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Encapsulated blends of essential oils and organic acids improved performance, intestinal morphology, cecal microflora, and jejunal enzyme activity of broilers

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Abstract: This study investigated effects of encapsulated blends of essential oils (EO) and organic acids (OA) on performance, intestinal morphology, cecal microflora, and jejunal mucosal enzyme activity and secretory IgA (s-IgA) level of broilers. Three hundred one-day-old male broilers were divided into 5 treatments with 5 replicates each. Control group received basal diet. Three additional groups received basal diets supplemented with 150, 200, or 250 mg/kg encapsulated blends of EO and OA. Antibiotic group received basal diet supplemented with 30 mg/kg bacitracin zinc. On days 21 and 70 of trial, 2 broilers from each replicate were weighed, and then blood, duodenum, jejunum, ileum, and cecum samples were collected. Results showed that broilers fed blends of EO and OA had greater average daily gain (ADG) (linear, $P = 0.043$) and gain-to-feed ratio (G : F) (linear, $P = 0.052$) at day 21 compared to broilers fed the control diet. Blends of EO and OA reduced cecal *Escherichia coli* and *Salmonella* level of 21- and 70-day-old broilers (linear, $P < 0.01$). Jejunum villus height-to-crypt depth ratio (VCR) of 21-day-old broilers was elevated by blends of EO and OA (linear, $P = 0.041$) compared to control. Blends of EO and OA promoted chymotrypsin activity at day 21 (quadratic, $P = 0.014$), jejunal mucosal s-IgA content at day 70 (linear, $P = 0.012$), α-amylase activity at day 70 (quadratic, $P = 0.043$), and chymotrypsin activity at day 70 (quadratic, $P = 0.037$). Compared to control, antibiotic group increased ADG at day 21 ($P = 0.039$), decreased cecal *Salmonella* level at day 21 ($P = 0.018$), enhanced jejunum VCR at day 21 ($P = 0.049$), and elevated jejunal mucosal s-IgA content at day 21 ($P = 0.016$). It can be stated that EO and OA blends enhanced performance, increased jejunal s-IgA level and enzyme activity, improved intestinal morphology, and balanced intestinal microflora of broilers.

Keywords: essential oils; organic acids; encapsulation; growth; microflora; secretory IgA; broilers

Recently, many countries imposed restrictions on antibiotics as growth promoters in animal production due to concerns that antibiotics in feed could lead to antibiotic-resistant pathogens and antibiotic residue problems. For example, use of antimicrobial growth promoters has been banned in the European Union since 2006 (Giannenas et al. 2014). Therefore, it is important and significant to find alternative strategies to control pathogenic microbes and intestinal microbial balance, prevent intestinal disorders, and promote animal performance in livestock. Essential oil (EO) and organic

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acid (OA) additives are the most commonly used non-antibiotic substances for improving animal performance and health (Mueller et al. 2012; Hafeez et al. 2016; Liu et al. 2017).

Studies have revealed the antimicrobial and growth-promoting properties of EO (cinnamaldehyde and thymol), suggesting EO may be used as a natural replacement for antibiotics in animal feeds (Amerah et al. 2011, 2012). EO (carvacrol, cinnamaldehyde and capsaicin mixture) stimulated mucus release on glandular stomach and wall of jejunum, which may exert villi-related protective properties and reduce adhesion of pathogens to epithelium (Jamroz et al. 2006). Besides EO, OA is widely used in monogastric animals to improve performance through modulation of the gut microflora. Diets supplemented with OA (butyric, fumaric, or lactic acids) improved body weight gains, feed conversion ratio (FCR), and villus height in the small intestines of broilers (Adil et al. 2010). Morevoer, OA (formic, propionic and butanoic acids) increased ileum *Lactobacillus* colonization of broilers (Nava et al. 2009). However, some studies also reported that EO (mainly thymol) or OA (formic acid and propionic acid) addition did not affect performance and intestinal absorptive function of broilers (Jang et al. 2007; Ruhnke et al. 2015). The variation may be due to their instability and volatility in storage, feed processing, and digestive tract.

