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RESEARCH ARTICLE

Effect of Supplementing a Blend of Essential Oils on the Growth Performance, Carcass Characteristics, Meat Quality, Serological Parameters and Gut Health in Broiler Chickens

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A B S T R A C T

For an extended period, the poultry industry has employed antibiotics as feed additives to enhance the growth of birds. Nevertheless, the utilization of antibiotics raises significant concerns regarding the development of antimicrobial resistance. Consequently, it is essential to identify effective and safe alternatives for feed additives, such as essential oils. The study was meticulously designed to assess the effects of essential oil blends as feed additives in broilers. A total of 600 day-old broiler chicks (n=600) were randomly assigned to five treatment groups, each comprising three replicates with 40 birds per replicate. Treatments were the following: Group A (basal diet + antibiotics) positive control, Group B (basal diet only) negative control, Groups C, D, and E consisted of essential oil blend at the dose rate of 0.12mL/kg, 0.25mL/kg, and 0.50mL/kg of feed, respectively. The duration of the trial was 35 days. The findings revealed significant improvement in body weight and feed conversion ratio (FCR), while feed consumption showed no notable changes. Body weight gain and FCR were significantly better in group E. There was no significant improvement in carcass characteristics like eviscerated, dressed, and giblet weights. Meat pH, meat color, and antibody titer against ND were affected significantly ($P<0.05$) by treatment group E. Villus length was significantly higher in group D, while crypt depth and villus width remained unaltered. Total bacteria count was noticed to be lower in treatment groups than in control groups. In conclusion, essential oil supplementation has enhanced performance, gut health, meat quality, immunity and decreased total bacterial count.

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INTRODUCTION

The poultry sector is well developed yet progressing day by day and achieving new heights in genetic selection, management, feed, and production. According to the Economic Survey of Pakistan (PES, 2024), the poultry sector is the 2nd largest meat producer after beef production, contributing 40.7% of total meat production and contributing

1.3% to the GDP of Pakistan. It is amenable to employing almost 1.5 million people living in Pakistan. Balanced formulation with the addition of effective feed additives is responsible for better growth and performance (Pirgozliev *et al.,* 2019a, b). Feed additives are non-nutritious substances but are essential for growth and production. They have been excessively used in Pakistani poultry farming, and antibiotics have been at the top of the list for many years. Antibiotics are supplemented in poultry diets as growth promoters with the mechanism of thinning the intestinal lining, ultimately enhancing nutrient absorption. Antibiotic growth promoters (AGPs) are usually used in lower doses than therapeutic doses to improve the growth and performance of broiler birds. Nevertheless, unluckily, it poses very harmful issues of antimicrobial resistance in poultry, livestock, and human beings (Ishaq *et al.,* 2022; Liu *et al*., 2023). The harmful bacteria develop resistance against antibiotics by interfering with the mechanism, either by modifying the cell wall structure or producing the enzymes that can inactivate antibiotics (Ali and Alsayeqh, 2022). Antimicrobial resistance has now become a universal health issue, leading to the ban of antibiotic growth promoters in European countries (Casewell *et al.,* 2003). Consequently, it is essential to identify effective alternatives to antibiotics that can replace them and offer solutions to the growing concerns surrounding antimicrobial resistance (Heydarian *et al.,* 2020; Ahmed *et al.,* 2022). Many effective alternatives to AGPs have been available with different modes of actions, including prebiotics, probiotics, organic acids, enzymes, phytogenic feed additives, essential oils and have proved their success (Lee *et al.,* 2020; Su *et al.,* 2020). The most effective alternative is the one that improves growth parallel to antibiotics and has fewer side effects (Upadhayay and Vishwa, 2014). Natural herbal products can serve as a safe alternative to antibiotics and may enhance growth performance (Rahman *et al.,* 2015; Asghar *et al.,* 2023; Yousaf *et al.,* 2024).

Essential oils represent a potentially advantageous alternative to antibiotics, contributing to improved growth performance and addressing the issue of antimicrobial resistance (Iqbal *et al.,* 2021; Asghar *et al.,* 2023). Plant based products including essential oils have several beneficial roles, which include antibacterial properties, antiparasitic properties, antioxidant capacity, digestion stimulant, immunity booster, bronchodilator and property to mitigate heat stress (Emami e*t al.,* 2012; Idris *et al*., 2017; Abbas and Alkheraije; 2023; Al-Hoshani *et al*., 2023; Aljohani, 2023) and may have a significant role against diseases (Al Syaad *et al*., 2023; Saeed *et al*., 2023). It has been reported that essential oils prevent the proliferation of *Clostridium perfringens* and play a defensive role against necrotic enteritis (Timbermont *et al.,* 2010). They also prevent respiratory diseases of poultry (for example, avian influenza) by soothing the cilium of the respiratory tract and are responsible for raising the antibody titer against the most prevalent disease of poultry, i.e., Newcastle disease (Barbour *et al.,* 2010). A different study noted a rise of about 4.5% in broiler weight when they were given a blend of essential oils (Tiihonen *et al.,* 2010), illustrating effectiveness in promoting growth and enhancing immunity in broiler birds (Abd El-Latif *et al.,* 2013). Furthermore, essential oils have a positive impact on gut microbiota by reducing the load of harmful pathogens and promoting beneficial bacterial counts (Yang *et al.,* 2018; Iqbal *et al.,* 2021). Conclusively, essential oils provide a natural source for growth and better performance and can yield antibiotic-free, safe and healthy poultry products (Symeon *et al.,* 2009; Ponnampalam *et al.,* 2022). Research on the synergistic effects of essential oil blends, their dosage levels, and combinations as natural alternatives to antibiotics in broiler production is notably scarce. Thus, this study aims to investigate the impact of a potent blend of essential oils (including eucalyptus oil, thymol, camphor, citrus oil, and bromhexine) as an antibiotic alternative on growth performance, carcass traits, meat quality, serological outcomes, gut morphology, and bacterial count in broilers.

MATERIALS AND METHODS

The research was carried out according to the university's rules and regulations after receiving ethical approval from the institutional guidelines of the ethical review committee. The trial was conducted for 5 weeks at the Research Center of the College of Veterinary and Animal Sciences, Jhang (Sub-campus UVAS, Lahore).

