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Effect of Essential Oil and Organic Acid on Performance, Gut Health, Bacterial Count and Serological Parameters in Broiler

ABSTRACT

A total of 1500 day old broiler chicks were grouped into five treatments; each treatment group further consisted of six replicates. Group A was given a control diet having antibiotics. Group B and C were offered essential oil blend at the proportion of 0.1 g/kg and organic acid at the dose rate of 1g/kg. Group D was given both essential oil 0.1 g/kg and organic acid 1g/kg in combination. Group E was offered only a basal diet without antibiotics and considered as a negative control. The trial lasted for 35 days. Results indicated that the body weight gain and feed conversion ratio (FCR) exhibited significant improvement but insignificant in the case of feed consumption. Bodyweight and FCR were better in group B, followed by C. Carcass characteristics like eviscerated weight and giblet weight were also improved in group B while dressed weight showed insignificant results. Total cholesterol and high-density lipoproteins (HDL) were unaffected by the treatments; however, increased blood glucose levels and decreased low-density lipoproteins (LDL) concentration were significantly noticed. Treatment groups have no effect on antibody titer against ND at the end of the trial. In gut morphology, significantly higher villus height was observed in group C, but villus width and crypt depth remained unaffected. In conclusion, essential oil and organic acids have improved performance, carcass parameters, serum biochemistry, gut health, and decreased bacterial count.

INTRODUCTION

The poultry sector is a progressing and well-flourishing business in the world. The poultry industry's present status is the result of improvement in genetics through selection and advances in poultry nutrition, especially through feed formulation. Feed additives are an essential part of feed formulation to increase performance, growth, and production. Proper use of feed additives in feed can improve feed conversion and production (Khan & Iqbal 2016).

For decades, the poultry industry has been using antibiotic feed additives to boost the birds' growth and production. A non-therapeutic dose of antibiotics being used as growth promoters has side effects of developing anti-microbial resistance in the birds (Robinson *et al.*, 2019). This resistance is restricted to microbe and its progeny and transmitted to other irrelevant microbial species through the transfer of special genes. These resistant bacteria are then transferred to human beings and birds via the environment. Additionally, antibiotics residues in animal products seem to be a potential factor of drug resistance in humans. Hence anti-microbial resistance has become a global issue (Agyare *et al.*, 2018). Owing to this anti-microbial resistance, the European Union officially prohibited the utilization of antibiotics for



improving birds' growth (Abudabos *et al.*, 2017). So, nutritionists' quest becomes more intensified to find efficient alternates of antibiotics to prevent human beings from such harmful effects of antibiotics (Chand *et al.*, 2014).

Various natural substitutes of antibiotics growth promoters have been found efficacious with different mechanisms of action, including enzymes (exogenous or recombinant), prebiotics, clay minerals, probiotics, phytobiotic, nucleotides, symbiotic, antibodies of egg yolk, polyunsaturated fatty acids, and organic acids. All these additives help improve the production and gastrointestinal health of the birds (Sethiya 2016). Herbs and their additives are safe for animals, humans using animal products, and the environment. Herbs or herbal extract positively affect the broiler's performance, immune system, and hematological characteristics. Due to these positive effects and zero safety issues, herbs and their derivatives are good replacers of antibiotics (Hassan *et al.*, 2015). Essential oils can be defined as volatile oils derived from different plants and have anti-microbial, antiviral, antifungal properties, immunomodulatory action, hypolipidemic effect, digestive stimulation effect and have a property to alleviate the heat stress (Gopi *et al.*, 2014). These essential oils are recently being used in poultry feed because the digestion process is also improved and the increase in performance. This may occur due to the positive modulation of gastrointestinal microbiota of the birds (Wade *et al.*, 2018).

Essential oils from Capsicum and cinnamaldehyde causes certain modifications in the expression of genetic material. Capsicum oleoresin caused the changes in the genes which are involved in the metabolism and immunity of birds. Moreover, Cinnamaldehyde also changed genes' expression in inflammatory conditions, antigen presentation, and immune response of humoral origin (O'Bryan *et al.*, 2015). Furthermore, increased thigh muscle percentage and less abdominal fat are the meat characteristics that are also improved by feeding essential oils to broilers (Mehdi *et al.*, 2018).

