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Research Article

Evaluation of an essential oil blend derived from thymol, cinnamaldehyde, and menthol on the growth performance and gut health of broiler chickens

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Abstract

The study aimed to assess the effects of different levels of essential oil blend on the growth performance, gut health, and immune response of broiler chickens. A total of 192 day-old straight run broiler chicks were randomly assigned to four experimental groups, each comprising of 48 chicks divided into six replicates with eight chicks in each. The dietary groups included: basal diet without any growth promoter (CON) (group-1), basal diet with antibiotic (Bacitracin methylene disalicylate - BMD) @500 g/ton feed (AGP) (group-2), basal diet with essential oil blend @100 mg/kg feed (EOB100) (group-3) and basal diet with essential oil blend @200 mg/kg feed (EOB200) (group-4). Body weight gain, feed consumption, and feed conversion ratio were monitored at various intervals. Blood samples were collected for analysis, and antibody titers were measured. Carcass traits, gut microbiome, and gut morphology were evaluated at the end of the trial. Results showed that the EOB200 group had significantly higher (P<0.05) final body weight and average daily gain compared to other groups. Both EOB200 and EOB100 groups showed significantly improved (P<0.05) feed conversion ratio. EOB200, EOB100, and AGP groups had lower *Escherichia coli* and *Salmonella* sp counts and higher *Lactobacillus* counts. Villi height in the duodenum and ileum was significantly higher (P<0.05) in the EOB200 group. Essential oil blends 200 mg/kg feed could be effective alternatives to antimicrobials in poultry production.

Keywords: Broiler, Growth performance, Gut health, Immune response, Oil blend

Highlights

- Final body weight and average daily gain were significantly higher in the EOB200 group.
- Both EOB200 and EOB100 groups exhibited a significant improvement in feed conversion ratio.
- *E. coli* and *Salmonella* counts were significantly lower in the EOB200, EOB100, and AGP groups.
- *Lactobacillus* counts were significantly higher in the EOB200, EOB100, and AGP groups.

INTRODUCTION

Antibiotic growth promoters (AGPs) have been traditionally used in poultry production to enhance growth (Mehdi et al., 2018). However, due to the rising issue of microbial resistance, there is mounting pressure to discontinue the use of AGPs in poultry farming (Stefanello et al., 2020). As potential alternatives, plant-derived essential oils (EOs) have garnered attention for their diverse biological properties, including antimicrobial, antioxidant, and immune-modulatory effects (Donsì & Ferrari, 2016; Han et al., 2017).

Essential oils are concentrated hydrophobic liquids containing volatile aroma compounds that are produced as secondary metabolites in plants. Typically, EOs consist of 20 to 60 compounds from various chemical classes, such as alcohols, aldehydes, esters, ethers, ketones, phenols, and terpenes. These oils are renowned for their significant biological activities, including antimicrobial, antioxidant, and anti-inflammatory properties (Steiner, 2009). The antibacterial efficacy of EOs is primarily attributed to their phenolic components, with interactions among different constituents playing a pivotal role (Burt, 2004).

Blends of essential oils or their major components target multiple biochemical processes in bacteria, resulting in synergistic, additive, or occasionally antagonistic effects. The mechanisms of action of EO blends are contingent on their chemical composition. Phenolic compounds like carvacrol and thymol, which are present in thyme oil, exert antimicrobial effects by penetrating the lipid bilayer of the cytoplasmic membrane, ultimately leading to cell death (Nazzaro et al., 2013). The effectiveness of EOs on gut microflora in poultry varies, with inconsistent results when

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supplemented in diets at levels ranging from 20-200 mg EO/kg diet, which are lower than concentrations shown to have antimicrobial activity in vitro studies (0.03 to 2.0%) (Hammer et al., 1999). Some studies have demonstrated a reduction in intestinal microbiota, including *Escherichia coli* and *Clostridium perfringens*, in broilers (Jamroz et al., 2005; Clavijo & Flórez,2018). Selecting appropriate plants, active components, and effective dietary doses are crucial for the suitability of these substances in influencing poultry performance. While essential oils have been extensively studied and used in aviculture to enhance feed safety, further research is needed to confirm their impact on productive parameters and poultry health (Diaz-Sanchez et al., 2015).