Experiment design, diet formulation, and bird management: A total of 600 one-day-old broiler chicks were acquired and allocated into five treatment groups, each containing 120 chicks, further split into three replicates. The replicates were assigned randomly, with each holding 40 chicks. Group A, the Positive Control, received a basal diet supplemented with antibiotics, while Group B, the Negative Control, was given a basal diet without antibiotics. Groups C, D, and E were supplemented with an antibiotics-free basal diet containing an essential oil blend (bromhexine hydrochloride, eucalyptus oil, citrus oil, thymol, and Camphor). The essential oil blend was sprayed on feed at the dose rate of 0.12mL/kg (C), 0.25mL/kg (D), and 0.50mL/kg (E), respectively. The experimental diets for the starter (0-14 days) and finisher phases (15-35 days) are shown in Table 1 and 2, respectively. The chemical composition of diets is provided in Table 3. The essential oil blend composition is shown in Table 4. The experimental poultry shed was ready (cleaned, disinfected, and fumigated) and functional 24 hours before the arrival of chicks. On arrival of chicks, managemental conditions were fulfilled according to standards (pre-brooding management; temperature 35°C, humidity 65±5 and light 24 hours). Rice husk, six inches thick layer, served as bedding for the chicks in the shed. Each pen was supplied with three nipple drinkers and two tube feeders. Marek's vaccine was done at the hatchery, followed by the vaccination against Newcastle disease (ND) on day one via drinking water. Moreover, second and third doses of ND vaccines were given on days 7 and 15, respectively. Vaccination for Gumboro (infectious bursal disease) occurred on the 11th day. The temperature gradually decreased by 2-3°C each week until stabilizing at 24°C for 35 days. Water and feed were provided *ad libitum*.

Growth performance: Data regarding body weight, feed intake, and feed conversion ratio (FCR) were recorded on a weekly basis. FCR was calculated using cumulative feed intake and body weight according to Waqas *et al.* (2018), as follows;

$$FCR = \text{feed consumed (g)/ gained body weight (g)}$$

All these measurements were taken under careful observation.

Table 1: Feed formulation of starter diet (percentages).

Ingredients (%)	Diet group A	Diet group B	Diet group C	Diet group D	Diet group E
Betaine HCL	0.078	0.078	0.078	0.078	0.078
Lysine HCL	0.32	0.32	0.32	0.32	0.32
DLM*	0.265	0.265	0.265	0.265	0.265
Soda	0.15	0.15	0.15	0.15	0.15
MCP**	0.4	0.4	0.4	0.4	0.4
Salt	0.24	0.24	0.24	0.24	0.24
Threonine	0.12	0.12	0.12	0.12	0.12
Phytase	0.02	0.02	0.02	0.02	0.02
Coxiril®***	0.02	0	0	0	0
Enramycin	0.04	0	0	0	0
Vitamin premix	0.058	0.058	0.058	0.058	0.058
Mineral premix	0.058	0.058	0.058	0.058	0.058
Rice polish	0.274	0.334	0.334	0.334	0.334
Limestone	1.1	1.1	1.1	1.1	1.1
Total	3.1	3.1	3.1	3.1	3.1
Raw material					
Maize	52.2	52.2	52.2	52.2	52.2
Soyabean Meal	29	29	29	29	29
Canola meal	3	3	3	3	3
Poultry-by-product meal	2.7	2.7	2.7	2.7	2.7
Rice Polish	10	10	10	10	10

*DL-Methionine; **MCP= Mono calcium phosphate; ***Coxiril®= Coccidiostat containing 0.5% Diclazuril as an active ingredient. Vitamins and minerals premix: Vitamin A (all-trans-retinyl acetate): 10,000 IU, Biotin: 0.04 mg, Calcium pantothenate: 10 mg, Choline chloride: 400 mg, Folic acid: 1 mg, Menadione: 1.3 mg, Nicotinamide: 40 mg, Pyridoxine HCl: 4 mg, Riboflavin: 8 mg, Thiamin: 2.2 mg, Vitamin B12 (cobalamin): 0.013 mg, Vitamin D3 (cholecalciferol): 3,000 IU, and Vitamin E (all-rac-α-tocopherol): 30 IU, Cu (from copper sulfate): 8.0 mg, Fe (from ferrous sulfate): 80 mg, I (from calcium iodate): 1.1 mg, Mn (from manganese sulfate): 110 mg, Se (from sodium selenite): 0.3 mg, and Zn (from zinc oxide): 60 mg

Table 2: Feed formulation of finisher diet (percentages)

Ingredients (%)	Diet group A	Diet group B	Diet group C	Diet group D	Diet group E
Betaine HCL	0.06	0.06	0.06	0.06	0.06
Lysine HCL	0.335	0.335	0.335	0.335	0.335
DL-Methionine*	0.228	0.228	0.228	0.228	0.228
Soda	0.15	0.15	0.15	0.15	0.15
MCP**	0.35	0.35	0.35	0.35	0.35
Salt	0.24	0.24	0.24	0.24	0.24
Threonine	0.07	0.07	0.07	0.07	0.07
Phytase	0.02	0.02	0.02	0.02	0.02
Coxiril***	0.01	0	0	0	0
Enramycin	0.03	0	0	0	0
Vitamin Premix	0.058	0.058	0.058	0.058	0.058
Mineral Premix	0.058	0.058	0.058	0.058	0.058
Rice polish	0.20	0.24	0.24	0.24	0.24
Limestone	0.9	0.9	0.9	0.9	0.9
Total	2.7	2.7	2.7	2.7	2.7
Raw material					
Maize	67	67	67	67	67
Soybean Meal	22	22	22	22	22
Rapeseed meal	2.9	2.9	2.9	2.9	2.9
Poultry-by-product meal	3.7	3.7	3.7	3.7	3.7
Corn gluten 60%	1.7	1.7	1.7	1.7	1.7

*DL-Methionine; **MCP= Mono calcium phosphate; ***Coxiril®= Coccidiostat containing 0.5% Diclazuril as an active ingredient. Vitamins and minerals premix: Vitamin A (all-trans-retinyl acetate): 10,000IU, Biotin: 0.04mg, Calcium pantothenate: 10mg, Choline chloride: 400mg, Folic acid: 1mg, Menadione: 1.3mg, Nicotinamide: 40mg, Pyridoxine HCl: 4mg, Riboflavin: 8mg, Thiamin: 2.2mg, Vitamin B12 (cobalamin): 0.013mg, Vitamin D3 (cholecalciferol): 3,000IU, and Vitamin E (all-rac-α-tocopherol): 30IU, Cu (from copper sulfate): 8.0mg, Fe (from ferrous sulfate): 80mg, I (from calcium iodate): 1.1mg, Mn (from manganese sulfate): 110mg, Se (from sodium selenite): 0.3mg, and Zn (from zinc oxide): 60mg.