Organic acids are another alternative that scientists have attracted because of their antibacterial effect against different pathogenic microorganisms. Moreover, it affects decreasing pH in the gastrointestinal tract, which leads to the improvement of the utilization of nutrients in the broiler birds (Kim *et al.*, 2015). Organic acids are safer feed additive, and the European Union has permitted to use of them in poultry feed (El-naggar & EL-Maaty 2017; Sultan *et al.*, 2015). Moreover, organic acids can increase the digestibility of

protein, increase the pancreas' secretion, improve gut morphology, and decrease gut microbes' (anti-microbial property) activity (Liu *et al.*, 2017). Phytogenic feed additives, as well as organic acids, are together given in the diet have the capability of enhancing nutrient digestion resulting in increased broiler performance. So, the utilization of both of these compounds can be a better substitute for antibiotic growth promoters (Yang *et al.*, 2018). The interlinked phenomena behind both of these compounds' synergism may be the positive modulation of gastrointestinal microflora. The hydrophobic nature of essential oils makes the bacterial cell membrane more permeable, resulting in an increased influx of organic acids into the cellular cytoplasm. Hence, the organic acids in their un-dissociated form make cellular pH more acidic, resulting in hampered bacteria's cellular metabolism (Stefanello *et al.*, 2020). The public concerns about the availability of antibiotic residues free meat has a significant role in the consumption of poultry products. Both essential oils and organic acids can provide this antibiotic residue-free meat and will positively contribute to the development of meat industry. Keeping in view all the above facts, this study was designed to evaluate the efficacy of essential oil or organic acids as effective substitutes of antibiotics by investigating their effects on performance, gut health, total bacterial count in intestine and biochemical effects.

MATERIALS AND METHODS

A total of 1500 broiler (Ross 308) chicks were obtained from a commercial hatchery (Jadeed Group), and they were divided into 5 groups randomly. Each treatment had 300 chicks. Each group was further consisted of 6 replicates having 50 birds in each. Group A was given a normal diet having antibiotics (Enramycine; Enradin 8% MSD; 0.3 grams/Kg). Group B was given a basal diet and a commercially available essential oils product named Activo™ (EW Nutrition Germany; Marketed by Ghazi brothers in Pakistan) at the dose rate of 0.1 grams/Kg of basal diet. Activo™ is a blend of essential oils containing oregano, rosemary, cinnamon, and chili pepper extract as active ingredients. Group C consisted of organic acid (Sodium diformate, Formi NDF; Addcon Germany; Marketed by Ghazi brothers in Pakistan) at the dose rate of 1g/Kg of the basal diet. Sodium diformate was composed of formic acid 40%, formate 40%, and sodium 20%. Group D was offered with the mixture of both essential oils and organic acid at the dose rate of 0.1 gram/Kg of "Activo™" essential oils and



1g/Kg of organic acid in the diet. Group E contained only a basal diet considering it as a negative control. The whole experimental layout is given in the table 1. The birds were kept in a controlled environment containing pan feeders for feed and nipple drinkers

for water availability. Pellet feed and fresh drinking water were given *ad libitum*. The shed's temperature was maintained at 32°C for the early five days then gradually declined according to standard management protocols up to 25°C. The humidity of the shed was

Table 1 – Experimental design of the study.

Diet group	Treatment	Dose rate	Number of birds	Replicates	Number of birds per replicate	
A	Basal diet (Formulated according to standards) with antibiotics (Positive control)	As recommended	300	6	50	Total groups: 5 Replicate/group: 6 Birds/replicate: 50 Total birds: 50×6×5 =1500
B	Activo™ Essential oil (EO) product in basal diet	100 grams/ton in basal diet or 0.1gram/kg	300	6	50	
C	Organic acid (OA) in basal diet	1 kg/ton or 1 gram/kg in basal diet	300	6	50	
D	Organic acid + essential oils in basal diet	100 g/ton of Activo™ and 1 kg/ton of organic acids in basal diet	300	6	50	
E	Only basal diet without antibiotics (Negative control)	As recommended	300	6	50	

A: Basal diet supplemented with antibiotics; B: EO 0.1gram/kg in basal diet; C: OA 1 gram/kg in basal diet; D: EO 0.1gram/kg in basal diet and OA 1 gram/kg in basal diet; E: Only basal diet (negative control).