Encapsulation can protect EO and OA from light, storage, loss during feed processing, and intestinal delivery (Hernandez-Hernandez et al. 2014; Yang et al. 2016). Furthermore, encapsulation can carry substances into specific sites of the gastrointestinal tract and allows the slow release of effective materials in a specific moment or environment. Therefore, encapsulation can improve stability, delay absorption, reach distal part of intestine and enhance efficacy of EO or OA in broilers (Smith et al. 2012; Hernandez-Hernandez et al. 2014). For example, encapsulated citral (lemon EO) showed a better retained antimicrobial activity compared to unencapsulated form in intestinal digesta of broilers. Diets with encapsulated citral added at both 250 and 650 mg/kg significantly reduced intestinal necrotic enteritis lesions, which was comparable to the effect of diet supplemented with antibiotics (bacitracin and salinomycin) (Yang et al. 2016).

There is little published information on the results of the combination of EO and OA supplementation on broiler performance in the literature (Basmacioglu-Malayoglu et al. 2016; Liu et al. 2017). Moreover, an additive effect between EO (thymol, eugenol, and piperine) and OA (benzoic acid) was suggested due to the fact that digestive enzymes work more efficiently under acidic conditions (Weber et al. 2012). Therefore, this study was conducted to evaluate the effects of supplementing encapsulated blends of EO and OA on growth performance, intestinal morphology, intestinal mucosal enzymatic activity and secretory IgA (s-IgA) level, and cecal microflora of broilers.

MATERIAL AND METHODS

Ethics statement. Experimental procedures for this study were approved by the Committee of Animal Experiments of Fujian Agriculture and Forestry University (approval ID 2015040616).

Animals and diets. Three hundred one-day-old male Chinese yellow broilers were obtained from Wens Food Group Co., Ltd, China. Birds were divided into 5 treatment groups. Each treatment contained five replicates of 12 male broilers. Control group received basal diet containing no feed additives. Antibiotic group received diet supplemented with 30 mg/kg bacitracin zinc. Three additional groups received diets supplemented with 150, 200, or 250 mg/kg encapsulated blends of EO and OA (Vetagro, Reggio Emilia, Italy). Active ingredients of EO were thymol (≥ 1.7%) and vanillin (≥ 1.0%), while OA were citric acid (≥ 25.0%) and sorbic acid (≥ 16.7%). Citric acid, sorbic acid, thymol and vanillin are mixed in a packaging material of hydrogenated vegetable oil, and then the embedded granular product is formed by spraying and cooling process. The diets were formulated according to Chinese Feeding Standard of Chicken (2004). Feedstuff and nutritional content of diets are provided in Table 1. Chicks were kept in cages and raised in a temperature-controlled room. Water and diet were provided ad libitum. The experiment lasted for 70 days. The temperature was gradually reduced from approximately 30 to 20°C. On days 21 and 70 of the experiment, 2 broilers from each replicate were weighed and blood samples were taken. Serum was collected for further measurement. Afterwards, these broilers were killed by cervical dislocation. Duodenum, jejunum, ileum, and cecum were sampled after

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Table 1. Basal dietary composition and calculated nutrient content for meat-type broilers, as-fed basis

Item	Starter diet (0–3 weeks)	Grower diet (4–6 weeks)	Finisher diet (7–10 weeks)
Ingredient (%)			
Corn	60.11	66.09	74.43
Soybean meal	25.5	20.0	14.0
Extruded soybean	10	10	8
Limestone powder	1.8	1.5	1.4
Calcium monohydrogen phosphate	1.1	1.0	0.76
Premix compound[1]	1	1	1
DL-Methionine	0.24	0.16	0.16
Salt	0.25	0.25	0.25
Total	100.00	100.00	100.00
Calculated composition			
Metabolizable energy (MJ/kg)	12.10	12.35	12.56
Crude protein (%)	20.7	18.5	15.8
Lysine (%)	1.09	0.94	0.83
Methionine (%)	0.52	0.42	0.39
Methionine + cysteine (%)	0.82	0.70	0.63
Non-phytate P (%)	0.45	0.40	0.35
Ca (%)	0.99	0.88	0.80