Various commercial combinations of EOs have been investigated in poultry diets, but their biological efficacy remains inconclusive. Therefore, a shift from performance-based, product-driven research to a mechanistic approach is necessary to fully understand how plant-derived compounds work in domestic livestock and poultry species (Steiner, 2009). In this study, a blend of essential oils consisting of cinnamaldehyde, thymol, and menthol in a 1:1:1 ratio was used for their synergistic effects. Cinnamaldehyde, a key component of cinnamon (*Cinnamomum verum*), exhibits antioxidant, antimicrobial, and anti-inflammatory properties due to its rich content of aldehydes, esters, terpenes, and phenolic compounds (Ali et al., 2021). Thyme (*Thymus vulgaris* L.) is a source of thymol, a monoterpene with beneficial effects on nutrient utilization and gut health in poultry (Bento et al., 2013). Menthol, found in mint family plants, possesses antibacterial, antifungal, and antiviral properties (Schuhmacher et al., 2003).

Therefore, the objective of the present study was to evaluate the effects of a blend of EO (cinnamaldehyde, thymol, and menthol in a 1:1:1 ratio) on growth performance, gut health, and immunity in broilers.

MATERIALS AND METHODS

Broiler chickens, experimental design and diets: A total of 192 one-day-old straight-run commercial broiler chickens (Vencobb 400, Venkys, Pune, India) were randomly divided into four dietary groups, each consisting of six replicated pens (n=6) with eight broiler chickens per pen. The dietary groups included: 1) basal diet without any growth promoter (CON), 2) basal diet with antibiotic (Bacitracin methylene disalicylate - BMD) @200 mg/MT feed (AGP), 3) basal diet with essential oil blend(cinnamaldehyde, thymol, and menthol in a 1:1:1 ratio) @ 100 mg/kg feed (EOB100), and 4) basal diet with essential oil blend (cinnamaldehyde, thymol, and menthol in a 1:1:1 ratio) @ 200 mg/kg feed (EOB200). The basal diet (Table 1) was formulated in mash form using maize and soybean to meet or exceed the nutritional requirements of broiler chickens at different stages (starter, grower, and finisher) based on the recommendations for Vencobb 400 broiler chickens (Venkys, 2017). The experimental diets were prepared weekly, packed in high-density polyethylene bags with inner liners, and provided *ad libitum* along with portable drinking water.

Management and rearing of birds: Before the experiment starts, the poultry shed, feeding, and watering troughs were thoroughly cleaned and disinfected. The chicks were housed in floor pens (1.22 m × 0.76 m) separated group-wise by plastic wire netting. Rice husk and chopped paddy straw were used as litter, and each pen was equipped with plastic feeders and water troughs. Initially, the chicks were provided with continuous lighting from fluorescent lamps for the first two days, followed by 23 hours light-dark photo schedule. The temperature in the poultry house was regulated using heating elements, starting at 32°C on day 1 and gradually decreasing to 24°C by day 22. Proper ventilation was ensured with exhaust fans throughout the 35 days trial period. All birds were immunized against Newcastle disease virus (NDV) at 5 and 21 days of age and infectious bursal disease virus (IBDV) at 12 days of age.

Measurement of performance traits: The initial body weight (BW) of individual chick was recorded on the first day of the trial using digital weighing balance. The chicks' weights were measured weekly, with a final measurement on the last day in the morning. Average daily gain (ADG) was calculated for each replicate. Weekly feed intake was determined by subtracting the remaining feed from the total offered per pen. Average daily feed intake (ADFI) was calculated by dividing the total feed consumed per day by the number of chicks per pen. Feed conversion ratio (FCR) was calculated by considering cumulative feed intake and weight gain for each replicate pen. Daily mortality was recorded, and post-mortem examinations were conducted to determine the cause of death. Mortality percentage in each replicate was calculated at the end of the trial and used to adjust BW, ADG, ADFI, and FCR calculations.

