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Essential Oils and Sodium Butyrate supplementation in Broilers: Effect on growth, Nutrients Digestibility, Intestinal Morphology, and Blood biochemistry

Mohamed I. El-Katcha, Mosaad A. Soltan, Mohamed El-Banoby, Karima El-Naggar*
Department of Nutrition and Veterinary Clinical Nutrition, Faculty of Veterinary Medicine, Alexandria University, Egypt.

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***Correspondence to:**
karima.muhammad@alexu.edu.eg

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ABSTRACT

The present study investigated the effects of essential oil mixture (EOM) supplementation without or with sodium butyrate (SB) on growth, blood biochemistry, nutrient digestibility, intestinal microbiology, and morphology of broilers. Birds were randomly distributed into eight groups: G1; as control; G2 and G3 were supplemented with EOM 72 and 144 mg/L respectively in water, G4 was supplemented with 20 mg EOM /kg diet, while groups 5-8 have the same design but combined with SB (750 mg/kg diet). The addition of EOM in water without or with SB non-significantly ($P > 0.05$) increased body weight. The lower level of EOM reduced feed intake, while supplementation in feed (20 mg /kg) or water (144 mg/l) increased it compared with control. This result was reversed when added with dietary SB. Nutrients digestibility including crude protein, ether extract, inorganic matter, Ca, and P was improved with EOM especially lower-level added in water or feed. The same response was obtained when EOM combined with SB especially inorganic matter. Intestinal villi length was increased with EOM supplementation without or with dietary SB. The total bacterial count of ileocecal content was reduced with SB or EOM supplementation in water. Combination of SB with EOM through water improved *lactobacillus* proliferation in the intestine. EOM supplementation numerically decreased blood serum triglyceride and total cholesterol concentrations while SB had no clear effect on them. EOM without or with SB decreased LDL and improved HDL concentrations. It could be summarized that EOM administration in water at 72 mg/L or feed (20 mg/Kg) improved performance, increased villi length, and *lactobacillus* counts. Sodium butyrate combination with EOM (lower dose) in water is considered more effective than supplementation alone.

1. INTRODUCTION

With the continuous expansion of the poultry production sector using modern systems, birds facing several challenges during their productive lifetime, especially after hatching. A newly hatched chick starts its life with an immature and sterile gastrointestinal tract (GIT). When consuming the solid feed, it allows GIT function and all its components, including its microflora. During this time, the chick becomes very vulnerable to pathogenic microorganisms affecting its growth performance (Adams, 2004). Antimicrobial additives as antibiotics have been traditionally used to suppress detrimental microorganisms, improve growth and feed efficiency utilization (Jin et al., 2007). The widespread use of these additives as growth promoters become associated with the emergence of antibiotic resistance. For this reason, the European Union restricted the use of such antibiotics by focusing on finding new safe alternatives to antibiotics (Griggs and Jacob, 2005). Some alternatives such as enzymes, organic acids, probiotics, prebiotics, herbs, and essential oils (Griggs and Jacob, 2005) attracted the attention to replace antibiotic growth promoters.

Essential oils (EOs) are oily liquids that originated from plants, have various biological functions as immune-stimulating effects (Hosseini et al., 2016), antimicrobial, antifungal, or antioxidant activities (Silva et al., 2012). Using of these compounds in poultry attracted attention due to their growth-promoting effect (Mountzouris et al., 2011), antioxidant activity (Ciftci et al., 2010), and nutrient digestibility improvements through stimulation of

digestive enzyme secretion (Basmacioglu Malayoglu et al., 2010).

Organic acids are weak, partially dissociated short-chain fatty acids (SCFA). These acids or their salt forms can be supplied in feed or water. Organic acids were found to improve bird performance by increasing the absorption of nutrients, improve nutrient utilization, reduce toxic bacterial byproducts, and secretion of immune intermediates (Dibner and Buttin, 2002; Mansoub et al., 2011). Moreover, supplementation of organic acids showed an immunostimulatory effect in poultry through higher antibody titers against infectious bursal disease vaccine (Lohakare et al., 2005), increase the weight of bursa and thymus, and higher serum globulin concentration, (Abdel-Fattah et al., 2008; Ghazala et al., 2011). Therefore, the current study aimed to investigate the effects of EOM supplementation without or with sodium butyrate on the growth performance, nutrient digestibility, intestinal microbiology, and morphology as well as blood biochemistry in broiler chickens.