[1]premix compound (mineral and vitamin mix) provided per kg of diet: vitamin A 9000 IU, cholecalciferol 2200 IU, vitamin E 22 IU, menadione 2.2 mg, thiamine mononitrate 2.2 mg, riboflavin 5.5 mg, D-calcium pantothenate 12 mg, niacin 35 mg, pyridoxine hydrochloride 3.5 mg, biotin 0.15 mg, folacin 1.2 mg, vitamin B12 0.015 mg, choline chloride 600 mg, 8.0 mg of Cu from $CuSO_4 \cdot 5\ H_2O$, 60 mg of Zn from $ZnSO_4 \cdot H_2O$, 80 mg of Mn from $MnSO_4 \cdot H_2O$, 80 mg of Fe from $FeSO_4 \cdot H_2O$, 0.36 mg of I from KI, 0.24 mg of Se from Na_2SeO_3

slaughter and stored at –80°C for further analysis. The demarcation between the duodenum and jejunum is the flexure of the distal duodenum after the entrance of the bile and pancreatic ducts. The Meckel's diverticulum (yolk stalk) marks the end of the jejunum and the onset of the ileum. Growth performance (average daily feed intake (ADFI), average daily gain (ADG), gain-to-feed ratio (G : F) and survival rate) of chicks were analysed when the experiment was finished.

Determination of cecal microflora. Cecal *Escherichia coli, Salmonella*, and *Lactobacillus* were determined by the method of Zhang and Kim (2013). Generally speaking, cecum samples were collected from 2 broilers from each replicate, then placed on ice to perform microbial analysis as soon as possible. One gram of the cecal excreta sample was mixed with 9 ml of 1% peptone broth and then homogenised (Ultra-Turrax T8 homogenizer; IKA Labortechnik, Germany). Afterwards, bacteria counts were measured by serial 10-fold dilutions (in 10 g/l peptone solution) onto *Lactobacillus* MRS agar plates (Sinopharm Chemical Reagent, China), MacConkey agar plates, and *Salmonella-Shigella* agar plates to isolate the *Lactobacillus*, *Escherichia coli,* and *Salmonella*, respectively. The bacteria colonies were counted immediately after the plates were cultivated at 37°C under anaerobic conditions.

Small intestinal morphology. Small intestinal morphology was determined as we previously described (Gao et al. 2011). Briefly, duodenum, jejunum, and ileum samples (2 cm) were taken and stored in 10% neutral buffered formalin for 24 h. Afterwards, samples were cut and histological slides were prepared. Each sample was treated with paraffin and stained with hematoxylin and eosin. Ten longest and straightest villi and their associated crypts from each sample were determined. The villus height was determined from the tip to the base. The crypt depth was determined from the base of the villus to the base of the crypt. The villus height-to-crypt depth ratio (VCR) was also calculated.

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Determination of duodenal mucosal s-IgA, α-amylase ***and chymotrypsin activity***. Duodenal mucosal samples were prepared as we previously described (Gao et al. 2011). Duodenum samples of broilers were taken, dissected longitudinally and rinsed with 4°C phosphate buffered saline (PBS). Duodenal mucosa was scratched with a glass slide. Then 9 ml of 4°C PBS was added to 1 g of duodenal mucosa, and the mixtures were homogenised for 1–2 min. The homogenates were centrifuged at 3000 *g* for 5 min. The supernatant was collected for measuring mucosal s-IgA, α-amylase, and chymotrypsin activity by commercial kits (Nanjing Jiancheng Bioengineering Institute, China). Briefly, the enzyme-linked immunosorbent assay method was used to determine s-IgA. The activity of α-amylase was measured by determining the hydrolyzation of starch to glucose, maltose, and dextrin. Unhydrolysed starch can form blue complex with added iodine solution, which can be measured by colorimetric assay at 660 nm. Chymotrypsin activity was measured by determining the hydrolyzation of casein to phenolic amino acids, which can form blue substance with phenolic reagents. All the samples were normalised to protein concentration as determined by the Coomassie Blue assay.