kept (65±5%). The lighting period was almost 23 hours per day. The bedding material was rice husk (8cm thickness), and floor space was 5.5×3.5×2ft (L×W×H) according to the standard requirement of the chicks. Birds were already vaccinated for Marek's disease by the hatchery. According to standard procedures, the vaccine for Newcastle disease, infectious bronchitis, and infectious bursal disease was given. The whole flock was kept under serious observation until 35 days to complete. All other management procedures were performed according to the recommended protocols. Feed was offered in two phases, starter (0-22 days) and finisher (22-35 days) only. The ingredient and nutrient composition of the formulated diet is given in Table 2. The growth performance of the birds was measured weekly. Feed intake was measured by subtracting refused feed from the total offered feed on weekly basis. Weekly weight gain was measured by subtracting initial body weight from the final body weight. Moreover, the weekly feed conversion ratio was calculated as feed consumed in grams divided by body weight in grams. Carcass parameters such as dressed weight, eviscerated weight, and giblet weight were studied at the trial period's termination. Four birds from each replicate were picked randomly and slaughtered. After slaughtering and removing blood, feathers, and visceral organs, the carcass was weighed to check the dressed weight. Eviscerated weight and giblet weight were recorded. For gut morphology, tissue samples from jejunum were taken from three birds per

replicate. The Jejunum was cut about 3cm at its central region. Then these samples were preserved in the neutral buffer formalin (SJQW03140 Sigma-Aldrich, Merck; 10%) for 48 hours. After that, tissue samples were washed by using tap water and then treated with an alcohol solution (L850107 BDH). After sectioning of the tissues, samples were embedded in paraffin with the help of cassettes. Then sample tissue was cut down into 4-micrometer sections via microtome, mounted on the slide, and appropriately stained with HE (hematoxylin and eosin) stain (Medilines modified H 0706; E 920-921); after that, a light microscope was used for the examination of tissue sections and to determine the villus height and crypts depth. The measurement from the apex (tip of the upper border) of the villus till lamina propria was taken and recorded as villus height. Crypts depth was examined as the length between the crypts and villi according to the recommended protocol (Panda *et al.*, 2009). At the study trial's termination, the day after 35th, blood samples were taken during the birds slaughtering in non-heparinized tubes. Then centrifugation was done at 3000 RPM for 15 minutes for serum separation and kept in the freezer at -20°C until use. Then serum was thawed at room temperature. Serum sample was analyzed to determine total cholesterol, HDL and LDL by the method as described by Kamal & Ragaa (2014) and Vinus *et al.* (2017) and glucose concentration was determined by the method as described by Adil *et al.* (2010). Humoral immunity was estimated by



Table 2 – Ingredient composition of starter and finisher phase diets.

Ingredients (%)	Diet group A	Diet group B	Diet group C	Diet group D	Diet group E
Starter Phase Diet					
MCP*	0.3	0.3	0.3	0.3	0.3
Lysine HCL	0.31	0.31	0.31	0.31	0.31
DLM	0.263	0.263	0.263	0.263	0.263
Threonine	0.1	0.1	0.1	0.1	0.1
Salt	0.22	0.22	0.22	0.22	0.22
Soda	0.1	0.1	0.1	0.1	0.1
Betaine HCL	0.075	0.075	0.075	0.075	0.075
Phytase	0.01	0.01	0.01	0.01	0.01
Coxiril@**	0.01	0	0	0	0
Enramycin	0.03	0	0	0	0
Vitamin premix	0.055	0.055	0.055	0.055	0.055
Mineral premix	0.055	0.055	0.055	0.055	0.055
Trial product	0	0.01	0.1	0.11	0
Rice polish	0.272	0.302	0.212	0.202	0.312
Limestone	1	1	1	1	1
Maize	53.8	53.8	53.8	53.8	53.8
Soyabean Meal	28	28	28	28	28
Canola meal	4.4	4.4	4.4	4.4	4.4
Poultry by-product meal	3	3	3	3	3
Rice Polish	8	8	8	8	8
Finisher Phase Diet					
MCP	0.2	0.2	0.2	0.2	0.2
Lysine HCL	0.333	0.333	0.333	0.333	0.333
DL-Methionine	0.224	0.224	0.224	0.224	0.224
Threonine	0.09	0.09	0.09	0.09	0.09
Salt	0.22	0.22	0.22	0.22	0.22
Soda	0.1	0.1	0.1	0.1	0.1
Betaine HCL	0.05	0.05	0.05	0.05	0.05
Phytase	0.01	0.01	0.01	0.01	0.01
Coxiril	0.01	0	0	0	0
Enramycin	0.03	0	0	0	0
Vitamin Premix	0.055	0.055	0.055	0.055	0.055
Mineral Premix	0.055	0.055	0.055	0.055	0.055
Trial product	0	0.01	0.1	0.11	0
Rice polish	0.2	0.3	0.2	0.2	0.3
Limestone	0.8	0.8	0.8	0.8	0.8
Maize	63	63	63	63	63
Soybean Meal	25	25	25	25	25
Rapeseed meal	5	5	5	5	5
Poultry by-product meal	3	3	3	3	3
Corn gluten 60%	1.6	1.6	1.6	1.6	1.6
Nutrient Composition of experimental diets					
Ingredients	Starter		Finisher		
Moisture (%)	11.5		11.5		
CP (%)	23		21		
Ash (%)	5		4		
Crude Fat (%)	4		4.5		
Crude Fiber (%)	3		4		
Metabolizable Energy (Kcal/Kg)	2900		2950		