1. MATERIAL AND METHODS

2.1. Birds care and experimental design

Three hundred and twelve, two day- old *Cobb* broiler chicks of mixed sex were used in this experiment. Chicks were individually weighed then randomly assigned into eight groups with three replicates/ group (13 chicks per each). Group 1 (G1) considered as control; G2 and G3 were supplemented with EOM 72 and 144 mg/L respectively in water, G4 was supplemented with 20 mg EOM /kg diet, while groups 5-8 have the same design but combined with SB (750 mg/kg diet). Additives used: Aeroment: produced by Top Line Co, Egypt. and composed of (Ethanol, 22.5%; Emulsifiers, 15.3%; Eucalyptus oil 7%; Menthol crystal 6.6%; Mint oil 2.0% and demineralized water up to 100%) with manufacturer recommendation to be used in the drinking water at 0.25 or 0.5 ml/liter water; Digestram: L-menthol (peppermint 8% - Eugenol (Clove) 2% - Anethole (Anise) 3.4% carrier sodium chloride up to 100%- Micro-plus Germany and the recommendation for usage is 0.15 kg /ton feed; Deng So: contain sodium butyrate 99%, produced by Koran company and the recommended dose is 0.75 Kg/ton feed.

Birds were housed in clean, well-ventilated, and thermally controlled compartments for six weeks. Chicks had free access to feed and water over the experiment. They were vaccinated against Newcastle disease and infectious bursal disease viruses according to the regular vaccination program. Basal diet (BD) used was formulated (Table 1) according to (NRC, 1994). Growth parameters as body weight (BW) and feed intake (FI) of chicks were recorded every week. Weight gain (WG) and feed conversion ratio (FCR) were calculated. All bird care procedures were approved by the local ethical committee of Animal use, Faculty of Veterinary Medicine, Alexandria University, Egypt.

2.2. Nutrient Digestibility

During the last week of the experimental period (6^{th} week), excreta was quantitatively collected for five successive days with recording FI. Excreta was dried in a hot air oven, ground, and stored until chemical analysis of nutrients. Nutrient contents of feed and fecal samples were analyzed according to (AOAC., 1990). Wet ashing was done for the preparation of samples for calcium (Ca) and phosphorus (P) analysis. Calcium and phosphorus were determined according to (Gericke and Kurmies, 1952; Slavin, 1968) using a flame photometer. Apparent digestibility coefficient = nutrient intake – nutrient in faces / nutrient intake * 100.

2.3. Intestinal Morphology and Microbiology

Six birds per treatment were randomly selected and slaughtered. The intestine was removed, and one gram of ileocecal content was collected for microbiological determination. 9 ml of peptone water was added to each one gram of sample (tube number one), and a ten-fold serial dilution was done according to (Mountzouris et al., 2007). Then the total bacterial count and lactobacillus count were determined following the method described by (Bivolarski et al., 2011). Approximately 5 cm of the middle portion of the duodenum and jejunum was excised, flushed with physiological saline, and fixed in 10% formalin for at least two days to examine the intestinal villi morphology. Slides were prepared according to (Bancroft et al., 2013), then the analysis was completed using Image J analysis software (NIH, MD, USA).

Table 1. Ingredient composition of the basal diet.

Ingredients %	Starter	Grower	Finisher
Yellow corn	53.6	58.1	61.6
Soybean meal (44%)	32.6	29.5	26.6
Corn gluten (60%)	8.0	6.50	5.5
Vegetable oil [a]	2.0	2.0	2.5
DCP [b]	1.7	1.5	1.7
Limestone [c]	1.3	1.6	1.3
Lysine [d]	0.05	0.05	0.05
DL-Methionine [e]	0.15	0.15	0.15
Common Salt	0.3	0.3	0.3
Premix (mineral and vitamin) [f]	0.3	0.3	0.3
Chemical analysis (%)			
Moisture	12.14	11.98	13.16
Crude protein	23.03	21.12	19.06
Ether extract	5.58	5.76	5.72
Crude fiber	2.72	2.65	2.67
Ash	6.77	6.55	6.66
NFE*	49.78	51.49	52.73
Calcium	1.10	1.09	0.98
Total Phosphorus	0.73	0.68	0.69
Metabolizable energy (ME, Kcal/kg diet)	3081.3	3122.41	316841
Calorie /protein ratio**	133.79	147.84	166.23