Table 3: Chemical analysis of basal diets.

Ingredients	Starter	Finisher
Metabolizable Energy (kcal/kg)	2950	3150
Moisture (%)	9	9.5
Crude Protein (%)	22	20
Crude Fiber (%)	2.5	3
Crude Fat (%)	5	4.5
Ash (%)	4	3

Carcass characteristics: At the end of the trial, four birds were picked randomly and slaughtered using the Halal method (Farouk *et al.*, 2014), following the guidelines of the ethics committee of the University of Veterinary and Animal Sciences, Lahore, Pakistan. Following the slaughtering process, the weight of the eviscerated bird was determined after bleeding, removal of feathers, and internal organs, except the giblet, head, and feet. The dressed weight of the carcass was determined by weighing it after bleeding, removal of feathers, internal organs, head and feet.

Table 4: Composition of essential oils used in the experimental diet.

Ingredient	Quantity (mg/mL)
Bromhexine HCL	20
Eucalyptus Oil	10
Citrus Oil	10
Thymol	10
Camphor	10

Meat quality: At the end of the experimental trial, the meat quality parameters were studied. The meat was finely ground, and water was added at a 1:9 ratio. The meat underwent homogenization, and its pH level was assessed with a pH meter electrode. A chroma meter was used to evaluate the color of the meat.

Serological/Immunity parameters: The level of humoral immunity was assessed by measuring the antibody titer against the Newcastle disease virus (NDV) vaccination. On days 1, 7, and 17, the birds received the NDV vaccine through their drinking water. Blood samples were taken after the birds were slaughtered on day 35 to analyze the antibody titer. A non-heparinized vacutainer was used to collect three milliliters of the blood sample, and the cold chain was carefully maintained. The blood sample was liquefied and centrifuged at 4000 RPM for 15 minutes at 4°C, after which the serum was separated and stored at -20°C for later use and storage. To evaluate the antibody titer against the NDV, a commercially available hemagglutination inhibition (HI) test, as delineated by Aksu and Bozkurt (2009), was employed.

Gut morphology: To determine gut morphology, tissue samples were collected from the jejunum, about 3 centimeters from its midpoint. These samples were preserved for 48 hours in a 10% neutral buffered formalin solution. Following preservation, the samples were rinsed under running water and treated with an alcohol solution. After slicing, the tissue samples were embedded in paraffin. The sample slices were then cut to a thickness of 4 micrometers with a microtome. Finally, the sections were mounted on slides and stained using HE (hematoxylin and eosin) stain. A light microscope was used to examine the slides and to measure the villus height (from the top of the villus to the opening of the crypt) and the crypt depth from the base of the crypt to the opening of the crypt. The morphology of the gut was examined in accordance with the methodology established by Panda (2009).

Count of gut microbiota: One gram of ingested material was taken from the gut and diluted with a standard saline solution. After a ten-fold dilution, one milliliter from each dilution was utilized to inoculate an agar plate employing the spread plate technique. Nutrient agar served as the medium for the cultivation of bacterial colonies. Total colony-forming units of various bacterial species, including *Salmonella*, *Bifidobacterium*, *Escherichia coli*, *Lactobacillus* species, *Enterobacter*, and others, were quantified to estimate the total intestinal bacteria according to the method proposed by Hartemink and Rombouts (1999). Subsequently, the bacterial population was documented as $\log_{10}$ colony-forming units (CFU) per gram.

Statistical analysis: Data analysis was conducted utilizing one-way analysis of variance (ANOVA) through the software SPSS 23.0. The probability value (P<0.05) indicates that the results are statistically significant. Duncan's comparison test was employed to evaluate the differences in the means of the treatments.

RESULTS

Growth performance

Feed intake: Feed intake has been presented in Table 5. The study found no significant difference (P>0.05) regarding feed intake, and there was no notable difference among the treatment groups. However, there was a significant difference in feed intake between the groups during the first week; significantly (P<0.05) higher feed intake was observed in group E compared to other groups.

Body weight: The body weight is shown in Table 6. The birds fed with different levels of blends of essential oils showed significantly (P<0.05) improved body weight during the week one and five. During weeks one and five, group E showed significantly higher body compared to the rest of the treatment groups, while during the second, third, and fourth weeks, no significant (P>0.05) body weight differences were perceived among the treatment groups.

Feed conversion ratio: The feed conversion ratio has been described in Table 7. It can be observed that in weeks three and five, the FCR of group E is significantly improved (P<0.05) compared to the rest of the treatment groups. No significant difference was observed between the groups in the first, second, and fourth weeks.

Carcass characteristics: The carcass traits have been exhibited in Table 8. The results exhibit that there is no significant (P>0.05) difference among the groups regarding eviscerated weight, dressed weight, and giblet weight. However, numerically, higher eviscerated weight and dressed weight were observed in group C compared to other treatment groups, while higher giblet weight was observed in group E.

Table 5: Effect of essential oil blend on feed intake of broilers.

Group	1st week	2nd week	3rd week	4th week	5th week
A	160.40±3.81^{b}	538.90±4.57	1180.40±6.96	2152.00±8.23	3324.08±6.40
B	167.70±2.84^{ab}	546.40±8.51	1200.00±9.71	2174.30±14.21	3297.40±16.29
C	168.30±1.35^{ab}	553.30±2.74	1185.10±11.08	2147.70±17.81	3302.13±22.71
D	167.00±0.38^{ab}	556.60±6.24	1196.40±9.91	2148.50±7.98	3295.50±13.28
E	174.80±2.79^{a}	557.20±2.94	1188.50±4.64	2152.80±10.44	3313.00±7.93
P-Value	0.020	0.141	0.520	0.548	0.619

^{a,b} Within a column, values with different superscripts differ statistically at P<0.05 with mean ± SE.

Table 6: Effect of essential oil blend on weekly body weight of broilers.

Group	1st week	2nd week	3rd week	4th week	5th week
A	176.20±3.02^{c}	453.00±6.22	839.35±3.94	1437.80±10.78	2135.38±12.64^{ab}
B	184.18±2.92^{bc}	454.25±10.31	844.95±11.84	1456.43±28.53	2174.63±32.52^{ab}
C	191.73±3.27^{ab}	467.68±7.15	843.28±10.27	1440.85±26.96	2168.65±43.28^{ab}
D	190.88±4.02^{ab}	469.78±7.63	842.40±13.30	1397.63±27.07	2090.50±18.39^{b}
E	194.70±1.47^{a}	474.35±3.94	876.83±8.26	1464.08±21.51	2203.13±36.00^{a}
P-Value	0.005	0.199	0.099	0.367	0.015

$^{a-c}$ Within a column, values with different superscripts differ statistically at P<0.05 with mean ± SE.