*MCP= Mono-calcium phosphate ** Coxiril@= Coccidiostat containing 0.5% Diclazuril as active ingredient; A: Basal diet supplemented with antibiotics; B: EO 0.1gram/kg in basal diet; C: OA 1 gram/kg in basal diet; D: EO 0.1gram/kg in basal diet and OA 1 gram/kg in basal diet; E: Only basal diet (negative control)



using antibody titer of IgG against the NDV vaccine by standard hemagglutination inhibition test. Briefly, birds were vaccinated for NDV on day 1st, followed by 7th and 17th day via drinking water. Samples of blood were taken through slaughtering on the day after the 35th to determine antibody titer. Three ml blood sample was taken in a non-heparinized vacutainer and placed on a cold chain. After thawing and centrifugation of the sample at 3000 RPM for 15 minutes at 23°C, serum was separated and kept at -20°C for storage and further procedure. The NDV antibody titer was determined through a commercial HI-based kit as described by (Aksu & Bozkurt, 2009). For bacterial count, three birds were chosen randomly from each replicate at the experimental period's termination, and slaughtering was performed. After slaughtering, part of the intestine from the duodenal distal end to the ileocaecal junction was severed and taken out. Digesta was taken out from the intestine of about one gram amount and diluted with 0.9%

sodium chloride solution. Dilution was made up to ten-fold, and a 1 ml diluted solution from each dilution was taken and inoculated on an agar plate with the help of the spread plate method and the count was done according to the standard procedure (Hartemink & Rombouts 1999; Hassan *et al.*, 2010); after that, the bacterial population was taken as log₁₀ CFU/gram. The data analysis was performed with the help of one-way ANOVA (Analysis of variance) using the SPSS version 23.0. The difference of means among the treatments was measured through Duncan's comparison test. The significance level was set at $p \leq 0.05$.

RESULTS

According to this study, non-significant results were noticed in case of feed intake in broilers. However, in 2nd-week feed intake manifested a remarkable difference among different groups (Table 3). Essential oils and organic acids increased the weight of broilers

Table 3 – Effect of essential oil and organic acid on mean daily feed intake (g) of broilers.

Group	1 st week	2 nd week	3 rd week	4 th week	5 th week
A	171.60±8.41	542.27 ^a ±3.20	1183.47±4.46	2158.93±6.19	3318.88±6.32
B	172.20±3.51	555.60 ^{ab} ±2.99	1193.67±4.28	2156.87±6.82	3315.88±7.04
C	169.67±0.55	559.27 ^b ±4.65	1195.13±8.04	2146.20±9.64	3298.40±8.75
D	168.13±2.05	540.33 ^a ±7.44	1190.07±10.85	2153.53±17.25	3285.85±14.34
E	178.20±7.02	560.07 ^b ±6.14	1190.20±8.37	2164.20±15.79	3308.28±14.63
P-Value	0.71	0.025	0.84	0.87	0.21

*Within a column, values with different superscripts differ statistically at $P < 0.05$ while the standard error of a particular group was written after \pm with mean value. A: Basal diet supplemented with antibiotics; B: EO 0.1gram/kg in basal diet; C: OA 1 gram/kg in basal diet; D: EO 0.1gram/kg in basal diet and OA 1 gram/kg in basal diet; E: Only basal diet (negative control).

significantly except at the 4th weeks of age. The maximum weight was measured in group B having essential oils followed by group C having organic acids. However, the organic acids treated group manifested

the highest weight in the first week, followed by an essential oil group. In the 4th week, no significant improvement was noticed, irrespective of the treatment group (Table 4).