Measurement of serum biochemical and antibody titer against Newcastle disease virus and Infectious bursal disease virus: Biochemical blood samples

Table 1. Composition of basal diets

Sl. No.	Ingredients	Starter (d1-14)	Grower (d15-28)	Finisher (d29-35)
1	Maize	55.947	59.111	62.335
2	Soya DOC (45%).	38.177	34.161	30.039
3	Oil-veg	2.103	3.217	4.270
4	Dicalcium phosphate	1.486	1.372	1.260
5	Limestone powder	0.871	0.891	0.884
6	salt (Nacl)	0.322	0.315	0.326
7	DL-methionine[1]	0.311	0.259	0.231
8	L-lysine HCL[2]	0.240	0.151	0.130
9	L-threonine[3]	0.074	0.053	0.055
10	Toxin binder[4]	0.050	0.050	0.050
11	Sodium bi-carbonate	0.100	0.100	0.100
12	Choline chloride, 60%[5]	0.050	0.050	0.050
13	Trace mineral mixture[6]	0.100	0.100	0.100
14	Vitamin premix[7]	0.100	0.100	0.100
15	Antioxidant[8]	0.010	0.010	0.010
16	Phytase 5000[9]	0.010	0.010	0.010
17	Emulsifier	0.050	0.050	0.050
Nutrient composition				
1	Metabolizable energy (kcal/kg)[10]	2999.00	3100.00	3200.00
2	Crude protein (%)[11]	22.50	20.83	19.20
3	Ether extract (%)[11]	4.71	5.90	7.03
4	Crude fiber (%)[11]	3.61	3.44	3.25
5	Calcium (%)[11]	0.94	0.91	0.87
6	Total phosphorus (%)[10]	0.75	0.71	0.67
7	Available phosphorus (%)[10]	0.45	0.42	0.39
8	Lysine (%)[10]	1.25	1.09	0.98
9	Methionine (%)[10]	0.60	0.53	0.49
10	Methinine + cysteine (%)[10]	0.88	0.80	0.74
11	Threonine (%)[10]	0.77	0.70	0.65
12	Sodium (%)[10]	0.16	0.16	0.16
13	Chloride (%)[10]	0.18	0.18	0.18

[1]Evonik Methinine SEA Pte. Ltd. Singapore.
[2]Qiqihar Longjiang Fufeng Biotechnologies Co., Ltd. Heilongjiang, China.
[3]Xinjiang Meihua Amino Acid Co., Ltd, Xinjiang, China.
[4]Niltox™, Zeus Biotech Limited, Mysore, India. (Toxin binder)
[5]Indian Herbs Specialitis Pvt. Ltd. Solan, Himachal Pradesh.
[6]Contains zinc 4.0%, manganese 4.0%, iron 1.5%, copper 0.8%, iodine 0.4%, selenium 300 ppm, chromium 200 ppm (Zenex animal health India Pvt. Ltd, Patiya, Ahmedabad, India)
[7]Contains vitamin E 100 g, vitamin A 40000000 IU, vitamin D_3 12000000 IU, pantothenic acid 60 g, vitamin K 8 g, vitamin B_1 120 g, vitamin B_2 24 g, vitamin B_6 10 g, vitamin B_{12} 0.10 g, biotin 0.40 g, folic acid 4 g, niacin 100 g (DSM Nutritional Products India Pvt. Ltd. Mahabubnagar, Telangana, India).
[8]Endox, Kemin Industries, Inc., USA.
[9]Quantamblue, AB Vista, Pune, India.
[10]Calculated values (based on the Asia South feed ingredients report 2016, Evonik Pte Ltd, Singapore).
[11]Analysed values (average of triplicate values).