Statistical analysis. Statistical analysis of the data was performed according to Almeida et al. (2013). All data were analysed using the GLM procedure of SAS software (Statistical Analysis System, Version 8.0, 1999). The model included diet as the fixed effect and block as the random effect. Replicate was used as the experimental unit. Contrasts were performed between antibiotic group vs control group, and antibiotic group vs diets added with encapsulated EO and OA blends. Orthogonal polynomial contrasts were used to determine significant linear and quadratic effects of the increasing dose of encapsulated EO and OA blends to the diets. Appropriate coefficients for unequally spaced concentrations of EO and OA supplemental blends were obtained using the interactive matrix language procedure (PROC IML) of SAS. Means and pooled standard error of the means (PSEM) were presented. An α-level of $P \leq 0.05$ was used for determining the statistical significance, and $P \leq 0.10$ was taken to indicate the statistical tendency.

RESULTS

Effects of EO and OA encapsulated blends on performance of broilers. At the end of the starter phase (days 0–21), ADFI was not affected by EO and OA encapsulated blends. However, compared to control group, broiler ADG was increased by antibiotic (P =

Table 2. Effects of encapsulated blends of EO and OA on growth performance of male broilers reared to 70 days of age[1]

Item	Blends of EO and OA (mg/kg)				AB	PSEM	*P*-value		*P*-value	
	0 (control)	150	200	250			AB vs control	AB vs blends	L	Q
Days 0–21										
ADFI	27.57	27.27	27.44	27.75	27.91	0.289	0.425	0.219	0.851	0.244
ADG	13.97	14.06	14.40	14.38	14.45	0.153	0.039	0.340	0.043	0.595
G : F	0.507	0.516	0.525	0.518	0.518	0.005	0.158	0.776	0.052	0.511
Days 22–70										
ADFI	104.6	104.9	103.8	102.2	107.7	2.305	0.360	0.144	0.519	0.540
ADG	38.83	39.54	39.13	38.66	40.38	0.728	0.148	0.147	0.987	0.372
G : F	0.371	0.377	0.377	0.378	0.375	0.004	0.507	0.693	0.239	0.801
Days 0–70										
ADFI	81.97	80.87	81.32	80.32	82.30	1.342	0.862	0.356	0.442	0.950
ADG	31.41	31.90	32.69	31.45	32.62	0.515	0.112	0.324	0.486	0.239
G : F	0.383	0.395	0.402	0.392	0.396	0.005	0.063	0.955	0.057	0.145

EO = essential oils, OA = organic acids, AB = antibiotic group, L = linear contrast, Q = quadratic contrast, ADFI = average daily feed intake, ADG = average daily gain, G : F = gain-to-feed ratio

[1]values are presented as means and pooled standard error of the means (PSEM). Data are means of 5 replicate pens with 12 broilers per pen. An α-level of $P \leq 0.05$ was used for determination of statistical significance, and $P \leq 0.10$ was taken to indicate a statistical tendency

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0.039) and EO and OA encapsulated blends (linear, $P = 0.043$; Table 2). Moreover, broilers fed EO and OA encapsulated blends had greater G : F (linear, $P = 0.052$) compared to broilers fed the control diet. At the grower and finisher phase (days 22–70), the addition of antibiotic and encapsulated blends had no effect on broiler performance. For the overall phase (days 0–70), G : F tended to be promoted by antibiotic ($P = 0.063$) and EO and OA encapsulated blends (linear, $P = 0.057$) compared to control. In starter, finisher, and overall phase, there was no difference in growth performance between treatments with antibiotic supplementation and EO and OA encapsulated blends. Mortality was not affected by antibiotic and EO and OA encapsulated blends compared to control (data not shown).