Table 4 – Effect of essential oil and organic acid on weekly body weight gain (g) of broilers.

Group	Week 0	1 st Week	2 nd Week	3 rd Week	4 th Week	5 th Week
A	42.00±0.59	177.52 ^a ±1.94	446.79 ^a ±6.13	816.51 ^{ab} ±15.82	1407.75±21.29	2096.26 ^a ±27.51
B	42.16±0.43	191.00 ^{bc} ±3.18	475.57 ^c ±2.70	872.84 ^c ±9.83	1461.86±14.32	2205.77 ^b ±23.42
C	41.67±0.55	192.60 ^c ±2.62	472.90 ^c ±5.03	849.66 ^{bc} ±10.30	1405.07±17.79	2133.19 ^{ab} ±23.11
D	41.83±0.52	186.42 ^{bc} ±2.66	466.18 ^{bc} ±1.92	831.44 ^b ±13.60	1419.93±29.85	2136.05 ^{ab} ±40.62
E	42.50±0.43	182.92 ^{ab} ±2.90	457.83 ^{ab} ±4.62	791.32 ^a ±12.88	1400.46±21.63	2062.97 ^a ±23.89
P-Value	0.808	0.004	0.001	0.002	0.283	0.02

Within a column, values with different superscripts differ statistically at $P < 0.05$ while the standard error of a particular group was written after \pm with mean value. A: Basal diet supplemented with antibiotics; B: EO 0.1gram/kg in basal diet; C: OA 1 gram/kg in basal diet; D: EO 0.1gram/kg in basal diet and OA 1 gram/kg in basal diet; E: Only basal diet (negative control).

The feed conversion ratio showed significant improvement in essential oil and organic acid groups compared with the antibiotic group. The most significant improvement was found in essential oil group B while the 4th week had no statistically

significant results among all groups. After essential oil, the best improved FCR was noticed in group C (organic acids) at 2nd and 3rd week. (Table 5).

Our study results exhibited a marked ($p < 0.05$) increase in carcass characteristics, especially in



Table 5 – Effect of essential oil and organic acid on feed conversion ratio in broilers.

Groups	Week 1	Week 2	Week 3	Week 4	Week 5
A	0.97 ^b ±0.04	1.22 ^{bc} ±0.02	1.45 ^{bc} ±0.03	1.54±0.03	1.59 ^{bc} ±0.02
B	0.90 ^{ab} ±0.01	1.17 ^a ±0.01	1.37 ^a ±0.02	1.48±0.01	1.50 ^a ±0.02
C	0.88 ^a ±0.01	1.18 ^{ab} ±0.01	1.41 ^{ab} ±0.01	1.53±0.02	1.55 ^{abc} ±0.01
D	0.90 ^{ab} ±0.01	1.16 ^a ±0.01	1.43 ^{ab} ±0.02	1.52±0.02	1.54 ^{ab} ±0.02
E	0.97 ^b ±0.02	1.22 ^c ±0.01	1.51 ^c ±0.02	1.55±0.03	1.61 ^c ±0.02
P-Value	0.041	0.002	0.004	0.235	0.012

Within a column, values with different superscripts differ statistically at P<0.05 while the standard error of a particular group was written after ± with mean value. A: Basal diet supplemented with antibiotics; B: EO 0.1gram/kg in basal diet; C: OA 1 gram/kg in basal diet; D: EO 0.1gram/kg in basal diet and OA 1 gram/kg in basal diet; E: Only basal diet (negative control).

eviscerated weight and giblet weight. Group B containing essential oils, revealed the highest eviscerated weight and giblet weight than all other

groups, followed by group C (organic acids). In dressing percentage or dressed weight, no significant difference was found among the groups (Table 6).

Table 6 – Effect of essential oils and organic acid on carcass characteristics of broiler.