Table 7: Effect of essential oils blend on weekly FCR of broilers.

Group	1st week	2nd week	3rd week	4th week	5th week
A	0.91±0.01	1.19±0.01	1.41±0.01a	1.50±0.01	1.56±0.01ab
B	0.91±0.01	1.20±0.02	1.42±0.02a	1.49±0.03	1.52±0.02b
C	0.88±0.01	1.18±0.02	1.41±0.02a	1.49±0.02	1.52±0.02ab
D	0.88±0.02	1.19±0.01	1.42±0.02a	1.54±0.03	1.58±0.01a
E	0.90±0.02	1.18±0.01	1.36±0.01b	1.47±0.02	1.51±0.03b
P-Value	0.258	0.657	0.044	0.390	0.043

a-b Within a column, values with different superscripts differ statistically at P<0.05 with mean ± SE; FCR: feed conversion ratio

Table 8: Effect of essential oil blend on carcass characteristics of broiler.

Groups	Live weight	Eviscerated weight	Dressed weight	Giblet weight
A	2135.38±12.64	1294.75±57.65	1190.25±54.90	104.50±5.58
B	2174.63±32.52	1326.50±59.24	1214.75±52.54	111.75±8.52
C	2168.65±43.28	1499.25±49.04	1375.50±50.05	123.75±3.71
D	2090.50±18.39	1404.00±38.84	1288.00±41.92	116.00±7.22
E	2203.13±36.00	1379.25±13.75	1245.25±17.19	134.00±14.43
P-Value	0.152	0.061	0.081	0.202

Values within the column have no superscripts as these do not differ statistically at P>0.05 with mean ± SE.

Meat Quality: Table 9 presents the meat quality parameters. The supplementation of a blend of essential oils positively influenced the meat quality. Significantly (P<0.05) lower pH is observed in group E compared to other treatment groups, while significantly (P<0.05) higher color is observed in groups A, B, and D, while the lowest color is observed in group E.

Serological Parameters: Fig. 1 presents a line graph illustrating the effects of various concentrations of essential oils on antibody titer. The concentrations of essential oils (labeled A to E) are represented on the X-axis, while antibody titer is displayed on the Y-axis. A P-value of less than 0.05 indicates that there is a statistically significant difference among the various concentrations of essential oils with respect to their effects on antibody titer. The solid line reflects a modest increase in antibody titer from A to E, and the error bars indicate considerable variability, suggesting that the observed changes are significantly distinct from one another. A statistically significant increase (P<0.05) in antibody titer is noted in group E, while the lowest titer is recorded in group B.

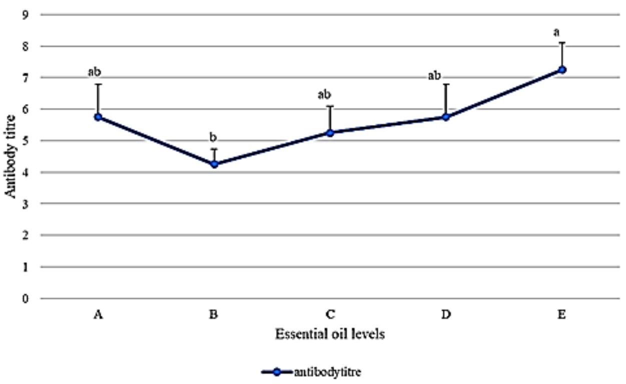

Fig. 1: Effect of an essential oil blend on antibody titer with P<0.05.

Table 9: Effect of essential oil blend on meat pH and meat color of broilers

Groups	Meat Ph	Meat color
A	6.24±0.04b	45.61±0.34a
B	6.56±0.08a	48.38±0.59a
C	6.00±0.10ab	42.32±1.6ab
D	5.89±0.10cd	47.73±3.87a
E	5.73±0.08d	39.06±0.6b
P-Value	<0.001	0.019

a-d Within a column, values with different superscripts differ statistically at P<0.05 with mean ± SE.

Gut morphology: The morphometric parameters of the gut are presented in Table 10. A statistically significant (P<0.05) enhancement in the villus height of the jejunum was observed in broilers subjected to a diet supplemented with essential oils. The greatest height was recorded in Groups D and E, followed by the other groups. Conversely, no significant differences were detected regarding villus width and crypt depth among the treatment groups.

Table 10: Effect of essential oils blend on gut morphology of broilers.

Groups	Villus length	Villus width	Crypts depth
A	714.8±1.66b	86.19±3.35	88.90±5.94
B	791.81±31.21b	87.19±5.81	92.30±6.00
C	767.62±33.89b	92.17±5.22	98.38±12.83
D	912.48±21.94a	93.19±11.58	96.69±8.69
E	869.90±22.3a	95.40±2.30	107.89±9.55
P-Value	<0.001	0.825	0.638

a-b Within a column, values with different superscripts differ statistically at P<0.05 with mean ± SE.

Count of Gut Microbiota: The bar chart in Fig. 2 illustrates the significant impact of different essential oil levels (A to E) on broiler gut microbiota count (P<0.021). levels of essential oil on the X-axis and TBC on the y-axis have been presented. Treatment B (negative control) shows a significantly (P<0.05) higher bacterial count (~8.4 log10 CFU/g) compared to all other treatment groups. Overall, numerically lower bacterial count was observed in essential oil groups than in positive control (group A) and negative control (group B).

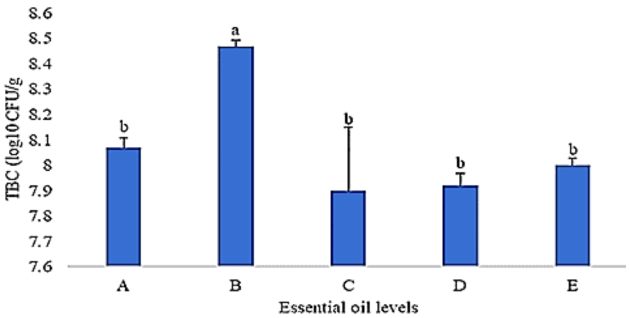

Fig. 2: Effect of an essential oil blend on total gut microbiota count of broilers with P<0.021; TBC: total bacterial count; CFU: colony farming unit.