Effects of EO and OA encapsulated blends on cecal microflora of broilers. For 21-day-old broilers, the EO and OA encapsulated blends reduced cecal *Escherichia coli* (linear, $P = 0.001$; quadratic, $P = 0.041$; Table 3) and *Salmonella* level (linear, $P = 0.001$), while tended to elevate *Lactobacillus* level (linear, $P = 0.071$) compared to control. Likewise, antibiotic addition repressed cecal *Salmonella* level ($P = 0.018$) compared to control. For 70-day-old broilers, EO and OA encapsulated blends reduced cecal *Escherichia coli* (linear, $P = 0.001$; quadratic, $P = 0.080$) and *Salmonella* level (linear, $P = 0.006$), while tended to elevate *Lactobacillus* level (linear, $P = 0.062$) compared to control. Besides, cecal *Escherichia coli* level was also decreased by EO and OA encapsulated blends compared to antibiotic group ($P = 0.035$).

Effects of EO and OA encapsulated blends on duodenum, jejunum, and ileum morphology of broilers. EO and OA encapsulated blends tended to decrease duodenum crypt depth of 21-day-old broilers compared to control group (linear, $P = 0.101$; Table 4). Duodenum VCR of 21-day-old broilers showed an uptrend by antibiotic addition ($P = 0.073$) and EO and OA encapsulated blends (linear, $P = 0.059$) compared to control. Likewise, jejunum crypt depth of 21-day-old broilers tended to be repressed by antibiotic ($P = 0.080$) and EO and OA encapsulated blends (linear, $P = 0.074$) compared to control. Jejunum VCR of 21-day-old broilers was elevated by antibiotic supplementation ($P = 0.049$) and encapsulated blends of EO and OA (linear, $P = 0.041$) compared to control. There was no difference of ileum morphology of 21-day-old broilers among treatments. Moreover, duodenum, jejunum, and ileum morphology of 70-day-old broilers was also not affected by antibiotic and EO and OA encapsulated blends (data not shown).

Effects of EO and OA encapsulated blends on jejunal mucosal s-IgA content, α-amylase and chymotrypsin activity of broilers. For 21-day-old broilers, jejunal mucosal s-IgA content was enhanced by antibiotic addition compared to control group ($P = 0.016$; Table 5), and tended to be increased by antibiotic addition compared to EO and OA encapsulated blends ($P = 0.090$). Encapsulated blends of

Table 3. Effects of encapsulated blends of EO and OA on cecal *Escherichia coli, Salmonella,* and *Lactobacillus* ($\log_{10}$ cfu/g) of male broilers[1]

Item	Blends of EO and OA (mg/kg)				AB	PSEM	*P*-value		*P*-value	
	0 (control)	150	200	250			AB vs control	AB vs blends	L	Q
Day 21										
Escherichia coli	7.11	7.05	6.80	6.57	6.98	0.097	0.347	0.135	0.001	0.041
Salmonella	6.77	6.63	6.59	6.49	6.61	0.046	0.018	0.484	0.001	0.515
Lactobacillus	7.99	8.01	8.08	8.18	8.15	0.066	0.110	0.477	0.071	0.253
Day 70										
Escherichia coli	7.33	7.12	6.94	7.11	7.21	0.057	0.152	0.035	0.001	0.080
Salmonella	6.92	6.88	6.71	6.80	6.85	0.040	0.230	0.239	0.006	0.882
Lactobacillus	7.83	7.86	8.08	7.98	7.89	0.075	0.566	0.352	0.062	0.877

EO = essential oils, OA = organic acids, AB = antibiotic group, L = linear contrast, Q = quadratic contrast

[1]values are presented as means and pooled standard error of the means (PSEM). Data are means of 5 replicate pens with 12 broilers per pen. An α-level of $P \leq 0.05$ was used for determination of statistical significance, and $P \leq 0.10$ was taken to indicate a statistical tendency

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Table 4. Effects of encapsulated blends of EO and OA on duodenum, jejunum, and ileum morphology of 21-day-old male broilers[1]