Groups	Live weight	Eviscerated weight	Dressed weight	Giblet weight*
A	1975.25±19.55	1337.38 ^a ±11.31	1265.75±19.00	108.67 ^a ±3.93
B	1959.38±11.88	1434.00 ^c ±9.10	1282.54±10.47	126.67 ^b ±4.26
C	1947.83±11.28	1383.92 ^b ±11.58	1263.71±11.55	118.13 ^{ab} ±3.07
D	1962.75±28.10	1416.13 ^{bc} ±14.58	1258.00±20.03	115.17 ^a ±2.65
E	1975.79±23.25	1323.58 ^a ±10.58	1306.71±20.39	109.25 ^a ±1.89
P-Value	0.846	0.000	0.247	0.001

*Giblet weight means the weight of three organs including liver, heart, and Gizzard. Within a column, values with different superscripts differ statistically at P<0.05 while the standard error of a particular group was written after ± with mean value. A: Basal diet supplemented with antibiotics; B: EO 0.1gram/kg in basal diet; C: OA 1 gram/kg in basal diet; D: EO 0.1gram/kg in basal diet and OA 1 gram/kg in basal diet; E: Only basal diet (negative control).

Our study revealed no remarkable change in total cholesterol and high-density lipoprotein levels when birds were offered essential oil and organic acids in the diet. Low-density lipoprotein was decreased by feeding essential oils and organic acids. Group B containing essential oil has the lowest LDL level as compared to other groups, followed by group C

and D (Table 9). Essential oil and organic acids raised the blood glucose level significantly compared to the control group. Glucose level was highest in the organic acid group C followed by group B having essential oil (Table 9). There was no significant effect on antibody titer noticed on the 35th days of age (Table 7).

Table 7 – Effect of essential oil and organic acid on serological parameters of broilers.

Groups	Cholesterol	LDL	HDL	Glucose	Antibody titer 35 th day
A	60.83±7.25	41.00 ^b ±2.37	53.67±3.37	215.00 ^a ±11.96	4.50±1.02
B	58.17±5.36	33.50 ^a ±1.61	50.83±3.36	244.83 ^b ±8.24	6.17±0.90
C	61.67±3.55	38.50 ^{ab} ±1.23	51.83±2.33	249.67 ^b ±3.78	4.83±0.48
D	48.83±4.56	38.83 ^{ab} ±2.06	44.33±3.58	233.33 ^{ab} ±11.67	5.33±0.95
E	46.17±4.04	41.67 ^b ±1.54	47.17±1.80	224.50 ^{ab} ±3.18	4.50±0.85
P-Value	0.133	0.031	0.204	0.051	0.624

Within a column, values with different superscripts differ statistically at P<0.05 while the standard error of a particular group was written after ± with mean value. A: Basal diet supplemented with antibiotics; B: EO 0.1gram/kg in basal diet; C: OA 1 gram/kg in basal diet; D: EO 0.1gram/kg in basal diet and OA 1 gram/kg in basal diet; E: Only basal diet (negative control).

A significant increase in jejunal villus height was observed by feeding essential oil and organic acids to the broilers. The maximum height was noticed in group C having organic acids followed by group B. In the case of villus width and crypts depth, and there was no remarkable difference among the groups (Table 8).

This study revealed that essential oil and organic acids significantly lower the total viable count in broilers' intestine. Our results are showing that the lowest number of aerobic bacteria was counted in group D having both essential oil and organic acid, followed by group C that contains organic acids in the diet (Table 9).



Table 8 – Effect of essential oil and organic acids on gut health of broilers.

Groups	Villi length	Villi width	Crypts depth
A	714.52 ^a ±1.50	85.69±4.23	85.31±14.98
B	864.30 ^{bc} ±30.13	94.35±1.46	112.20±12.67
C	917.20 ^c ±30.57	92.71±16.24	93.10±10.44
D	827.53 ^{bc} ±44.22	103.12±14.01	118.47±3.42
E	756.03 ^{ab} ±44.49	89.86±10.46	92.87±15.63
P-Value	0.011	0.835	0.321

Within a column, values with different superscripts differ statistically at $P < 0.05$ while the standard error of a particular group was written after \pm with mean value. A: Basal diet supplemented with antibiotics; B: EO 0.1gram/kg in basal diet; C: OA 1 gram/kg in basal diet; D: EO 0.1gram/kg in basal diet and OA 1 gram/kg in basal diet; E: Only basal diet (negative control).

Table 9 – Effect of essential oil and organic acids on total bacterial count in broilers.