DISCUSSION

This research found that supplementation of essential oils did not impact the feed consumption of broilers. However, feed consumption showed a striking difference across groups in the first week. Our study's findings are consistent with earlier studies that found no appreciable effect on weekly feed intake between groups supplemented with essential oils and antibiotics (Bozkurt *et al.,* 2012; Cabuk *et al.,* 2014; Mohammadi Gheisar *et al.,* 2015; Basmacioğlu-Malayoğlu *et al.,* 2016).

The birds fed with different levels of blends of essential oils showed improved body weight significantly during the first and fifth weeks. Our findings are supported by several earlier studies, which showed that essential oils can function as growth promoters by thinning the intestine, ultimately responsible for more nutrient uptake (Iqbal *et al.,* 2021; Su *et al.,* 2021). Nutrient uptake is directly proportional to body weight gain (Basmacioğlu-Malayoğlu *et al.,* 2016; Fathi *et al.,* 2016; Peng *et al.,* 2016; ELnaggar and EL-Maaty, 2017; Su *et al.,* 2021; Yousaf *et al.,* 2024). Essential oils addition increases the digestion by stimulating the secretion of more digestive enzymes (Zhang *et al.,* 2020) and this would in return increase the weight gain.

The feed conversion ratio significantly improved in essential oil groups compared to the antibiotic group (positive control). After using the essential oils blend, group E showed better FCR results during weeks three and five. Undoubtedly, essential oils are responsible for improving the feed conversion ratio, as they continue to increase weight gain on constant feed intake. Our findings are in line with earlier research that found essential oils improved FCR when compared to the control group (Asghar *et al.,* 2023; Fascina *et al.,* 2012; Basmacioğlu-Malayoğlu *et al.,* 2016), although some studies contradict our findings and found no improvement in growth performance when essential oils were used (Belenli *et al.,* 2015; Pathak *et al.,* 2017).

Nevertheless, the enhancement of digestion, the regulation of intestinal microbiota, and the augmented diversity of endogenous digestive enzymes attributable to essential oils are considered to be among the potential explanations for the observed improved performance (Popović *et al*., 2016). The present study is consistent with numerous other research investigations indicating that essential oils significantly contribute to production performance by enhancing the feed conversion ratio (FCR). In addition to the antibacterial effects, herbal extracts have antioxidant properties that may contribute to a better version of broilers (El-Shenway and Ali, 2016). Various studies conducted in the past on broilers have produced contradictory findings on the effects of essential oils on growth performance. While there is research that backs us up, there is also research that contradicts us. Resolving these polemics and producing biologically reliable results is sufficiently challenging due to the numerous variations in inclusion levels, active ingredients, concentrations, types of essential oils utilized, environmental conditions, basal feed composition, outbreaks of infectious diseases, and the foundational aspects of the herbal extracts (Bozkurt *et al.,* 2012; Basmacioğlu-Malayoğlu *et al.,* 2016). In addition, the positive effects of essential oils on gut microflora and their bactericidal effects may be responsible for the increased broiler weight and improved FCR. This may be the case because crucial oils contain acids that interfere with the cell membranes and cell macromolecules of bacteria, which disrupts the energy metabolism and the transport of nutrients (ELnaggar and EL-Maaty, 2017).

According to the current study's findings, carcass parameters, eviscerated weight, dressed weight, and giblet weight have not been affected by different levels of essential oil significantly (P>0.05). However, numerically, group C, containing essential oils, had the greatest eviscerated and dressed weights compared to control and other the treatment groups, and giblet weight was observed to be numerically higher in group E compared to control and other treatment groups. No discernible difference was seen between the groups for dressing % or dressed weight. Rehman *et al.* (2016), who discovered minor outcomes in the case of dressing percentage, support this as well. Additionally, prior research supports our findings, demonstrating that essential oils had more favorable impacts on all carcass parameters (Khattak *et al.,* 2013; Peng *et al.,* 2016; Ragaa and Korany, 2016). While some studies contradict our results, indicating no discernible impact on carcass characteristics (El-Shenway and Ali, 2016; Özsoy *et al.,* 2017; Gomathi *et al.,* 2018), others support them. The presence of phenolic compounds and antioxidants in essential oils, which reduced the detrimental bacteria population in birds' digestive tracts and raised the number of amino acids ingested, is responsible for improving carcass features. Additionally, an essential oil's active component triggers pancreatic secretions. This increased digestive output may lead to better digestion and nutrient absorption, which can enhance the quality of the carcass (Ragaa *et al.,* 2016). Moreover, better digestion of proteins and amino acids results in improved muscle. This might be another factor contributing to the better carcass metrics of essential oils (Hossain and Nargis, 2016).

The current research study showed significant results regarding meat quality. The findings regarding meat pH and color are consistent with other research showing an appreciable effect of dietary essential oils. Essential oils significantly deal with the meat pH, responsible for many meat traits. Meat pH highly controls the palatability, tenderness and shelf life of meat. Myoglobin contents increased with essential oils, as shown by our research findings and supported by many other researchers (Yesilbag, 2011). Essential oils have some extra beneficial properties, i.e., preservation effect. It was observed that the meat of broiler birds supplemented with essential oil had a more preserving effect than the control group (Hwang *et al.,* 2015).

The findings pertaining to antibody titers are of considerable significance, with a markedly higher antibody titer observed in Group E, while Group B exhibited the lowest titer. In concordance with our findings, El-Sayed *et al.* (2024) documented that antibody titers against Newcastle disease were significantly elevated (P<0.05) in broiler chickens that were administered essential oils at both 18 and 28 days, in comparison to those provided with probiotics and control groups. This enhancement in immune response may be associated with a notable increase in the bursa of Fabricius weight index, which was observed in chickens receiving essential oils and phytobiotics relative to the control group (Al-Mufarrej, 2014). Conversely, other studies have indicated no significant effect of dietary essential oils on antibody titers (Fascina *et al.,* 2017). The presence of terpenoids and phenolic compounds, which offer protection against DNA oxidation, may contribute to the improvement in humoral immunity associated with the consumption of essential oils. We may infer that such substances have a scavenging effect on free radicals, which immediately strengthens an immune system (ELnaggar and El-Tahawy, 2018). Another study presented no significant differences between essential oils-treated and vaccinated groups against Newcastle disease. However, it was observed that if essential oils were added to the Newcastle disease vaccine, the shedding of the virus significantly decreased. Thus, the epidemiological load of Newcastle disease virus in the environment has reduced enough (Lebdah, 2022).