Item		Blends of EO and OA (mg/kg)				AB	PSEM	*P*-value			
		0 (control)	150	200	250			AB vs control	AB vs blends	L	Q
Duodenum	VH (μm)	1276	1288	1319	1291	1297	19.95	0.470	0.916	0.346	0.626
	CD (μm)	145.5	137.3	130.9	132.8	131.4	6.234	0.124	0.751	0.101	0.820
	VCR	8.86	9.46	10.13	9.77	9.96	0.413	0.073	0.712	0.059	0.693
Jejunum	VH (μm)	955.3	977.4	984.6	981.1	984.4	17.17	0.245	0.865	0.223	0.705
	CD (μm)	137.3	135.4	132.2	133.3	132.4	1.876	0.080	0.590	0.074	0.963
	VCR	6.97	7.22	7.46	7.37	7.43a	0.158	0.049	0.635	0.041	0.774
Ileum	VH (μm)	775.5	793.7	799.6	785.1	796.6	9.631	0.138	0.737	0.246	0.210
	CD (μm)	126.5	125.0	122.8	123.6	123.1	3.926	0.553	0.888	0.526	0.964
	VCR	6.17	6.39	6.52	6.41	6.47	0.244	0.383	0.913	0.363	0.709

EO = essential oils, OA = organic acids, AB = antibiotic group, L = linear contrast, Q = quadratic contrast, VH = villus height, CD = crypt depth, VCR = villus height-to-crypt depth ratio

[1]values are presented as means and pooled standard error of the means (PSEM). Data are means of 5 replicate pens with 12 broilers per pen. An α-level of $P \leq 0.05$ was used for determination of statistical significance, and $P \leq 0.10$ was taken to indicate a statistical tendency

EO and OA improved jejunal mucosal chymotrypsin activity of 21-day-old broilers compared to control (quadratic, $P = 0.014$). For 70-day-old broilers, EO and OA encapsulated blends promoted jejunal mucosal s-IgA content (linear, $P = 0.012$), α-amylase activity (quadratic, $P = 0.043$), and chymotrypsin activity (quadratic, $P = 0.037$) compared to control.

DISCUSSION

Effects of EO and OA encapsulated blends on performance of broilers. Previous results showed that male chicks did benefit more from supplementation with encapsulated blends of EO and OA than the female chicks. Perhaps due to fast growth, the

Table 5. Effects of encapsulated blends of EO and OA on jejunal mucosal s-IgA content, α-amylase, and chymotrypsin activity of male broilers[1]

Item	Blends of EO and OA (mg/kg)				AB	PSEM	*P*-value			
	0 (control)	150	200	250			AB vs control	AB vs blends	L	Q
Day 21										
s-IgA (mg/g protein)	171.4	197.9	222.5	200.7	251.1	21.42	0.016	0.090	0.187	0.560
α-Amylase activity (U/mg protein)	0.86	1.14	1.03	1.00	0.98	0.100	0.341	0.479	0.187	0.117
Chymotrypsin activity (U/mg protein)	69.62	109.91	89.41	78.13	84.25	10.19	0.322	0.492	0.346	0.014
Day 70										
s-IgA (mg/g protein)	195.0	213.2	306.1	281.4	230.4	27.54	0.375	0.264	0.012	0.638
α-Amylase activity (U/mg protein)	0.76	1.00	1.03	0.78	0.86	0.088	0.503	0.530	0.447	0.043
Chymotrypsin activity (U/mg protein)	58.78	77.41	86.12	60.65	69.14	8.142	0.379	0.559	0.320	0.037

EO = essential oils, OA = organic acids, AB = antibiotic group, L = linear contrast, Q = quadratic contrast, s-IgA = secretory IgA

[1]values are presented as means and pooled standard error of the means (PSEM). Data are means of 5 replicate pens with 12 broilers per pen. An α-level of $P \leq 0.05$ was used for determination of statistical significance, and $P \leq 0.10$ was taken to indicate a statistical tendency