Groups	TBC (log ₁₀ cfu/g)
A	8.07 ^c ±0.06
B	7.97 ^c ±0.01
C	7.85 ^b ±0.03
D	7.65 ^a ±0.03
E	8.48 ^d ±0.03
P-Value	0.000

Within a column, values with different superscripts differ statistically at $P < 0.05$ while the standard error of a particular group was written after \pm with mean value. A: Basal diet supplemented with antibiotics; B: EO 0.1gram/kg in basal diet; C: OA 1 gram/kg in basal diet; D: EO 0.1gram/kg in basal diet and OA 1 gram/kg in basal diet; E: Only basal diet (negative control).

DISCUSSION

According to this study, non-significant results were noticed in feed intake in broilers except for 2nd-week. Our study results follow earlier research, which revealed no noticeable influence on weekly feed consumption among organic acid, essential oils, and antibiotics supplemented group (Bozkurt *et al.*, 2012; Cabuk *et al.*, 2014; Mohammadi Gheisar *et al.*, 2015; Basmacioğlu-Malayoğlu *et al.*, 2016). Essential oils and organic acids increased the weight of broilers significantly except for 4th week than that of the antibiotic group. Several previous studies favor our results, showing the increased body weight by feeding essential oils and organic acid groups rather than a control group containing antibiotics (Basmacioğlu-Malayoğlu *et al.*, 2016; Fathi *et al.*, 2016; Peng *et al.*, 2016; Elnaggar & EL-Maaty 2017). The feed conversion ratio manifested the significant improvement in essential oil and organic acid groups compared with the antibiotics group. Our results also match with previous studies which show that essential oils, as well as organic acids, have improved FCR in comparison with the control group (Fascina *et al.*, 2012; Basmacioğlu-Malayoğlu *et al.*, 2016), while some studies are against us showing no improvement in growth performance with essential oils as well as organic acids (Belenli *et al.*, 2015; Pathak *et al.*, 2017).

Stimulation of digestion, modulation of gut flora, and increased release of different endogenous digestive enzymes due to essential oils are the possible reasons for improved performance (Popović *et al.*, 2016). Along with the antibacterial effect, herbal extract's antioxidant property may also be the possible reason for enhanced broiler's performance (El-Shenway & Ali, 2016). In the case of growth performance, essential oils manifested controversy in several previous studies on broilers. Some studies are in favor, while others are against us. It is difficult enough to resolve these controversies and to generate biologically consistent results due to a lot of variations in inclusion levels, active ingredient concentrations, types of essential oil used, surrounding environment conditions, basal feed composition, infectious diseases outbreak, and origin of that herbal extract (Bozkurt *et al.*, 2012; Basmacioğlu-Malayoğlu *et al.*, 2016). Additionally, enhanced broilers' weight and improved FCR may be due to the beneficial impact of organic acids on gut microflora and the bactericidal effect of organic acids because organic acids cause the interference of bacterial cell membrane and macromolecules of cells leading to hindrance in energy metabolism and nutrients transport (Elnaggar & EL-Maaty, 2017).

Our study results exhibited a marked ($p < 0.05$) increase in carcass characteristics, especially in eviscerated weight and giblet weight. This is also favored by Rehman *et al.* (2016), who found insignificant results in dressing percentage. Previous studies are also in favor of us, showing that essential oils and organic acids exert better overall carcass traits (Khattak *et al.*, 2013; Peng *et al.*, 2016; Ragaa *et al.*, 2016). While some researches are against our findings, no significant effect on carcass parameters was noticed (El-Shenway & Ali, 2016; Özsoy *et al.*, 2017; Gomathi *et al.*, 2018). This conflict of results may be due to variation in diet composition and breeds of broilers (Peng *et al.*, 2016). The improvement in carcass characteristics is due to the existence of antioxidants and phenolic compounds in essential oil, decreasing



the harmful microbial population in the digestive tract of birds and increasing the absorbed amino acid level. Moreover, an active ingredient of essential oil like carvacrol has triggering action on secretions of the pancreas. This high digestive secretion can improve digestion and absorption of certain nutrients (amino acids), leading to improved carcass characteristics (Ragaa *et al.*, 2016). Better digestion and slower transit rate through the intestine due to organic acid feeding make certain improvements in the absorption of specific nutrients leading to better carcass parameters. Moreover, better proteolysis and digestion of proteins and amino acids cause better musculature. This may be the other reason for improved carcass parameters by organic acids (Hossain & Nargis, 2016).