were collected from broiler chickens on day 35 after a 12-hour fasting period. The samples were drawn from the wing vein. Twelve birds per treatment were randomly selected, with two birds chosen from each pen. 2 mL blood was collected without anticoagulant, and the serum was stored at -20°C until analysis. The concentrations of glucose, total protein, albumin, uric acid, triglycerides, and cholesterol in the serum were measured using commercial kits from DiaSys Diagnostic India Pvt. Ltd., Mumbai, India. Antibody titers for NDV and IBDV were determined following manufacturer's instruction of ELISA kit from IDEXX Laboratories Inc., USA. The optical density (OD) was measured twice for each sample in a Microplate reader from Meril Life, India, and the mean OD values were used for analysis.

Measurements of carcass characteristics: On day 35, two birds from each sex close to the average BW from the same replication were slaughtered by cervical disarticulation for the evaluation of carcass trait. Cut-up parts and internal organs were accurately weighed and recorded using a digital weighing balance.

Enumeration of caecal bacterial count: The caecal contents of all the slaughtered birds were collected aseptically in a sterile sample collection bag (HiMedia, India) and processed on the same day for the evaluation of bacteriological count. A standard colony counting procedure by Quinn et al. (1994) was followed to quantify *E. coli*, *Salmonella*, and *Lactobacillus* in the caecal content. One gram of caecal content was diluted tenfold with sterile phosphate-buffered saline (PBS). Subsequently, 10 µL of the diluted sample was spread onto specific agar plates for each bacterium: *E. coli* on sorbitol-MacConkey agar, *Salmonella* on Xylose Lysine Deoxycholate agar, and *Lactobacillus* on Lactobacillus agar (HiMedia, India). The agar plates were incubated at 37°C for 24 to 48 hours, and the characteristic colonies for each bacterial group were counted using a digital colony counter (HiMedia, India).

The results were expressed as Log_{10} colony-forming units (cfu) per gram of the sample.

Morphological study of small intestine: On day 35, 6 chickens from each dietary group were slaughtered, and small intestinal tissue samples were collected to measure villus height, villus width, and crypt depth. Sections of the duodenum, jejunum, and ileum were prepared as per standard procedure and it was taken and fixed in buffered formaldehyde solution, embedded in paraffin wax, stained with Delafield's Hematoxylin and Eosin, and mounted on DPX. Measurements were taken using an ocular micrometer and image analysis software. Villus height was measured from the tip to the villus-crypt junction, and crypt depth was measured as the depth between two villi. Twelve intact villi per section were selected based on the lamina propria. Each sample was observed in at least three sections with ten measurements each, and the values were averaged for analysis.

Chemical analysis of feeds samples: The feed samples were analyzed according to (Association of Official Analytical Chemists [AOAC], 2005) methods. Dry matter (DM) was determined using method 934.01. Crude protein (CP) was analyzed using method 968.06 using Kelplus, Pelican Equipments, Chennai, India. Crude fiber (CF) was measured using the Foss Fiber Cap 2021 Fiber Analysis System, Foss Analytical, Hilleroed, Denmark. Ether extract (EE) was determined using method 920.39 by Socsplus, Pelican Equipments, Chennai, India. Calcium content was determined following the procedure described by Talapatra et al. (1940). Additionally, the AIA levels of the diets were measured using the technique outlined by Furuichi and Takahashi (1981).

Statistical analysis: The data were analyzed using one-way analysis of variance (ANOVA) with the statistical software SPSS (Statistical Package for the Social Sciences [SPSS] Inc, 1997). The study followed a completely randomized design, with treatment as the main factor and pen as the experimental unit for body weight gain, feed intake, and FCR. Individual birds were considered as the experimental units for other parameters. Mortality data met homogeneity criteria and did not require transformation for statistical analysis. A significance level of $P \leq 0.05$ was used to determine significance, while $P \leq 0.01$ was considered a trend. When treatment had a significant effect, Duncan test was used to detect differences among treatment means.