[a] Vegetable oil (mixture of sunflower oil and cottonseed oil). [b] DCP= dicalcium phosphate (contain 18% P and 25% Ca). [c] Limestone (contain 34% calcium). [d] Lysine = lysine hydrochloride (contain 98.5% Lysine). [e] DL-Methionine (Produced by Evonic Co and contain 99.5% methionine).[f] The premix used was produced by Heropharm and composed of (per 3 kg) vitamin A 12000000 IU, vitamin D3 2500000 IU, vitamin E 10000 mg, vitamin K3 2000 mg, thiamin 1000 mg, riboflavin 5000 mg, pyridoxine 1500 mg, cyanocobalamin 10 mg, niacin 30000 mg, biotin 50 mg, folic acid 1000 mg, pantothenic acid 10000 mg, manganese 60000 mg, zinc 50000 mg, iron 30000 mg, copper 4000 mg, iodine 300 mg, selenium 100 mg and cobalt 100 mg. * NFE= Nitrogen free extract (calculated by difference "100- (moisture % + CP % + EE % + CF % + ash %)". **Calorie/protein ratio = ME/ CP.

2.4. Phagocytosis and blood Biochemistry

Six birds/ group were randomly selected, and two blood samples were gathered from each bird. One sample was collected without anticoagulant and used for obtaining serum, as blood was then separated by centrifugation at 3000 rpm / 10 minutes. Serum samples were kept at 20 °c for analysis of some blood biochemical constituents including glucose, total protein, albumin, triglycerides, total cholesterol, low and high-density lipoprotein (LDL and HDL), aspartate aminotransferase (AST), alanine aminotransferase (ALT), by spectrophotometer using commercial kits from using kits of Vitro Scient company. The other blood sample was collected in anticoagulant-containing Eppendorf tubes for the determination of phagocytic activity (PA) and phagocytic index (PI) according to (Kawahara et al., 1991). From the same birds, immune organs (bursa and spleen) were collected and weighed. The obtained results were analyzed using analysis of variance (two-way ANOVA) using (SAS, 1996) to measure the effect of EOM supplementation and sodium butyrate addition. The difference was considered significant at $P< 0.05$.

2. RESULTS

As presented in table 2, EOM supplementation through drinking water or feed non-significantly ($P> 0.05$) improved BW. Likewise, dietary SB supplementation alone or combined with EOM in water non-significantly ($P> 0.05$) increased BW than birds fed the same diet without SB. Combination of SB with EOM supplemented in diet non-significantly reduced BW ($P> 0.05$). The lower level of EOM (72 mg/L) numerically reduced FI, while the higher level (144 mg/L) in drinking water and ration numerically increased it when compared with control. Additionally, SB supplementation with EOM (higher level added in water or those doses mixed with feed) lowered FI compared with their control groups without SB addition. Supplementation of each additive separately had no significant effect on FCR compared with control, however EOM supplementation at a low level in water or feed non-significantly improved FCR ($P> 0.05$). The same response was obtained when the higher level of EOM added in water combined with SB ($P> 0.05$) compared with birds reared without SB addition. EOM supplementation at a low level in water or feed improved the digestibility of crude protein (CP) and ether extract, while the higher level of EOM reduced it (table 3). The same result was obtained with birds that received SB compared with control. EOM supplementation at 72 mg/L water or 20 mg/Kg diet significantly increased ($P< 0.05$) ash, Ca, and P digestibility. Additionally, SB addition improved Ca

and P digestibility compared with control while did not affect when combined with EOM.

EOM supplementation significantly (P< 0.05) increased villi length, with the highest length recorded in those supplemented in water than in feed (Table 4 and Fig. 1). On the other hand, villi width nearly showed inverse results. Additionally, the combination of a low level of EOM in water or added in feed with SB significantly increased villi length (P< 0.05). On the contrary, villi width was reduced when SB combined with EOM added in water. Therefore, EOM at 72 mg/ L water or 20 mg /kg diet better combined with butyrate and was considered more effective on intestinal development. EOM supplementation reduced the total bacterial count of ileocecal content (table 4). SB supplementation showed the same effect. The combination of both additives was more effective in reducing bacterial colonies than using each one separately. The lower dose of EOM supplemented in water increased *lactobacillus* counts while was decreased with the higher level of EOM added in water (144 mg/ L) and feed (20 mg /kg diet). On the other hand, SB combined with EOM through water improved the proliferation of *lactobacillus* in the intestine. The lower level of EOM in water plus butyrate was more effective as a *lactobacillus* stimulant than other treatments. Moreover, EOM supplementation in feed without or with SB negatively affects *lactobacillus* proliferation.