Our study revealed no remarkable change in total cholesterol and high-density lipoprotein levels due to feeding essential oil and organic acids in the diet. Low-density lipoprotein was decreased by feeding essential oils and organic acids. Our results are according to previous research in which essential oil and organic acids decreased the serum LDL concentration. At the same time, HDL remained unchanged (Elnaggar & El-Tahawy, 2018; Yildirim *et al.*, 2018), and the total cholesterol level also remained unchanged (Popović *et al.*, 2016; Vinus *et al.*, 2017). The reduction in low-density cholesterol is the inhibition of specific enzymes like peroxidase and dehydrogenase by herbal extracts (essential oils). Moreover, active ingredients of these essential oils like carvacrol and thymol have a limiting effect on lethal metabolites, resulting in increased blood cholesterol levels (Hedayati & Manafi, 2018).

Essential oil and organic acids raised the blood glucose level significantly compared to the control group. Our findings match several past studies showing that essential oil and organic acid increased glucose levels compared with the control group (Rahman *et al.*, 2015; Elnaggar & EL-Maaty, 2017; Elnaggar & El-Tahawy, 2018). However, some studies are against our findings showing no noticeable influence on broilers' glucose level (Belenli *et al.*, 2015). There was no significant effect on antibody titer noticed at 35th days of age (Table 9). Our results match previous studies with no significant influence of dietary essential oils and organic acids separately or in combination with antibody titer at the end of the trial (Fascina *et al.*, 2017).

A significant increase in jejunal villus height was observed by feeding essential oil and organic acids to the broilers. Several previous studies' results are similar to our results showing improvement in villus height, i.e. (Liu *et al.*, 2017; Sukandhiya *et al.*, 2017). If villus length and crypt depth are shorter, the bird has

a lower nutrient absorption rate due to less surface area available for absorption, and enterocytes are less mature. Thus less nutrient absorption leads to the poor performance of the bird (Paiva *et al.*, 2014). Moreover, essential oils and organic acids cause regulation and balancing effect on gastrointestinal microflora. This effect may be beneficial in improving gut morphology (Zeng *et al.*, 2015). Furthermore, improved intestinal morphology may be due to the anti-inflammatory and anti-oxidation mechanism of essential oils and organic acids (Du *et al.*, 2016; Gao *et al.*, 2019).

This study revealed that essential oil and organic acids have a significant lowering effect on the total viable count in broilers' intestine. Our results are showing that the lowest number of aerobic bacteria was counted in the group "D," which contains both essential oil and organic acid, followed by group "C" that contains organic acids in the basal diet. Our results are matching with previous researches, which exhibited that essential oil and organic acids have a significant decreasing effect on bacterial gut count (Basmacioğlu-Malayoğlu *et al.*, 2016; Ndelekwute *et al.*, 2018) and Chowdhury *et al.* (2018) also observed that pathogenic bacterial count like *Escherichia coli* was reduced by feeding cinnamon essential oil, but beneficial bacterial species like *Lactobacillus spp.* was unaffected. However, Pathak *et al.* (2016) observed no noticeable effect on a total viable bacterial count by feeding cinnamaldehyde and formic acid to the broilers. The lipophilic nature of organic acids in their un-dissociated form causes them to enter the bacterial cell through passive diffusion. This intracellular acid affects cells' normal physiology because the bacterial cell cannot bear acidic pH, causing the stoppage of microbial enzymes and transport systems, leading to inhibition of bacterial growth (Ragaa & Korany, 2016). This may be the reason behind the antibacterial activity of organic acids.

CONCLUSION

Hence, we concluded that essential oil and organic acids have improved performance, carcass parameters, serum biochemistry, gut health, and decreased bacterial count. The consequences of group B (Essential oil supplemented) were the best among all treatment groups. According to our research, almost no synergism between essential oil and organic acids was noticed except for the gut microbial count parameter. So essential oil or organic acids can be a better substitute for antibiotics separately rather than in combination.



CONFLICT OF INTERESTS STATEMENT

The authors declare that there is no conflict of interest regarding this article's publication.

FINANCIAL DISCLOSURE STATEMENT

There was financial support for this research.

ANIMAL RIGHTS STATEMENT

The experiments on animals were conducted following the local Ethical Committee laws and regulations regarding the care and use of experimental animals.

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