Essential oils possess the potential to influence the intestinal wall and its structure, particularly the villi, which serve as an indicator of intestinal absorptive capacity (Heydarian *et al.,* 2020). A significantly greater villus length was observed in groups D and E in comparison to the other groups receiving essential oil. In alignment with our findings, El-Sayed *et al.* (2024) reported significant increases ($P<0.05$) in villus length, width, and crypt depth among broiler chickens that were administered essential oils and probiotics, as opposed to the control groups. The present study is also supported by previous research findings that have demonstrated an increase in villus height (Liu *et al.*, 2017; Sukandhiya *et al.*, 2017; Yarmohammadi *et al.,* 2020; Su *et al.*, 2021). With a reduced surface area available for absorption and immature enterocytes, a bird will absorb nutrients slower if its villus length and crypt depth are less. As a result, the bird performs poorly due to decreased nutritional absorption (Paiva *et al.*, 2014). Additionally, essential oils balance and regulate the internal gut surface and microbiota. This impact could be advantageous for enhancing gut morphology and ultimately lead to better growth performance (Zeng *et al.*, 2015). Furthermore, essential oils' anti-inflammatory and antioxidant characteristics may be accountable for better intestinal morphology (Du *et al.*, 2016; Gao *et al.*, 2019).

This research indicates that essential oils substantially diminish the total viable count within the gastrointestinal tract of broilers. Our findings reveal that Group C, which comprises essential oils administered at the lowest dosage, exhibited the least number of aerobic bacteria. Conversely, a significantly higher total bacterial count was noted in the negative control (Group B), followed by the positive control (Group A). Our findings align with previous studies which indicate that essential oils markedly diminish the population of bacteria in the gastrointestinal tract (Basmacioğlu-Malayoğlu *et al.,* 2016; Ndelekwute *et al.,* 2018). Furthermore, Chowdhury *et al.* (2018) discovered that administering cinnamon essential oil to mice resulted in a reduction of pathogenic bacteria, such as Escherichia coli, while leaving the population of beneficial bacterial species, including Lactobacillus, unaffected. Conversely, Pathak *et al.* (2016) determined that the administration of cinnamaldehyde and formic acid to broiler chickens did not yield a significant effect on the overall viable bacterial population. These essential oils disrupt the integrity of bacterial cell walls by converting their fragmented active ingredients into molecular forms that can readily penetrate bacterial cells and induce damage.

Conclusions: The utilization of essential oils has been demonstrated to enhance the growth performance of broilers, improve carcass characteristics, increase meat quality, optimize gut morphology, bolster immunity, and reduce total bacterial count. Among the treatment groups, the dose levels of 0.50mL/kg (group E) demonstrated the most favorable effects, while group D (0.25mL/kg) showed positive results in gut health. Group E (0.50mL/kg) effectively improved body weight, FCR, and meat quality. These results suggest that essential oils may be more effective than antibiotics in promoting broiler health. The study indicates that essential oils have a significant impact on broilers and should be considered as an alternative to antibiotics on an industrial scale. The outcomes with essential oils were even better than those with antibiotics. Therefore, their widespread use is necessary, and further research is needed to explore new combinations of these herbal extracts. Essential oils are reliable alternatives to antibiotics and can be considered another antibiotic substitute.

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REFERENCES

Abbas A and Alkheraije KA, 2023. Immunomodulatory Effects of Carica papaya extract against Experimentally Induced Coccidiosis in broiler chickens. Pak Vet J 43(3): 628-632.

Abd El-Latif AS, Saleh NS, Allam TS, *et al.,* 2013. The effects of rosemary (*Rosemarinus afficinalis*) and garlic (*Allium sativum*) essential oils on performance, hematological, biochemical and immunological parameters of broiler chickens. Br J Poult Sci 2(2):16-24.

Ahmed I, Li Z, Shahzad S, *et al.,* 2022. Potential probiotics role in excluding antibiotic resistance. J Fd Qual 2022(1): p.5590004.

Aksu T and Bozkurt AS, 2009. Effect of dietary essential oils and/or humic acids on broiler performance, microbial population of intestinal content and antibody titres in the summer season. Kafkas Univ Vet Fak Derg 15(2):185–190.

Aljohani ASM, 2023. Botanical Compounds: A Promising Approach to Control Mycobacterium Species of Veterinary and Zoonotic Importance. Pak Vet J 43: 633-642.

Al-Hoshani N, Al Syaad KM, Saeed Z, *et al.*, 2023. Anticoccidial activity of Star anise (Illicium verum) essential oil in broiler chicks. Pak Vet J 43(3): 553-558.

Al-Mufarrej S, 2014. Immune-responsiveness and performance of broiler chickens fed black cumin (*Nigella sativa L.*) powder. J Saudi Soc Agricultural Sci 13(1):75–80.

Al Syaad KM, Salman M, Abbas RZ, *et al*., 2023. Anticoccidial effect of Cinnamomum verum essential oil and its impact on hematological and serum biochemical parameters in broilers. Adv Life Sci 10(3):457-463.

Ali S and Alsayeqh AF, 2022. Review of major meat-borne zoonotic bacterial pathogens. Front Pub Health 10: p.1045599.

Asghar MU, Rahmana A, Hayata Z, *et al.,* 2023. Exploration of *Zingiber officinale* effects on growth performance, immunity and gut morphology in broilers. Braz J Biol 83: e250296

Barbour EK, Yaghi RH, Jaber LS, *et al.,* 2010. Safety and antiviral activity of essential oil against avian influenza and Newcastle disease viruses. Int J Appl Res Vet Med 8(1):60–64.

Basmacioğlu-Malayoğlu H, Ozdemir P and Bağriyanik H, 2016. Influence of an organic acid blend and essential oil blend, individually or in combination, on growth performance, carcass parameters, apparent digestibility, intestinal microflora and intestinal morphology of broilers. Br Poult Sci 57(2):227–234.

Belenli D, Udum D, Cengiz SŞ, *et al.,* 2015. Influence of various volatile oils as a dietary supplement on biochemical and performance parameters in broilers. J Environ Sci 9(25):47–55.

Bozkurt M, Küçükyılmaz K, Çatlı A, *et al.,* 2012. Effects of administering an essential oil mixture and an organic acid blend separately and combined to diets on broiler performance. Arch Geflugelkd 76(2):81–87.

Cabuk M, Eratak S, Alçiçek A, *et al.,* 2014. Effect of herbal essential oil mixture on intestinal mucosal development, growth performance, and weights of internal organs of quails. J Essent Oil-Bear Plants 17(4):599-606.

