

2.6. Statistical analysis

The data were analyzed by One-way ANOVA using the SPSS 20.0 (SPSS Inc., Chicago, IL, USA). Results were expressed as treatment means with their pooled standard error of the mean (SEM). A probability value of P < 0.05 was described to be statistically significant and the notable differences between the treatments were determined by Duncan’s multiple comparisons test.

| Table 1 | Ingredients and composition (g/kg diet) of basal diets. |
|---------|------------------------------------------------------|
| **Item** | **Growing phases** | **Finisher (d 22 to 42)** |
| Ingredients | | |
| Corn | 572.7 | 609.9 |
| Soybean meal | 352.0 | 300.0 |
| Cottonseed meal | 20.0 | 40.0 |
| Soybean oil | 20.0 | 20.0 |
| Sodium chloride | 3.6 | 3.5 |
| Limestone | 20.0 | 17.0 |
| Calcium hydrogen phosphate | 3.0 | 3.0 |
| Choline chloride | 0.5 | 0.5 |
| L-lysine HCl | 1.4 | 0.6 |
| Minerals premix1 | 3.0 | 3.0 |
| Phytase | 1.0 | 1.0 |
| DL-methionine | 2.5 | 1.2 |
| Vitamin premix2 | 0.3 | 0.3 |
| Analyzed nutrient composition | | |
| Crude protein | 219.1 | 198.9 |
| Calcium | 9.6 | 9.1 |
| Total phosphorous | 6.1 | 6.0 |
| Lysine | 12.1 | 10.5 |
| Methionine | 5.7 | 4.5 |
| Methionine + Cysteine | 9.0 | 7.5 |
| Calculated nutrient composition | | |
| Metabolizable energy, MJ/kg | 12.34 | 12.74 |
| Available phosphorus | 4.0 | 4.0 |

1 The mineral premix provided per kilogram of diets: Cu (as copper sulfate) 10 mg, Fe (as ferrous sulfate) 80 mg, Mn (as manganese sulfate) 80 mg, Zn (as zinc sulfate) 75 mg, I (as potassium iodide) 0.40 mg, Se (as sodium selenite) 0.30 mg.
2 The vitamin premix provided per kilogram of diets: vitamin A, 250,000 IU; vitamin D3, 50,000 IU; vitamin K3, 53 mg; vitamin B6, 40 mg; vitamin B12, 120 mg; vitamin B12, 0.50 mg; vitamin E, 600 IU; biotin, 0.65 mg; folic acid, 25 mg; pantothenic acid, 240 mg; niacin, 1,000 mg.
3. Results

3.1. Growth performance

The ADFI and ADG among the 4 treatment groups were similar (P > 0.05; Table 2). However, at d 42, FCR was significantly (P < 0.05) reduced for birds fed diets with EOA supplementation for grower period compared with other groups. During the grower period, the addition of EOA tended to reduce FCR (P = 0.057).

3.2. Immune organ index

No treatment effect due to EOA supplementation was observed for immune organ index of broilers at d 21 (Table 3). There were no differences (P > 0.05) in the thymus index and the bursa of Fabricius index among the treatment groups at d 42. Both EG and EF groups showed a higher (P < 0.05) spleen index than CON groups at d 42. However, the spleen index of EG, EF and AG groups were similar (P > 0.05).

3.3. Intestinal morphology

There was no difference (P > 0.05) in villus height, crypt depth, and villus height to crypt depth ratio of the duodenal morphology among the treatment groups at d 21 (Table 4). Birds in AG group showed the lowest (P < 0.05) thickness of the muscular layers at d 21. Birds in EG and AG groups showed a higher (P < 0.05) villus height than the birds in CON and EF groups at d 42. The crypt depth of birds in EF group was higher (P < 0.05) than those of birds in CON and AG groups. There was no difference (P > 0.05) in crypt depth between the birds in EG and AG groups. Birds in EG and EF groups showed a higher (P < 0.05) muscular layer thickness than those in CON and AG groups at d 42.

There was no difference (P > 0.05) in the jejunal morphology of broilers among the treatment groups at d 21 (Table 5). Birds in EG group showed a higher (P < 0.05) villus height than those in CON and AG groups at d 42. The thickness of the muscular layers for birds in EF group was higher (P < 0.05) than that AG group. Birds in EG group showed a higher (P < 0.05) crypt depth and villus height to crypt depth ratio of the jejunal morphology than the others.

There were no significant differences (P > 0.05) in the ileal morphology of broilers among the 4 groups at d 21 (Table 6). Compared with other groups, crypt depth of the jejunum and ileum was markedly affected (P < 0.05) by EOA supplementation of the finisher period. Birds in EF group showed a higher crypt depth than the others (P < 0.05) at d 42. The product supplementation of the grower period significantly increased muscular layers of the ileum in broiler chickens compared with CON and AG groups (P < 0.05). No differences were observed (P > 0.05) across the treatments in the villus height and the villus height to crypt depth ratio of the ileal morphology of broiler chickens.