As shown in table 5, EOM supplementation in drinking water at low level (72mg/l) non-significantly improved PA and PI (P> 0.05) compared with the control, while the higher level added in water non-significantly reduced them. Moreover, sodium butyrate without or with EOM had no significant effect on PA and PI or bursa weight. EOM supplementation at 72 mg /L water and 20 mg/Kg diet significantly increased bursa weight while the higher level non significantly reduced it when compared with control. Moreover, EOM supplementation in water or feed non-significantly increased (P> 0.05) spleen weight compared with control, while this weight was reduced when added with sodium butyrate.

EOM supplementation in drinking water (higher level) non-significantly increased (P> 0.05) serum total protein and albumin concentrations (table 6). Sodium butyrate addition with the low level of EOM in water significantly increased total protein concentration when compared with birds reared without SB addition. Blood serum AST concentrations were significantly reduced (*P*< 0.05) in birds supplemented with EOM in drinking water or diet while non-significantly reduced with sodium butyrate addition. EOM supplementation numerically decreased blood serum triglyceride and total cholesterol concentrations while SB addition had no clear effect (table 7). On the other hand, EOM supplementation single or combined with SB decreased blood serum LDL and improved HDL concentrations (P> 0.05).

Table 2. Growth performance of broiler chicken supplemented with essential oil mixture (EOM) without or with sodium butyrate.

	EOM supplementation	Sodium butyrate	
		0.0	0.75g/kg diet
Initial Body weight (g)	Control	58.1±0.6	56.4±0.2
	72 mg /L	56.7±0.6	57.7±0.6
	144 mg /L	57.7±0.6	57.1±0.6
	20 mg /Kg	56.7±0.5	56.9±0.5
Final Body weight (g)	Control	2202.7±58.6	2267.8±52.2
	72 mg /L	2204.2±106.5	2257.9±127.3
	144 mg /L	2253.7±50.2	2303.6±46.8
	20 mg /Kg	2357.1±45.2	2212.4±39.6
Total Body gain (g)	Control	2144.6±58.2	2211.4±51.9
	72 mg /L	2147.5±67.6	2200.2±36.4
	144 mg /L	2196.0±49.7	2246.6±46.3
	20 mg /Kg	2300.4±44.7	2155.5±39.1
Total feed intake (g)	Control	4111.7	4177.0
	72 mg /L	3872.1	4087.3
	144 mg /L	4166.7	4041.0
	20 mg /Kg	4156.5	4092.8
Feed conversion ratio	Control	1.91±0.05	1.88±0.04
	72 mg /L	1.80±0.06	1.85±0.22
	144 mg /L	1.89±0.05	1.79±0.04
	20 mg /Kg	1.80±0.04	1.89±0.04

Values are means ± SE. Letters at the same column (a-c) represent the statistical difference in EOM supplementation, or row (x-z) represents the statistical difference regarding the sodium butyrate free or supplemented groups. Difference considered significant at $P < 0.05$.

Table 3. Nutrient digestibility of broiler chicken supplemented with essential oil mixture (EOM) without or with sodium butyrate.

Nutrient	EOM supplementation	Sodium butyrate	
		0.0	0.75g/kg diet
Crude protein	Control	82.5±3.3^{bx}	84.3±3.5^{ax}
	72 mg /L	88.2±4.1 ^{ax}	89.1±2.9 ^{ax}
	144 mg /L	81.6±4.0^{bx}	85.6±3.4^{ax}
	20 mg /Kg	86.7±3.1 ^{ax}	88.1±4.1 ^{ax}
Ether extract	Control	86.8±3.5	87.8±4.1
	72 mg /L	89.5±3.7	90.8±3.0
	144 mg /L	84.2±2.6	89.6±2.1
	20 mg /Kg	88.1±2.2	86.7±3.1
Inorganic matter (ash)	Control	55.2±8.9^{by}	69.8±6.2^{bx}
	72 mg /L	65.8±5.5^{ay}	71.1±4.5^{abx}
	144 mg /L	59.1±4.7^{by}	76.2±5.2^{ax}
	20 mg /Kg	69.1±5.8^{ax}	66.2±4.2^{bx}
Calcium	Control	66.1±6.6^{by}	71.23±6.6^{ax}
	72 mg /L	71.3±6.2 ^{ax}	71.11±5.9 ^{ax}
	144 mg/L	69.1±4.2^{abx}	69.99±4.6^{ax}
	20 mg /Kg	72.9 ±5.5 ^{ax}	70.11±6.2 ^{ax}
Phosphorus	Control	59.9±6.2^{by}	64.2±6.0^{ax}
	72 mg /L	67.9±5.2^{ax}	69.3±5.1^{ax}
	144 mg /L	62.2±5.9^{abx}	63.1±5.9^{ax}
	20 mg /Kg	69.2±6.1^{ax}	65.2±5.2 ^{ax}

Values are means ± SE. Letters at the same column (a-c) represent the statistical difference in EOM supplementation or row (x -z) represents the statistical difference regarding the sodium butyrate free or supplemented groups. Difference considered significant at $P < 0.05$.