Table 2. Effect of essential oil blend derived from thymol, cinnamaldehyde, and menthol on average daily gain (ADG), final body weight, average daily feed intake (ADFI) and feed conversion ratio (FCR) of broiler chickens

	Treatment[1]					
Attribute	**CON**	**AGP**	**EOB100**	**EOB200**	**SEM[2]**	**P-Value**
ADG (g/d)						
Day 1-14	37.39	41.91	43.34	44.61	1.283	0.214
Day15-28	68.86	69.79	72.94	74.31	1.857	0.724
Day 29-35	83.77	95.82	98.33	103.96	4.566	0.482
Day 1-35	59.25^{c}	63.84^{b}	66.18^{ab}	68.36^{a}	0.969	0.002
Final body weight	2121.25^{c}	2282.29^{b}	2363.96^{ab}	2439.81^{a}	33.891	0.002
ADFI (g/d)						
Day 1-14	41.27	41.73	44.87	44.42	0.618	0.074
Day15-28	101.51	109.15	99.72	101.56	1.546	0.131
Day 29-35	147.92	146.46	143.08	156.39	2.278	0.203
Day 1-35	86.69	89.64	86.45	89.67	0.892	0.412
FCR (g intake/g gain)						
Day 1-14	1.10	1.01	1.05	1.01	0.019	0.247
Day15-28	1.48	1.59	1.38	1.39	0.034	0.116
Day 29-35	1.77	1.74	1.60	1.52	0.098	0.792
Day 1-35	1.46^{a}	1.41^{ab}	1.31^{b}	1.31^{b}	0.023	0.024

^{abc}means bearing different superscripts in the same row differ significantly ($P \leq 0.05$). [1]The control diet (CON), control diet was supplemented with Antibiotic (BMD) at 200 mg/MT feed (AGP), control diet with of essential oil blend at the rate of 100 mg/kg (EOB100), control diet with of essential oil blend at the rate of 200 mg/kg (EOB200). [2]SEM, standard error of means (n=6).

Table 3. Effect of essential oil blend derived from thymol, cinnamaldehyde, and menthol on blood biochemical profile of broiler chickens

	Treatment[1]					
Attribute	CON	AGP	EOB100	EOB200	SEM[2]	P-Value
Glucose (mg/dL)	259.60	209.80	238.40	232.60	14.942	0.736
Total protein (mg/dL)	4.22	5.82	6.78	5.70	0.477	0.315
Albumin (mg/dL)	1.78	1.40	2.06	2.02	0.108	0.105
Uric acid (mg/dL)	2.98	2.88	3.04	2.78	0.172	0.962
Triglyceride (mg/dL)	46.20	56.40	48.00	47.00	3.380	0.724
Cholesterol (mg/dL)	134.40	127.60	124.20	128.60	5.213	0.933

[1]The control diet (CON), control diet was supplemented with Antibiotic (BMD) at 200 mg/MT feed (AGP), control diet with of essential oil blend at the rate of 100 mg/kg (EOB100), control diet with of essential oil blend at the rate of 200 mg/kg (EOB200).
[2]SEM, standard error of means (n=6).

Table 4. Effect of essential oil blend derived from thymol, cinnamaldehyde, and menthol on antibody titer ($\log_{10}$) against infectious bursal disease vaccine (IBDV) and Newcastle disease vaccine (NDV) of broiler chickens

	Treatment[1]					
Attribute	CON	AGP	EOB100	EOB200	SEM[2]	P-Value
IBDV						
Day 28	1.82	1.87	2.16	2.21	0.110	0.509
Day 35	3.10	3.15	3.11	3.15	0.080	0.994
NDV						
Day 28	3.93	3.96	4.03	4.04	0.065	0.933
Day 35	2.23	2.48	2.33	2.41	0.062	0.542

[1]The control diet (CON), control diet was supplemented with Antibiotic (BMD) at 200 mg/MT feed (AGP), control diet with of essential oil blend at the rate of 100 mg/kg (EOB100), control diet with of essential oil blend at the rate of 200 mg/kg (EOB200).
[2]SEM, standard error of means (n=6).