3.4. Digestive enzyme activity

There were no differences in amylase activity of intestinal tract among the 4 groups at d 21 and d 42 (Tables 7–9). Birds in EG and AG groups showed a higher (P < 0.05) lipase activity in the duodenum than those in CON and EF groups at d 21. The EOA supplementation of the grower period (EG) increased trypsin and chymotrypsin activity of the duodenum in broilers at d 21 compared with other dietary groups (P < 0.05). Birds in AG group showed a higher (P < 0.05) trypsin and chymotrypsin activity in the duodenum than the birds in CON and EF groups at d 21.

Birds in EG group showed a higher (P < 0.05) lipase and trypsin at d 21, and trypsin and chymotrypsin at d 42 in the jejunal morphology of broilers than the others. Birds in AG group showed a higher (P < 0.05) lipase and trypsin at d 21, and trypsin and chymotrypsin at d 42 in the jejunum of broilers than those in CON and EF groups.

Table 2

| Item | Experimental diets2 | SEM | P-value |
|------|---------------------|-----|---------|
| Day 1 to 21 | | | |
| ADFI, g | 45.64 | 46.04 | 47.10 | 46.47 | 0.354 | 0.530 |
| ADG, g | 31.46 | 32.60 | 32.08 | 32.75 | 0.229 | 0.480 |
| FCR | 1.45 | 1.42 | 1.44 | 1.45 | 0.005 | 0.057 |
| Day 1 to 42 | | | |
| ADFI, g | 83.08 | 85.09 | 86.75 | 85.17 | 0.650 | 0.278 |
| ADG, g | 49.46 | 52.02 | 50.79 | 50.44 | 0.443 | 0.006 |
| FCR | 1.68a | 1.64b | 1.70a | 1.68a | 0.007 | 0.017 |
| SEM = standard error of the mean; ADFI = average daily feed intake; ADG = average daily gain; FCR = feed conversion ratio (feed intake to BW gain ratio). |

Table 3

| Item | Experimental diets2 | SEM | P-value |
|------|---------------------|-----|---------|
| Day 21 | | | |
| Thymus | 4.20 | 4.26 | 4.24 | 4.27 | 0.158 | 0.672 |
| Spleen | 0.80 | 0.81 | 0.94 | 0.84 | 0.039 | 0.508 |
| Bursa of Fabricius | 2.18 | 1.79 | 2.25 | 1.96 | 0.105 | 0.147 |
| Day 42 | | | |
| Thymus | 3.65 | 2.85 | 2.70 | 2.91 | 0.148 | 0.747 |
| Spleen | 0.64b | 0.96a | 0.92a | 0.78ab | 0.049 | 0.049 |
| Bursa of Fabricius | 0.43 | 0.46 | 0.63 | 0.48 | 0.058 | 0.736 |

SEM = standard error of the mean. a,b Within a row, numbers with different superscripts differ statistically at P ≤ 0.05.

Table 4

| Item | Experimental diets2 | SEM | P-value |
|------|---------------------|-----|---------|
| Day 21 | | | |
| VH, μm | 1,026.51 | 980.93 | 1,105.60 | 1,086.31 | 24.711 | 0.283 |
| CD, μm | 89.79 | 93.50 | 92.92 | 91.39 | 2.015 | 0.940 |
| ML, μm | 102.97a | 101.25a | 101.08a | 88.61a | 1.953 | 0.033 |
| VH:CD | 11.47 | 10.49 | 11.91 | 12.09 | 0.596 | 0.547 |
| Day 42 | | | |
| VH, μm | 1,086.28b | 1,259.99a | 1,090.54b | 1,270.43a | 37.434 | 0.001 |
| CD, μm | 91.41c | 97.62c | 111.24a | 107.79ab | 3.165 | 0.010 |
| ML, μm | 98.01b | 123.13a | 124.94a | 97.55b | 4.296 | 0.006 |
| VH:CD | 11.98 | 11.26 | 10.23 | 11.88 | 0.602 | 0.271 |

SEM = standard error of the mean; VH = villus height; CD = crypt height; ML = muscular layers; VH:CD = villus height to crypt depth ratio. a,b Within a row, numbers with different superscripts differ statistically at P ≤ 0.05.