Table 4. Intestinal morphology and microbiology of broiler chicken supplemented with essential oil mixture (EOM) without or with sodium butyrate.

	EOM supplementation	**Sodium butyrate**	
		0.0	0.75g/kg diet
Intestinal Morphology			
Villi length (µm/bird)	Control	1644.7±17.1^{cx}	1750.8±61.9^{cx}
	72 mg L	2197.1±53.4^{ay}	2667.1±47.6^{ax}
	144 mg L	1982.9±25.3^{bx}	2077.8±31.8^{bx}
	20 mg Kg	1747.4±67.1^{cy}	2084.7±64.2^{bx}
Villi width (µm/bird)	Control	274.7±19.7^{bx}	221.4±17.7^{bx}
	72 mg /L	278.8±17.7^{abx}	158.1±8.2^{cy}
	144 mg/L	290.1±7.5^{ax}	163.0±10.6^{cy}
	20 mg /Kg	311.3±17.7^{ax}	340.9±33.6^{ax}
Intestinal Microbiology			
Total bacterial counts (cfuX10^{6})	Control	160.0±37.8^{ax}	12.0±5.69^{ay}
	72 mg L	67.3±43.5^{bx}	14.6±2.91^{ax}
	144 mg/L	51.3±27.7^{bx}	1.3±0.33^{ax}
	20 mg Kg	62.0±44.7^{bx}	2.6±1.20^{ax}
Lactobacillus counts (cfuX10^{4})	Control	0.73±0.1^{bx}	0.0±0.00^{bx}
	72 mg /L	15.6±2.3^{ax}	20.0±7.94^{ax}
	144 mg/L	0.0±0.0^{bx}	6.0±0.00^{bx}
	20 mg /Kg	0.0±0.0^{bx}	0.0±0.00^{bx}

Values are means ± SE. Letters at the same column (a-c) represent the statistical difference in EOM supplementation, or row (x – z) represents the statistical difference regarding the sodium butyrate free or supplemented groups. Difference considered significant at $P < 0.05$.

Table 5. Phagocytosis and immune organ weight of broiler chicken supplemented with essential oil mixture (EOM) without or with sodium butyrate.

	Essential oil supplementation	Sodium butyrate supplementation	
		0.0	0.75g/kg diet
Phagocytic activity	Control	43.47±1.72	43.73±1.68
	72 mg /L	44.90±1.47	44.23±1.76
	144 mg /L	40.73±0.96	45.53±0.63
	20 mg /Kg diet	43.65±2.21	43.87±0.54
Phagocytic index	Control	1.48±0.06	1.53±0.00
	72 mg /L	1.51±0.04	1.57±0.22
	144 mg /L	1.37±0.12	1.55±0.08
	20 mg /Kg diet	1.37±0.06	1.39±0.15
Bursa weight (g)	Control	1.40 ± 0.12^{abx}	1.46 ± 0.19^{ax}
	72 mg /L	1.70 ± 0.26^{abx}	1.47 ± 0.50^{ax}
	144 mg /L	1.17 ± 0.07^{bx}	1.27 ± 0.22^{ax}
	20 mg /Kg diet	2.83 ± 1.18^{ax}	1.57 ± 0.09^{ax}
Spleen weight (g)	Control	1.87 ± 0.35^{ax}	2.17 ± 0.27^{ax}
	72 mg /L	2.47 ± 0.09^{ax}	2.37 ± 0.18^{ax}
	144 mg /L	2.23 ± 0.52^{ax}	1.73 ± 0.13^{ax}
	20 mg /Kg diet	2.00 ± 0.29^{ax}	1.47 ± 0.26^{bx}

Values are means ± SE. Letters at the same column (a-c) represent the statistical difference in EOM supplementation, or row (x – z) represents the statistical difference regarding the sodium butyrate free or supplemented groups. Difference considered significant at $P < 0.05$.

Table 6. Blood biochemistry of broiler chicken supplemented with essential oil mixture (EOM) without or with sodium butyrate.