RESULTS

Growth performance: Table 2 presents the final body weight, average daily gain, average daily feed intake, and feed conversion ratio data. The final body weight of birds significantly (P<0.05) increased in the EOB200 group compared to the CON and AGP groups, but did not differ with the EOB100 group. The average daily gain (ADG) was significantly (P<0.05) higher in the EO200 group throughout the 35-day experiment period compared to the CON and AGP groups, but did not differ with the EOB100 group. However, no significant differences were observed during the starter (1-14 days), grower (15-28 days), and finisher (29-35 days) phases. The average daily feed intake (ADFI) did not vary significantly (P=0.412) among the treatment groups during different phases or over the entire 35-day period. The feed conversion ratio (FCR) showed no significant differences during the starter, grower, and finisher phases but was significantly (P<0.05) improved in the EOB100 and EOB200 groups compared to the CON group over the entire 35-day period, however did not differ with the AGP group.

Blood serum biochemical profile: The concentration of glucose, total protein, albumin, uric acid, triglyceride, and cholesterol in serum did not vary significantly (P>0.05) based on dietary treatments in this study (Table 3).

Immune response: There were no significant differences (P>0.05) in antibody titers to the infectious bursal disease (IBD) and Newcastle disease (ND) vaccines between the dietary treatment groups on days 28 and 35 (Table 4).

Carcass characteristics: No significant differences (P>0.05) were observed in slaughter weight, dressed weight, dressing percentage, breast, frame, thigh, drumstick, wing, neck, gizzard, liver, heart, spleen, bursa, and abdominal fat weight in grams across the various treatment groups (Table 5).

Gut microbes: The count of *E. coli* and *Salmonella* sp were significantly lower (P<0.05) in the EOB100, EOB200 and AGP groups compared to the control group. The count of *Lactobacillus* was significantly higher (P<0.05) in the EOB100 and EOB200 groups compared to the control and antibiotic supplemented groups (Table 6).

Gut morphology: There were no significant differences (P>0.05) in the crypt depth (CD), and the ratio of villi

Table 5. Effect of essential oil blend derived from thymol, cinnamaldehyde, and menthol on carcass characteristics of broiler chickens

Attribute	Treatment[1] CON	AGP	EOB100	EOB200	SEM[2]	P-Value
Slaughter BW (g)	2146.33	2146.33	2108.83	2189.33	18.004	0.539
Eviscerated BW (g)	1430.17	1401.17	1467.67	1459.50	14.782	0.390
Dressing Percentage (%)	66.64	65.25	66.45	66.66	0.312	0.340
Breast (g)	542.83	549.17	575.67	566.33	8.044	0.472
Frame (g)	275.17	273.0	277.67	281.33	3.548	0.875
Thigh (g)	175.33	170.67	178.67	177.0	3.698	0.899
Drumstick (g)	207.67	199.0	211.67	208.67	3.417	0.622
Wing (g)	108.67	110.67	111.67	110.67	2.474	0.982
Neck (g)	54.83	53.67	55.83	54.83	1.241	0.952
Gizzard (g)	47.44	47.65	49.33	47.50	0.810	0.837
Liver (g)	41.16	42.90	42.91	40.73	0.877	0.757
Heart (g)	9.60	10.42	10.47	10.30	0.190	0.347
Spleen (g)	2.19	1.79	2.32	2.23	0.112	0.357
Bursa (g)	4.29	4.39	4.45	4.39	0.162	0.989
Abdominal Fat (g)	30.28	27.09	34.32	35.26	1.997	0.468

[1]The control diet (CON), control diet was supplemented with Antibiotic (BMD) at 200 mg/MT feed (AGP), control diet with of essential oil blend at the rate of 100 mg/kg (EOB100), control diet with of essential oil blend at the rate of 200 mg/kg (EOB200).
[2]SEM, standard error of means (n=6).