Casewell M, Friis C, Marco E, *et al.,* 2003. The European ban on growth-promoting antibiotics and emerging consequences for human and animal health. J Antimicrob Chemother 52(2):159–161.

Chowdhury S, Mandal GP, Patra AK, *et al.,* 2018. Different essential oils in diets of broiler chickens: 2. Gut microbes and morphology, immune response, and some blood profile and antioxidant enzymes. Anim Feed Sci Technol 236:39–47.

Du E, Wang W, Gan L, *et al.,* 2016. Effects of thymol and carvacrol supplementation on intestinal integrity and immune responses of broiler chickens challenged with *Clostridium perfringens*. J Anim Sci Biotechnol 7(1):1–10.

ELnaggar AS and EL-Maaty HMA, 2017. Impact of using organic acids on growth performance, blood biochemical and hematological traits and immune response of ducks (*Cairina moschata*). Egypt Poult Sci J 37(3):907–925.

ELnaggar AS and El-Tahawy W, 2018. Productive performance, physiological and immunological response of broiler chicks as affected by dietary aromatic plants and their essential oils. Egypt Poult Sci J 38(3):773–795.

El-Sayed Y, Khalil W, Fayez N, *et al.,* 2024. Enhancing effect of oregano essential oil and *Bacillus subtilis* on broiler immune function, intestinal morphology and growth performance. BMC Vet Res 20(1):112.

El-Shenway AM and Ali GI, 2016. Effect of some organic acids and essential oils as feed additives on growth performance, immune response and carcass quality of Japanese quail. Alex J Vet Sci 51(1):68–77.

Emami NK, Samie A, Rahmani H, *et al.,* 2012. The effect of peppermint essential oil and fructooligosaccharides, as alternatives to virginiamycin, on growth performance, digestibility, gut morphology and immune response of male broilers. Anim Feed Sci Technol 175(1-2):57–64.

Farouk MM, Al-Mazeedi HM, Sabow AB, *et al.,* 2014. Halal and kosher slaughter methods and meat quality: A review. Meat Sci 98(3):505–519.

Fascina VB, Pasquali G, Carvalho F, *et al.,* 2017. Effects of phytogenic additives and organic acids, alone or in combination, on the performance, intestinal quality and immune responses of broiler chickens. Braz J Poult Sci 19(3):497–508.

Fascina VB, Sartori JR, Gonzales E, *et al.,* 2012. Phytogenic additives and organic acids in broiler chicken diets. R Bras Zootec 41(10):2189–2197.

Fathi R, Samadi MS, Qotbi A, *et al.,* 2016. Effects of feed supplementation with increasing levels of organic acids on growth performance, carcass traits, gut microbiota and pH, plasma metabolites, and immune response of broilers. Anim Sci Pap Rep 34(2):195–206.

Gao Y-Y, Zhang X-L, Xu L-H, *et al.,* 2019. Encapsulated blends of essential oils and organic acids improved performance, intestinal morphology, cecal microflora, and jejunal enzyme activity of broilers. Czech J Anim Sci 64(5):189–198.

Gomathi G, Senthilkumar S, Natarajan A, *et al.,* 2018. Effect of dietary supplementation of cinnamon oil and sodium butyrate on carcass characteristics and meat quality of broiler chicken. Vet World 11(7):959–964.

Hartemink R and Rombouts FM, 1999. Comparison of media for the detection of bifidobacteria, lactobacilli and total anaerobes from fecal samples. J Microbiol Methods 36(3):181–192.

Heydarian M, Ebrahimnezhad Y, Meimandipour A, *et al.,* 2020. Effects of Dietary inclusion of the encapsulated thyme and oregano essential oils mixture and probiotic on growth performance, immune response and intestinal morphology of broiler chickens. Poult Sci J 8: 17–25.

Hossain M and Nargis F, 2016. Supplementation of organic acid blends in water improves growth, meat yield, dressing parameters and bone development of broilers. Bang J Anim Sci 45(1):7–18.

Hwang A, Cheng-An and Xuetong Fan, 2015. Processing, quality, and safety of irradiated and high-pressure-processed meat and seafood products. Minim Process Foods Technol Saf Qual Convenience 251–278.

Idris M, Abbas RZ, Masood S, *et al*., 2017. The potential of antioxidant rich essential oils against avian coccidiosis. World's Poult Sci J 73 (1): 89-104.

Iqbal H, Rahman A, Khanum S *et al.,* 2021. Effect of essential oil and organic acid on performance, gut health, bacterial count and serological parameters in broiler. Braz J Poult Sci 23 (3):1–10.

Ishaq K, Ahmad A, Rafique A, *et al*., 2022. Occurrence and antimicrobial susceptibility of *Proteus mirabilis* from chicken carcass. Pak Vet J 42(4):576–579.

Khattak F, Ronchi A, Castelli P, *et al*., 2013. Effects of a natural blend of essential oil on growth performance, blood biochemistry, cecal morphology, and carcass quality of broiler chickens. Poult Sci 93(1):132–137.

Lebdah M, 2022. The natural antiviral and immune stimulant effects of *Allium cepa* essential oil onion extract against virulent Newcastle disease virus. Saudi J Biol Sci 29(2):1239–1245.

Lee JW, Kim DH, Kim YB, *et al.,* 2020. Dietary encapsulated essential oils improve production performance of coccidiosis-vaccine-challenged broiler chickens. Animals 10 (3): 481.

Liu Y, Yang X, Xin H, *et al.,* 2017. Effects of a protected inclusion of organic acids and essential oils as an antibiotic growth promoter alternative on growth performance, intestinal morphology, and gut microflora in broilers. Anim Sci J 88(9):1414–1424.

Liu J, Zhang X, Niu J, *et al*., 2023. Complete genome of multi-drug resistant Staphylococcus Aureus in bovine mastitic milk in Anhui, China. Pak Vet J 43(3): 456-462.

Mohammadi Gheisar M, Hosseindoust A and Kim I, 2015. Evaluating the effect of microencapsulated blends of organic acids and essential oils in broiler chickens diet. J Appl Poult Res 24(4):511–519.

Ndelekwute EK, Enyenihi GE and Essien EB, 2018. Effect of organic acid-treated diets fed during finisher phase on gut microbiota and blood profile of broiler chickens. J Dairy Vet Sci 5(4):1–6.