	EOM supplementation	Sodium butyrate supplementation	
		0.0	0.75g/kg diet
Total protein (g/dl)	Control	5.73 ± 0.2^{ax}	5.67 ± 0.1^{bx}
	72 mg/ L	5.63 ± 0.1^{ay}	6.57 ± 0.3^{ax}
	144 mg/ L	6.03 ± 0.3^{ax}	5.87 ± 0.0^{bx}
	20 mg /Kg	5.87 ± 0.1^{ax}	5.80 ± 0.0^{bx}
Albumin (g/dl)	Control	3.80±0.21	3.60±0.1
	72 mg /L	3.43±0.24	3.63±0.2
	144 mg/L	4.20±0.46	3.83±0.1
	20 mg /Kg	3.40±0.40	3.40±0.3
Glucose (mg/dl)	Control	95.7±2.8	94.8±3.7
	72 mg /L	92.1±5.6	99.1±3.6
	144 mg/L	92.0±2.3	93.8±1.8
	20 mg /Kg	94.1±1.5	99.7±4.9
Aspartate aminotransferase (AST), (µ/L)	Control	111.3 ± 4.9^{ax}	74.0 ± 2.5^{ay}
	72 mg /L	86.3 ± 3.7^{bx}	78.3 ± 4.8^{ax}
	144 mg /L	73.0 ± 4.0^{bcx}	72.6 ± 4.1^{ax}
	20 mg /Kg	66.0 ± 4.3^{cx}	68.3 ± 5.8^{ax}
Alanine aminotransferase (ALT) (µ/L)	Control	8.3 ± 1.4^{ax}	7.0 ± 0.2^{ax}
	72 mg /L	7.1 ± 0.1^{abx}	7.0 ± 0.2^{ax}
	144 mg/L	5.3 ± 0.6^{bx}	6.7 ± 0.5^{ax}
	20 mg /Kg	6.8 ± 0.8^{abx}	7.5 ± 0.2^{ax}

Values are means ± SE. Letters at the same column (a-c) represent the statistical difference in EOM supplementation or row (x-z) represents the statistical difference regarding the sodium butyrate free or supplemented groups. Difference considered significant at $P < 0.05$.

Table 7. Serum lipid profile of broiler chicken supplemented with essential oil mixture (EOM) without or with sodium butyrate.

Items	EOM supplementation	Sodium butyrate supplementation	
		0.0	0.75g/kg diet
Triglycerides (mg/dl)	Control	170.3±3.5	175.5±4.8
	72 mg /L	159.5±9.0	177.4±6.2
	144 mg /L	166.6±8.2	165.6±7.7
	20 mg /Kg	151.6±7.8	190.3±6.8
Total cholesterol (mg/dl)	Control	229.3±12.8	243.2±7.4
	72 mg /L	215.1±6.2	229.3±3.4
	144 mg/L	225.7±10.2	226.1±2.1
	20 mg /Kg	220.4±7.2	239.3±11.8
High-density lipoprotein. (mg/dl)	Control	58.6±1.5	74.5±1.0
	72 mg /L	75.8±0.3	73.3±2.8
	144 mg/L	74.6±4.9	75.6±6.1
	20 mg /Kg	69.2±0.3	72.6±3.1
Low-density lipoprotein. (mg/dl)	Control	136.6±14.9	133.6±8.1
	72 mg /L	127.3±8.14	120.4±6.5
	144 mg/L	116.6±14.8	117.3±3.6
	20 mg /Kg	120.9±7.3	128.6±10.8

Values are means ± SE. Letters at the same column (a-c) represent the statistical difference in EOM supplementation, or row (x – z) represents the statistical difference regarding the sodium butyrate free or supplemented groups. Difference considered significant at $P < 0.05$.