SEM = standard error of the mean; VH = villus index; CD = crypt index; ML = muscular layers; VH:CD = villus height to crypt depth ratio.
Effects of feeding blends of essential oils and organic acids (EOA) on digestive enzyme activity (U/mg protein) in jejunum of broilers.\(^2\)

| Item | Experimental diets\(^3\) | SEM | P-value |
|------|--------------------------|-----|---------|
|      | CON | EG | EF | AG |
| **Day 21** | | | | |
| VH, \(\mu m\) | 720.58 | 697.18 | 757.01 | 714.46 | 10.269 | 0.216 |
| CD, \(\mu m\) | 97.95 | 103.55 | 106.56 | 87.75 | 3.097 | 0.130 |
| ML, \(\mu m\) | 121.70 | 125.51 | 117.48 | 104.45 | 4.923 | 0.516 |
| VH/CD | 7.35 | 6.80 | 7.14 | 8.22 | 0.254 | 0.248 |
| **Day 42** | | | | |
| VH, \(\mu m\) | 753.71\(^b\) | 965.80\(^b\) | 874.98\(^b\) | 730.11\(^a\) | 32.821 | 0.007 |
| CD, \(\mu m\) | 101.99\(^b\) | 107.65\(^b\) | 125.21\(^b\) | 105.08\(^b\) | 3.355 | 0.025 |
| ML, \(\mu m\) | 128.74\(^b\) | 138.86\(^b\) | 154.98\(^b\) | 117.47\(^a\) | 5.672 | 0.009 |
| VH/CD | 7.42\(^b\) | 9.03\(^a\) | 6.98\(^b\) | 6.93\(^a\) | 0.297 | 0.009 |

SEM – standard error of the mean. VH – villus height; CD – crypt depth; ML – muscular layers; VH/CD – villus height to crypt depth ratio.

\(^{a,b}\) Within a row, numbers with different superscripts differ statistically at \(P \leq 0.05\).

\(^1\) Ten replicates per treatment group (\(n = 10\)).

\(^2\) Dietary treatments were as follows: CON – basal diet; EG – basal diet + 0.30 g/kg product EOA during the grower period; EF – basal diet + 0.30 g/kg product EOA during the finisher period; AG – basal diet + 0.15 g/kg enramycin during the grower period.

Effects of feeding blends of essential oils and organic acids (EOA) on digestive enzyme activity (U/mg protein) in ileum of broilers.

| Item | Experimental diets\(^3\) | SEM | P-value |
|------|--------------------------|-----|---------|
|      | CON | EG | EF | AG |
| **Day 21** | | | | |
| Amylase | 4.10 | 3.67 | 3.76 | 3.81 | 0.096 | 0.964 |
| Lipase, U/g protein | 49.77\(^b\) | 53.34\(^a\) | 49.96\(^b\) | 51.81\(^a\) | 0.416 | 0.001 |
| Trypsin | 273.90\(^b\) | 297.37\(^a\) | 273.99\(^b\) | 285.62\(^a\) | 2.501 | <0.001 |
| Chymotrypsin | 108.13\(^a\) | 125.89\(^b\) | 107.88\(^a\) | 120.65\(^b\) | 2.050 | <0.001 |
| **Day 42** | | | | |
| Amylase | 3.71 | 3.73 | 3.78 | 3.56 | 0.063 | 0.716 |
| Lipase, U/g protein | 41.83\(^b\) | 50.45\(^b\) | 43.61\(^b\) | 44.47\(^b\) | 0.671 | <0.001 |
| Trypsin | 254.83\(^b\) | 284.89\(^a\) | 258.14\(^b\) | 277.92\(^a\) | 3.134 | <0.001 |
| Chymotrypsin | 98.01\(^b\) | 113.61\(^a\) | 100.90\(^b\) | 109.87\(^a\) | 1.750 | 0.003 |

SEM – standard error of the mean.

\(^{a,b}\) Within a row, numbers with different superscripts differ statistically at \(P \leq 0.05\).

\(^1\) Ten replicates per treatment group (\(n = 10\)).

\(^2\) Dietary treatments were as follows: CON – basal diet; EG – basal diet + 0.30 g/kg product EOA during the grower period; EF – basal diet + 0.30 g/kg product EOA during the finisher period; AG – basal diet + 0.15 g/kg enramycin during the grower period.

Trypsin and chymotrypsin activity in the ileum of broilers was significantly increased in EG group at d 21 compared with CON group (\(P < 0.05\)). Birds in AG group showed a higher (\(P < 0.05\)) trypsin in the ileum of broilers at d 21 than those in CON and EF groups. However, there was no difference (\(P > 0.05\)) in digestive enzyme activity in ileum of broilers at d 42 among the treatments.

3.5. Secretary Immunoglobulin A (SIgA)

There were no effects on SIgA level of intestinal mucosa among the 4 groups at d 21 (Table 10). The level of SIgA in duodenal and ileal mucosa was significantly increased in EF group at d 42 compared with other groups (\(P < 0.05\)).