Özsoy B, Ölmez M, Karadağoğlu Ö, *et al.,* 2017. Determination of the effect of a mixture of essential oil and organic acid containing at different levels on broiler rations on fattening performance, carcass parameters, and some internal organ weights. Lalahan Hayvancılık Araştırma Enstitüsü Dergisi 57(2):77–82.

Paiva D, Walk C and McElroy A, 2014. Dietary calcium, phosphorus, and phytase effects on bird performance, intestinal morphology, mineral digestibility, and bone ash during a natural necrotic enteritis episode. Poult Sci 93(11):2752–2762.

Pakistan Economic Survey (PES) 2023-2024. Agriculture. Ministry of Finance, Pakistan.

Panda AK, 2009. Effect of butyric acid on performance, gastrointestinal tract health, and carcass characteristics in broiler chickens. Asian-Australas J Anim Sci 22(7):1026–1031.

Pathak M, Mandal G, Patra A, *et al.,* 2016. Effects of dietary supplementation of cinnamaldehyde and formic acid on growth performance, intestinal microbiota, and immune response in broiler chickens. Anim Prod Sci 57(5):821–827.

Pathak M, Mandal G, Patra A, *et al.,* 2017. Effects of dietary supplementation of cinnamaldehyde and formic acid on growth performance, intestinal microbiota, and immune response in broiler chickens. Anim Prod Sci 57(5):821–827.

Peng Q, Li J, Li Z, *et al.,* 2016. Effects of dietary supplementation with oregano essential oil on growth performance, carcass traits, and jejunal morphology in broiler chickens. Anim Feed Sci Technol 214:148–153.

Pirgozliev V, Mansbridge S, Rose S, *et al.,* 2019a. Dietary essential oils improve feed efficiency and hepatic antioxidant content of broiler chickens. Anim 13(3):502–508.

Pirgozliev V, Rose SP and Ivanova S, 2019b. Feed additives in poultry nutrition. Bulg J Agric Sci 25(1):8–11.

Ponnampalam, EN, Kiani A, Santhiravel S, *et al.,* 2022. The importance of dietary antioxidants on oxidative stress, meat and milk production, and their preservative aspects in farm animals: Antioxidant action, animal health, and product quality-Invited review. Animals 12: 3279.

Popović S, Puvača N, Kostadinović L, *et al.,* 2016. Effects of dietary essential oils on productive performance, blood lipid profile, enzyme activity and immunological response of broiler chickens. Eur Poult Sci 80:1–12.

Ragaa NM and Korany RM, 2016. Studying the effect of formic acid and potassium diformate on performance, immunity and gut health of broiler chickens. Anim Nutr 2(4):296–302.

Ragaa NM, Korany RM and Mohamed F, 2016. Effect of thyme and/or formic acid dietary supplementation on broiler performance and immunity. Agric Agric Sci Procedia 10:270–279.

Rahman A, Saima, Pasha TN, *et al.,* 2015. Supplementation of exogenous enzymes and organic acid in broiler diets. Eurasian J Vet Sci 31(3):163-169.

Rehman ZU, Haq AU, Akram N, *et al.,* 2016. Growth performance, intestinal histomorphology, blood hematology and serum metabolites of broilers chickens fed diet supplemented with graded levels of acetic acid. Int J Pharmacol 12(8):874–883.

Saeed Z, Abbas RZ, Khan MK, *et al.*, 2023. Anticoccidial activities of essential oil of Amomum subulatum in broiler chicks. Pak J Agric Sci 60(2): 377-384.

Su G, Wang L, Zhou X, *et al.,* 2021. Effects of essential oil on growth performance, digestibility, immunity, and intestinal health in broilers. Poult Sci 100(8): 101242.

Su X, Zhou Y, Wang D, *et al.,* 2020. Dietary supplementation of plant essential oil improves growth performance, intestinal morphology and health in weaned pigs. J Anim Physiol Anim Nutr 104:579–589.

Sukandhiya K, Mani K, Rajendran K, *et al.,* 2017. Influence of dietary supplementation of sodium diformate on the intestinal histo: morphology of broilers in environmentally controlled housing system. Int J Chem Stud 5(6):842–843.

Symeon G, Zintilas C, Ayoutanti A, *et al.,* 2009. Effect of dietary oregano essential oil supplementation for an extensive fattening period on growth performance and breast meat quality of female medium-growing broilers. Can J Anim Sci 89(3):331–334.

Tiihonen K, Kettunen H, Bento MHL, *et al.,* 2010. The effect of feeding essential oils on broiler performance and gut microbiota. Br Poult Sci 51(3):381–392.

Timbermont L, Lanckriet A, Dewulf J, *et al.,* 2010. Control of Clostridium perfringens-induced necrotic enteritis in broilers by target-released butyric acid, fatty acids and essential oils. Avian Pathol 39(2):117–121.

Upadhayay U and Vishwa PCV, 2014. Growth promoters and novel feed additives improving poultry production and health, bioactive principles and beneficial applications: the trends and advances-a review. Int J Pharmacol 10(3):129–159.

Waqas M, Mehmood S, Mahmud A, *et al.,* 2018. Effect of yeast-based mannan oligosaccharide (Actigen™) supplementation on growth, carcass characteristics and physiological response in broiler chickens. Indian J Anim Res 53(11):1475–79.

Yang X, Xin H, Yang C, *et al.,* 2018. Impact of essential oils and organic acids on the growth performance, digestive functions and immunity of broiler chickens. Anim Nutr 4(4):388–393.

Yarmohammadi B, Jazi V, Mohebodini H, *et al.,* 2020. ffects of dietary lavender essential oil on growth performance, intestinal function, and antioxidant status of broiler chickens. Livest Sci 233: 103958.

Yesilbag D, 2011. Effects of dietary rosemary, rosemary volatile oil and vitamin E on broiler performance, meat quality and serum SOD activity. Br Poult Sci. 52(4):472–482.

Yousaf M, Sarwar M, Ahmed I, *et al.,* 2024. Effect of aqueous extract of *Cichorium intybus* as alternative antibiotic growth promotor on growth performance, lipid and mineral profiles of broilers. Pak J Zool. 1-8.

Zeng Z, Zhang S, Wang H *et al.,* 2015. Essential oil and aromatic plants as feed additives in non-ruminant Fnutrition: a review. J Anim Sci Biotechnol 6(1):1-10.

Zhang R, Wang XW, Liu LL, *et al.,* 2020. Dietary oregano essential oil improved the immune response, activity of digestive enzymes, and intestinal microbiota of the koi carp, Cyprinus carpio. Aquaculture 518:734781.