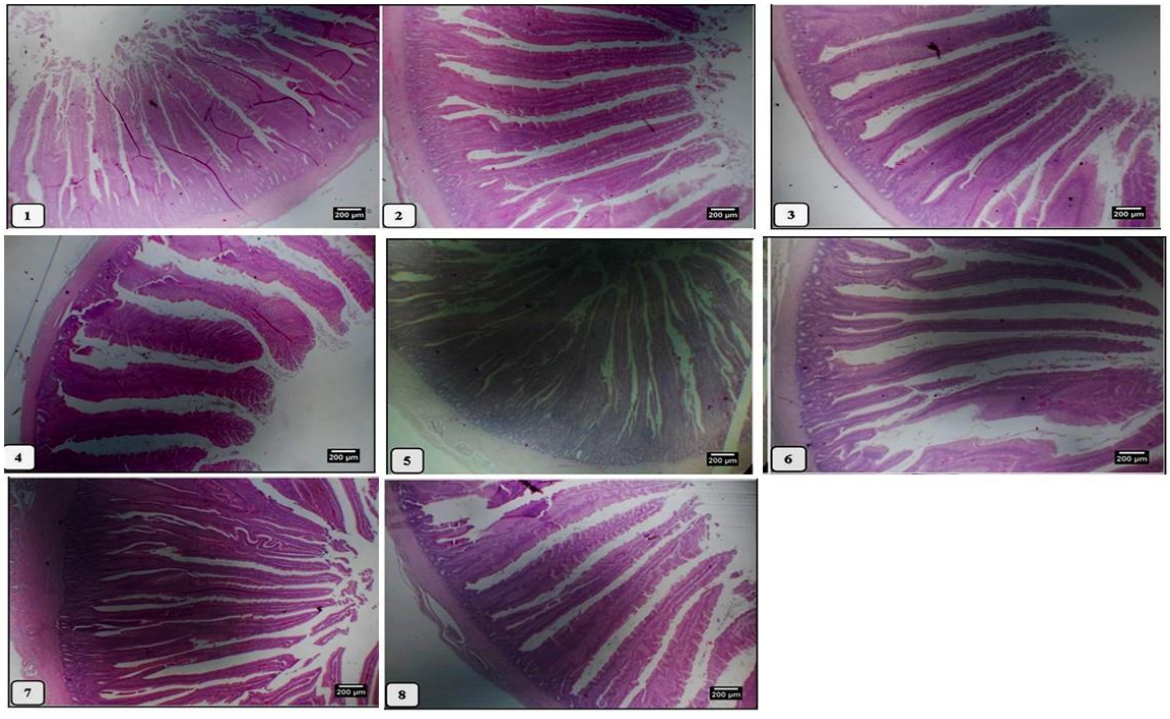

Figure 1. Broiler intestine stained with Hematoxylin and Eosin. G1; as control; G2 and G3 were supplemented with essential oil mixture (EOM) 72 and 144 mg/L respectively in water, G4 was supplemented with 20 mg EOM /kg diet; G5-G8 as G1-G4 but with the addition of sodium butyrate.

4. DISCUSSION

EOM supplementation improved broiler growth performance. This could be attributed to their role in stimulating digestive enzyme secretion, which enhanced the digestion of nutrients (Lee et al., 2004a). Similar findings were documented, especially with oil combinations having synergistic effects on growth performance (Abou-elkhair et al., 2014; Alcicek et al., 2003; Amad et al., 2011; Denli et al., 2004). However, other studies reported no effect on performance (Botsoglou et al., 2002; Cerisuelo et al., 2014). Inconsistency in results may be associated with EOM type present in the blend and their potential effects, hygienic conditions surrounding animals, flock health condition, and diet ingredients. The combination of EOM addition in water with SB showed the same response of non-significantly improved BW. Additionally, SB supplementation without or with EOM (lower level added in water) slightly increased FI, which could be associated with their effect on appetite and palatability (Cave, 1982). EOM supplementation non-significantly improved FCR while SB had no apparent effect on FCR except with the higher level of EOM supplemented in water which improved FCR. In this study, the addition of EOM in water at a low level or feed improved FCR, which is consistent with (Basmacioglu et al., 2004; Lee et al., 2004a) while in contrast with (Abdel-Wareth et al., 2012), who reported decreased FCR of birds supplemented with essential oils in the diet. Differences in results between different experiments could be due to differences in management practices applied and the physiological status of birds.

EOM supplementation at a low level in water or feed improved digestibility of CP and EE, while the higher level of EOM reduced it. Earlier studies had reported the effect of spices or their active components on bile salt secretion (Ganesh Bhat and Chandrasekhara, 1987; Sambaiah and Srinivasan, 1991), digestive enzyme activities of the intestinal mucosa and pancreas stimulation (Platel and Srinivasan, 2000), which in turn affected the digestibility of nutrients. On the other hand, the lower availability of different nutrients with higher EOM supplementation may be related to impaired enzyme secretion, as supported by (Kreydiyyeh et al., 2000). They suggested that EOs used inhibited the activity of Na+-K+ATPase located in enterocytes and consequently impair transport processes in the intestine. EOM supplementation at a lower level in water or diet significantly increased ash, Ca, and P digestibility. These results are in line with (Mountzouris et al., 2011; Olgun and Yildiz, 2014).