4. Discussion

China’s government does more to phase out the use of antibiotic growth promoters. Essential oils are multifarious mixtures of volatile and lipophilic substance obtained from plants. In addition to antimicrobial effects (Upadhyaya et al., 2013; Wlodarska et al., 2015; Du et al., 2016), dietary essential oils supplementation has been testified to improve intestinal morphology and increase digestive enzyme activity (X. Yang et al., 2018; 2019).
growth performance (Habibi et al., 2014; Placha et al., 2014; Pirgozliev et al., 2015). Correspondingly, organic acids were commonly used in the field of animal feeding, as it is well known to improve intestinal health (Paul et al., 2007). Therefore, using the combination of organic acids and essential oils to improve growth performance would be of great interest to the poultry industry.

In the present study, feed conversion ratio was reduced at d 42 after supplementation with EOA during the grower period. During the grower period, EG group tended to reduce FCR compared with the control treatment. The results showed nutrition intervention in the grower period increased feed efficiency of broiler chickens at market age. Previous findings have documented an increase in growth performance due to the use of single acids such as formic acid, citric acid and fumaric acid (Vieira et al., 2008; Abdelfattah et al., 2008; Al-Kassi and Mohssen, 2009; Asgar et al., 2013; Lakshmi and Sunder, 2015). Several studies revealed that adding essential oil to broiler diets improved production performances traits such as body weight, FCR and ADG (Abou-elkhair et al., 2014; Habibi et al., 2014). Du et al. (2016) reported adding thymol and carvacrol broiler diet tended to linearly reduce the FCR from 1.74 to 1.58 with no reduction in ADFI and ADG.

The FCR was improved for the birds fed diet with EOA with no change in ADFI and ADG. It is likely due to improving the digestive enzyme activity and intestinal morphology. Using EOA supplements during the grower period resulted of increasing lipase, trypsin and chymotrypsin activities of the small intestine. These results are in agreement with several reports that mentioned of using EOA will improve the digestive enzyme activities in broiler chickens (Lee et al., 2003; Jang et al., 2007; Basmacioglu et al., 2010; Emami et al., 2012). Added organic acids added to poultry diets could potentially enhance growth performance by improving digestive function through many modes of action such as reduction of intestinal pH, promoting the beneficial bacterial growth, or inhibiting growth of pathogenic microbes.

Nutrient digestion and absorption primarily occurs in the small intestine (Kawalliak et al., 2011). The enhancement of feed efficiency in poultry by EOA partly explained by improved intestinal morphology to increase the capacity for absorbing nutrients. Our results found that EOA supplementation during the finisher period increased villus height and muscular layers of the duodenum, as well as villus height and villus height to crypt depth ratio of the jejunum, and this lead to improve the digestibility and absorption efficiency. Similarly, previous studies indicated that the organic acids increased height of villus and width of crypts in ileum in chicken (Pelciano et al., 2005; Paul et al., 2007; Mohamed et al., 2014). Other research suggested that essential oils significantly improved gut morphology (Du et al., 2016). Birds developed larger intestinal villi, resulting in faster growth rates. Deeper crypts indicated faster cellular turnover as needed in response to the phenomenon of rapid growth or stimulation by microorganisms. Deeper crypts of the duodenum in EG group and of the jejunum and ileum in EF group were possibly explained by changes in microbial populations caused by diets with or without EOA, because decreased intestinal bacteria in the small intestine may improve the proliferation ability of epithelial cells and thus enhance intestinal absorptive capacity (Zeng et al., 2015).

The immune system plays an important role in regulation of poultry body health. The present study showed birds in the EOA groups showed higher spleen index than CON and AG groups at d 42. It has been suggested that SIgA is a primary protective tool of the gastrointestinal mucosal immune response to antigen defense by directly binding to the surface of the antigen (Holmgren and Czerkinsky, 2005; Yang et al., 2011). The results of the current study showed that diet supplementation with EOA increased SIgA levels of the duodenal and ileal mucosa in broilers at d 42. Supplementing EOA during the finisher period improved the immune status of animals, as indicated by an increase in SIgA. Nevertheless, it is necessary to study the effects of EOA on intestinal bacteria and bacterial metabolic products to determine mechanisms of action of EOA on performance and the digestive tract functions in broiler chickens.

5. Conclusions

The present study clearly showed that the addition of EOA mixture during the grower phase increased efficiency, possibly by improving intestinal morphology and increasing digestive enzyme activities of broiler chickens. Therefore, the addition of EOA could replace the antibiotic growth promoter in the poultry industry.

Conflict of interest

We declare that we have no financial and personal relationships with other people or organizations that can inappropriately influence our work. We confirm that the manuscript has been read and approved by all named authors. The manuscript has not been published previously.

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