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male chicks were more vulnerable for disturbances of the gut microflora, which might have been modulated by EO and OA blends (Weber et al. 2012). Therefore, we chose male broilers in our study. Blends of EO and OA can be considered as safe in poultry nutrition because mortality was not affected by blends of EO and OA as observed in our as well as other researches (Weber et al. 2012). Our results showed that ADG and G : F were improved in starter period while growth performance in finisher period was not affected by EO and OA encapsulated blends. The growth-promoting effect in the starter period revealed that EO and OA improved nutrient utilization in young animal, which is important considering secretion of endogenous enzymes is insufficient in this crucial phase (Noy and Sklan 1995). The same results were reported by researchers who carried out four growth trials and revealed that blends of EO and benzoic acid improved ADG and FCR of broiler chicks in different geographical locations and under various husbandry conditions (Weber et al. 2012). Other studies also demonstrated that feed efficiency and ADG were increased by blends of EO and OA in broilers (Basmacioglu-Malayoglu et al. 2016), in turkey (Giannenas et al. 2014), and in weanling pigs (Grilli et al. 2010). In fact, the effects of non-encapsulated EO or OA on the growth performance of poultry were variable (Botsoglou et al. 2002; Islam et al. 2012; Goodarzi Boroojeni et al. 2014a; Karadas et al. 2014; Placha et al. 2014). However, the experimental results indicated that encapsulated EO or OA improved growth performance of broilers (Hafeez et al. 2016; Levy et al. 2015). This may be due to the fact that non-encapsulated additives are mainly metabolised and absorbed in the proximal part of the digestive tract and rarely reach its distal part (Goodarzi Boroojeni et al. 2014b), while the encapsulated EO and OA can be slowly released along the intestine, which results in improved intestine health and performance (Piva et al. 2007).

Effects of EO and OA encapsulated blends on cecal microflora of broilers. The ceca are considered the major site of microbial fermentation in the avian gastrointestinal tract (Levy et al. 2015). Therefore, cecal microflora (*Escherichia coli, Salmonella*, and *Lactobacillus*) of broilers were determined in our study. Gut microbiota plays an important role for animal health, performance, and product safety. Decreased numbers of pathogenic bacteria in the gut may improve the ability of epithelial cells to regenerate villus and thus enhance intestinal absorptive capacity (Zeng et al. 2015). Results showed that *Escherichia coli* and *Salmonella* were decreased while *Lactobacillus* of 21- and 70-day-old broilers tended to be increased by encapsulated blends of EO and OA. Our results were in accord with others. For example, combinations of EO and OA have been reported to reduce ileum *Escherichia coli* counts of broilers (Basmacioglu-Malayoglu et al. 2016), increase lactic acid bacteria populations and decrease coliform bacteria in the cecum of turkey (Giannenas et al. 2014), as well as result in less coliforms and more lactic acid bacteria in the swine cecum (Piva et al. 2007). The antimicrobial effect of the EO and OA treatment appears to be selective for certain potentially harmful microbes, while beneficial bacteria such as *Lactobacillus* population were not negatively affected (Tiihonen et al. 2010). The occurrence of human salmonellosis caused by poultry meat contamination is of great importance for both producers and consumers. Reduction of *Salmonella* numbers in the intestine and the number of contaminated carcasses may improve food safety. Therefore, the decrease of pathogenic bacteria numbers in the intestine would be of great significance to the poultry industry.

Effects of EO and OA encapsulated blends on duodenum, jejunum, and ileum morphology of broilers. Shorter villi are associated with poor nutrient absorption and digestion because of a decrease in the absorptive area and less mature enterocytes (Paiva et al. 2014). Villus height increases twofold in the 48 h after hatching in chicks, which could improve nutrient absorption (Choct 2009). Deeper crypt depth indicates faster cellular turnover, which is likely in response to epithelial cell destruction, inflammation, and sloughing. Increased villus height and decreased crypt depth, which resulted in significantly elevated VCR, indicate mature enterocytes and efficient ability of nutrient absorption (Paiva et al. 2014; Zeng et al. 2015). Therefore, intestinal morphology can reveal intestinal health and integrity (Paiva et al. 2014). Our study showed that encapsulated blends of EO and OA tended to decrease duodenum and jejunum crypt depth, while increased duodenum and jejunum VCR of 21-day-old broilers compared to control group. Duodenum, jejunum, and ileum morphology of 70-day-old broilers were not af-