EOM supplementation had the most remarkable effect on intestinal morphology, causing an increase in villi length. Earlier investigations supported the present results as it concluded that the incorporation of EO in the diet resulted in a powerful stimulation of intestinal mucous membrane exhibited by the development of intestinal villi (Lavinia et al., 2009), deeper villi crypt (Reisinger et al., 2011), increased villus length and surface area, indicative of improved nutrient absorption (Geyra et al., 2001; Yang et al., 2018) and performance (Choct, 2009). The increase in villus surface area may be an explanation why birds supplemented with EOM had higher BW. Moreover, the addition of SB with EOM improved the intestinal villi length, and this could be due to butyrate functions as an energy source for the epithelial cells helping in the development and maintenance of their structure (Kien et al., 2007). EOM supplementation reduced the total bacterial count, especially with the higher dose in water, which was more effective. These findings are supported by (Jamroz et al., 2005; Rahimi et al., 2011). Also, butyrate addition was more effective than EOM in reducing total bacterial count. Kwon and Ricke (1998) showed that butyrate had the highest bactericidal efficacy among the SCFA against the acid-intolerant species such as *E. coli* and *Salmonella*. This effect is attributed to the ability of organic acids to diffuse into microorganisms and suppress their enzymes and nutrient transport (Huyghebaert et al., 2011; Van Immerseel et al., 2006). Conner (1993) suggested that essential oil suppression of microbes is related to the lipophilic character of the active principles of EO, which infuse into microorganisms and inhibit the membrane-bound electron flow and alter the energy metabolism. In support, (Marchese et al., 2017; Solórzano-Santos and Miranda-Novales, 2012) reported that EO contains various compounds which possess antimicrobial activity as phenols, ketones, hydrocarbons.

In the current study, EOM supplementation in the water (lower level) or feed non-significantly improved the PA and increased the weight of bursa and spleen. The present result suggests that EOM had an immune-stimulatory effect. Previous studies reported the immunomodulatory effect of essential oil in poultry including peppermint and eucalyptus essential oils, which enhance the immune response in chickens (Barbour and Danker, 2005). Peppermint helps to maintain the structural integrity of immune system cells and protects cell membranes against free radical-induced oxidation, thus enhancing the immune response (Arab Ameri et al., 2016; Awaad et al., 2016).

EOM supplementation non-significantly increased serum protein and albumin concentrations. Sodium butyrate addition combined with EOM improved the previous serum constituents. The obtained results are in line with (Al-Kassie, 2009); Toghyani et al. (2010) as serum total protein concentrations was significantly increased in birds supplemented with thyme powder which was attributed to the antioxidant properties of EOM components which consequently elevated the immune response of chicks. Blood serum AST concentration was reduced with both additives. The obtained data revealed that EOM without or with butyrate supplementation didn't affect the hepatic cell function which was supported by (Tollba et al., 2010), who found that the addition of aromatic herbal extract and organic acids to broiler diets did not change the AST and ALT enzyme activity. On the contrary, Ghazalah and Ali (2008) detected increased serum AST levels in broiler chicken fed 0.5% of dried rosemary leaf meal in the diet. This inconsistency reported in different studies may be associated with different essential oil and doses used.

Regarding the serum lipid profile, EOM supplementation reduced serum triglyceride and total cholesterol concentrations. On the other hand, EOM supplementation without or with SB decreased blood serum LDL and improved HDL concentrations. The higher level of EOM in drinking water was more effective in the improvement of blood serum HDL and reduction of LDL concentration compared with the lower level of EOM in water or diet. These findings align with Ghazalah and Faten Ibrahim (1996) and Osman et al. (2010). The decrease of total lipid and cholesterol may be attributed to the lowering effect of peppermint and eucalyptus on hepatic 3-hydroxy-3-methylglutaryl coenzyme A (HMG-COA), which is the key regulatory enzyme for cholesterol synthesis in the liver (Crowell, 1999) and consequently hypo-cholesterolemic effect (Lee et al., 2004b). The lack of sodium butyrate effect on blood serum triglycerides is in line with (Irani et al., 2011), who found no marked difference in serum TG in birds supplemented with butyric acid.

Conclusion

According to the obtained results, EOM supplementation through drinking water at 72 mg/L or feed (20 mg/Kg) improved growth performance, intestinal villi length, and increased *lactobacillus* counts. Moreover, the addition of sodium butyrate with the lower level of EOM in drinking water is considered more effective than supplementation alone.

Conflicts of interest

The authors declare that they have no conflicts of interest.

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