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Original Research Article

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 ($P < 0.05$) 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.

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).

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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 [EG], basal diet + 0.30 g/kg EOA during the finisher period [EF]). 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 glycerides) 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 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.

Table 1
Ingredients and composition (g/kg diet) of basal diets.

Item	Growing phases	
	Grower (d 1 to 21)	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 premix[1]	3.0	3.0
Phytase	1.0	1.0
DL-methionine	2.5	1.2
Vitamin premix[2]	0.3	0.3
Analyzed nutrient composition		
Crude protein	219.1	198.9
Calcium	9.6	9.1
Total phosphorus	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 D, 50,000 IU; vitamin K_3, 53 mg; vitamin B_1, 40 mg; vitamin B_2, 120 mg; vitamin B_{12}, 0.50 mg; vitamin E, 600 IU; biotin, 0.65 mg; folic acid, 25 mg; pantothenic acid, 240 mg; niacin, 1,000 mg.

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.

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

Table 2
Effects of feeding blends of essential oils and organic acids (EOA) on growth performance of broilers[1].

Item	Experimental diets[2]				SEM	*P*-value
	CON	EG	EF	AG		
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	0.606
FCR	1.68[a]	1.64[b]	1.70[a]	1.68[a]	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).
[a,b] Within a row, numbers with different superscripts differ statistically at $P < 0.05$.
[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.

Table 3
Effects of feeding blends of essential oils and organic acids (EOA) on the immune organ index (g/kg) of broilers[1].

Item	Experimental diets[2]				SEM	*P*-value
	CON	EG	EF	AG		
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	0.64[b]	0.96[a]	0.92[a]	0.78[ab]	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 $P \leq 0.05$.
[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.

Table 4
Effects of feeding blends of essential oils and organic acids (EOA) on the duodenal morphology of broilers[1].

Item	Experimental diets[2]				SEM	*P*-value
	CON	EG	EF	AG		
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	102.97[a]	101.25[a]	101.08[a]	88.61[b]	1.953	0.033
VH:CD	11.47	10.49	11.91	12.09	0.596	0.547
Day 42						
VH, μm	1,086.28[b]	1,259.99[a]	1,090.54[b]	1,270.43[a]	37.434	0.001
CD, μm	91.41[c]	97.62[bc]	111.24[a]	107.79[ab]	3.165	0.010
ML, μm	98.01[b]	123.13[a]	124.94[a]	97.55[b]	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 depth; ML = muscular layers; VH:CD = villus height to crypt depth ratio.
[a–c] 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.

Table 5
Effects of feeding blends of essential oils and organic acids (EOA) on the jejunal morphology of broilers[1].

Item	Experimental diets[2]				SEM	*P*-value
	CON	EG	EF	AG		
Day 21						
VH, μm	720.58	697.18	757.01	714.46	10.269	0.216
CD, μm	97.95	103.55	106.56	87.75	3.097	0.130
ML, μm	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, μm	753.71^{bc}	965.80^{a}	874.98^{ab}	730.11^{c}	32.821	0.007
CD, μm	101.59^{b}	107.65^{b}	125.21^{a}	105.08^{b}	3.355	0.025
ML, μm	128.74^{ab}	138.66^{ab}	154.98^{a}	117.47^{b}	5.672	0.090
VH:CD	7.42^{b}	9.03^{a}	6.98^{b}	6.95^{b}	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–c] 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.

Table 6
Effects of feeding blends of essential oils and organic acids (EOA) on the ileal morphology of broilers[1].

Item	Experimental diets[2]				SEM	*P*-value
	CON	EG	EF	AG		
Day 21						
VH, μm	555.31	583.61	570.31	563.73	17.965	0.967
CD, μm	83.45	95.23	94.99	85.44	2.986	0.398
ML, μm	110.88	113.50	130.96	104.02	4.448	0.165
VH:CD	6.60	6.12	6.06	6.59	0.129	0.305
Day 42						
VH, μm	717.06	810.04	954.89	707.04	46.473	0.207
CD, μm	89.80^{b}	96.06^{b}	125.64^{a}	104.35^{b}	4.879	0.017
ML, μm	132.03^{c}	152.72^{b}	165.76^{a}	137.74^{c}	4.294	0.001
VH:CD	8.02	7.84	7.55	6.76	0.278	0.439

SEM = standard error of the mean; VH = villus height; CD = crypt depth; ML = muscular layers; VH:CD = villus height to crypt depth ratio.
[a–c] 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.