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fected by EO and OA encapsulated blends. This is consistent with performance results in our research. Encapsulation strategy designed to enhance delivery of ingredients to improve intestinal health is effective (Smith et al. 2012). The beneficial effects of encapsulated blends of EO and OA on intestinal morphology might be due to regulation and balance of intestinal microflora (Zeng et al. 2015). Furthermore, the anti-inflammatory and antioxidant functions in gut of EO might also help to improve the intestinal morphology and health (Mueller et al. 2012; Karadas et al. 2014; Du et al. 2016). Our findings were consistent with other researches. For example, blend of EO and OA reduced crypt depth and elevated villus height in the ileum, and the use of the combination of EO and OA was more effective, in some respects, than their individual use (Basmacioglu-Malayoglu et al. 2016; Liu et al. 2017). In the *Clostridium perfringens*-challenged broiler chickens, dietary EO supplementation also improved the villus height and VCR and decreased the crypt depth, suggesting EO addition could alleviate intestinal injury by improving intestinal integrity in the *Clostridium perfringens*-challenged broiler chickens (Du et al. 2016).

Effects of EO and OA encapsulated blends on jejunal mucosal s-IgA content, α-amylase and chymotrypsin activity of broilers. To explore the effects of EO and OA on humoral immunity and digestive capacity, jejunal mucosal s-IgA level, α-amylase and chymotrypsin activity of broilers were analyzed. Our results showed that the encapsulated blends of EO and OA improved s-IgA level, α-amylase and chymotrypsin activity. The increased activity of digestive enzymes could result in enhanced digestion of nutrients in the intestine, leading to improved growth performance. The effects of encapsulated blends of EO and OA on intestinal s-IgA and digestive enzymes are rarely reported. Therefore, the possible and underlying mechanisms of EO and OA blends in modulating humoral immunity and digestive enzymes are barely understood. The results of improved digestive enzymes in our study may be partly explained by dietary EO addition, because lots of researches showed that EO improved intestinal amylase activity (Lee et al. 2003), lipase activity in pancreas and intestine wall (Jamroz et al. 2005), and pancreatic trypsin, pancreatic α-amylase and intestinal maltase of broilers (Jang et al. 2007). However, studies of EO on intestinal s-IgA are conflicting. Placha et al. (2014) showed EO supplementation increased s-IgA concentration in duodenal mucosa of chickens, but Du et al. (2016) revealed that s-IgA production was not affected by EO supplementation.

The effects of antibiotic addition and EO and OA encapsulated blends had similar effects on performance, intestinal morphology and jejunal mucosal s-IgA level, but the EO and OA encapsulated blends showed better results on intestinal microflora and jejunal mucosal enzyme activity. Therefore, encapsulated blends of EO and OA, based on our results, can replace antibiotic effectively. The mechanisms involved in causing growth promotion of EO and OA blends are far from being elucidated, since data on the complex gut ecosystem, gut function and *in vivo* immune system are still lacking (Zeng et al. 2015). Our study suggested that encapsulated blends of EO and OA enhanced the digestive enzyme activity, balanced microflora in the gut, therefore, improved intestinal morphology and reinforced immune status, which helps to explain the enhanced performance observed in broilers.

CONCLUSION

Encapsulated blends of EO and OA enhanced jejunal mucosal s-IgA level, α-amylase and chymotrypsin activity, regulated and balanced intestinal microflora (decreased *Escherichia coli* and *Salmonella* and increased *Lactobacillus*), improved intestinal morphology, and promoted the performance of broilers. Based on the results obtained in our study, the 200 mg/kg of encapsulated blends of EO and OA was an optimum supplementation dose for broilers. Besides, our results also suggested that the encapsulated blends of EO and OA can be used as an effective antibiotic alternative.

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