Table 7
Effects of feeding blends of essential oils and organic acids (EOA) on digestive enzyme activity (U/mg protein) in duodenum of broilers[1].

Item	Experimental diets[2]				SEM	*P*-value
	CON	EG	EF	AG		
Day 21						
Amylase	3.67	3.76	3.68	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^{c}	297.37^{a}	273.99^{c}	285.62^{b}	2.501	<0.001
Chymotrypsin	108.132^{c}	125.89^{a}	107.88^{c}	117.38^{b}	2.050	<0.001
Day 42						
Amylase	3.71	3.73	3.78	3.56	0.063	0.716
Lipase, U/g protein	43.83^{b}	50.45^{a}	43.61^{b}	44.47^{b}	0.671	<0.001
Trypsin	254.83^{b}	284.89^{a}	258.14^{b}	277.923^{a}	3.134	<0.001
Chymotrypsin	98.01^{b}	111.61^{a}	100.99^{b}	109.87^{a}	1.750	0.003

SEM = standard error of the mean.
[a–c] 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.

Table 8
Effects of feeding blends of essential oils and organic acids (EOA) on digestive enzyme activity (U/mg protein) in jejunum of broilers[1].

Item	Experimental diets[2]				SEM	*P*-value
	CON	EG	EF	AG		
Day 21						
Amylase	2.12	2.14	2.25	2.14	0.037	0.622
Lipase, U/g protein	25.92^{c}	46.48^{a}	25.98^{c}	36.55^{b}	2.361	<0.001
Trypsin	233.90^{c}	254.49^{a}	233.99^{c}	244.53^{b}	2.363	<0.001
Chymotrypsin	87.65	98.93	91.74	96.45	1.558	0.175
Day 42						
Amylase	2.20	2.14	2.13	2.05	0.034	0.515
Lipase, U/g protein	30.47	29.58	26.69	29.41	0.905	0.532
Trypsin	204.47^{c}	235.58^{a}	200.96^{c}	214.39^{b}	3.278	<0.001
Chymotrypsin	78.69^{b}	94.10^{a}	77.95^{b}	84.73^{b}	1.831	0.001

SEM = standard error of the mean.
[a–c] 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. Secretory 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

Table 9
Effects of feeding blends of essential oils and organic acids (EOA) on digestive enzyme activity (U/mg protein) in ileum of broilers[1].

Item	Experimental diets[2]				SEM	*P*-value
	CON	EG	EF	AG		
Day 21						
Amylase	0.77	0.86	0.78	0.76	0.022	0.412
Lipase, U/g protein	19.47	20.05	17.74	18.86	0.893	0.846
Trypsin	154.47^{c}	180.56^{a}	150.74^{c}	164.46^{b}	2.852	<0.001
Chymotrypsin	70.70^{b}	79.68^{a}	70.97^{b}	77.53^{ab}	1.400	0.029
Day 42						
Amylase	0.94	0.97	0.89	0.84	0.019	0.079
Lipase, U/g protein	17.26	19.19	19.91	18.23	1.300	0.914
Trypsin	153.26	160.69	153.92	154.23	1.453	0.241
Chymotrypsin	57.43	64.73	58.06	60.36	1.447	0.283

SEM = standard error of the mean.
[a–c] 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.

Table 10
Effects of feeding blends of essential oils and organic acids (EOA) on the intestinal mucosa SIgA (mg/100 mg protein) of broilers[1].

Items	Experimental diets[2]				SEM	*P*-value
	CON	EG	EF	AG		
Day 21						
Duodenum	4.43	4.45	6.15	4.62	0.329	0.186
Jejunum	5.15	5.50	6.00	6.03	0.535	0.304
Ileum	6.36	6.33	5.43	5.75	0.274	0.423
Day 42						
Duodenum	5.29^{b}	6.06^{b}	9.09^{a}	6.94^{b}	0.591	0.049
Jejunum	7.96	7.19	9.06	8.19	0.622	0.818
Ileum	7.36^{b}	6.32^{b}	10.51^{a}	6.80^{b}	0.603	0.029

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.

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 add 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 add thymol and carvacrol broiler diet tended to linearly reduce the FCR from 1.74 to 1.68 during d 14 to 28. Nevertheless, other researchers reported no effects on the growth performance of broiler chickens (Jang et al., 2007; Cerisuelo et al., 2014). Various effects of feed additives among studies may be associated with the health conditions of the flock, the basal diet type, and environmental conditions.

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 (Kawalilak et al., 2011). The enhancement of feed efficiency in poultry may be 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 (Pelicano 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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