Table 6. Effect of essential oil blend derived from thymol, cinnamaldehyde, and menthol on viable bacteria numbers (log_{10} cfu/g) in caecal content of broiler chickens

Attribute	Treatment[1] CON	AGP	EOB100	EOB200	SEM[2]	P-Value
Escherichia coli	5.63^a	4.99^b	4.98^b	5.04^b	0.080	0.003
Salmonella sp	5.38^a	5.04^b	5.01^b	5.10^b	0.041	0.001
Lactobacillus sp	6.90^b	7.03^b	7.52^a	7.47^a	0.062	0.00

[ab]means bearing different superscripts in the same row differ significantly ($P \leq 0.05$).
[1]The control diet (CON), control diet was supplemented with Antibiotic (BMD) at 200 mg/MT feed (AGP), control diet with of essential oil blend at the rate of 100 mg/kg (EOB100), control diet with of essential oil blend at the rate of 200 mg/kg (EOB200).
[2]SEM, standard error of means (n=6).

height to crypt depth (VH/CD) in the duodenum, jejunum and ileum among the treatment groups. However, the EOB100, EOB200 and AGP groups showed significantly (P<0.05) higher villi height in the duodenum compared to the control group. Additionally, the EOB200 group exhibited significantly (P<0.05) higher villi height compared to the EOB100, AGP and CON groups in the ileum (Table 7).

DISCUSSION

In this study, supplementing EOB200 significantly influenced the final body weight and average daily gain over the 35-day experiment. 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 (Abdel-Wareth et al., 2019; Nouri, 2019; Dong et al., 2024; Ibigbami et al., 2024). Nutrient uptake is directly proportional to body weight gain (Yousaf et al., 2024). Essential oil 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.

There were no significant changes in phase-wise average daily feed intake of broiler chickens in this study, consisting with the findings by Saied et al. (2022) and Sabir et al. (2023). In contrast, Waleed et al. (2024) observed that combinations of peppermint oil and chromium-l-methionine led to the best performance in weekly feed intake for broilers.

Feed conversion ratios were significantly improved in the EOB100 and EOB200-supplemented groups throughout the 35-days experimental period, in line with previous research. Hosseini and Meimandipour, (2018), and Waleed et al. (2024), reported

Table 7. Effect of essential oil blend derived from thymol, cinnamaldehyde, and menthol on gut morphology of broiler chickens

Attribute	Treatment[1] CON	AGP	EOB100	EOB200	SEM[2]	P-Value
Duodenum						
Villi height (VH; µm)	1247.33^{b}	1425.00^{a}	1439.33^{a}	1462.66^{a}	32.332	0.036
Crypt depth (CD; µm)	227	260	262.33	282	11.049	0.407
VH/CD ratio	4.42	5.53	5.65	6.52	1.069	0.094
Jejunum						
Villi height (VH; µm)	1116.66	1128	1148	1178.66	33.071	0.94
Crypt depth (CD; µm)	254.33	260.33	1148	265.33	4.202	0.867
VH/CD ratio	4.28	4.30	4.39	4.63	0.147	0.865
Ileum						
Villi height (VH; µm)	735^{c}	829^{b}	886.66^{b}	967.66^{a}	27.162	0.00
Crypt depth (CD; µm)	192	208	216.66	222.33	12.602	0.882
VH/CD ratio	3.53	4.25	4.35	4.71	0.260	0.498

[ab]means bearing different superscripts in the same row differ significantly (P≤0.05).
[1]The control diet (CON), control diet was supplemented with Antibiotic (BMD) at 200 mg/MT feed (AGP), control diet with of essential oil blend at the rate of 100 mg/kg (EOB100), control diet with of essential oil blend at the rate of 200 mg/kg (EOB200).
[2]SEM, standard error of means (n=6).

improvements in feed conversion with essential oil supplementation. Petrièeviæ et al. (2021) found that 0.4% peppermint in a broiler diet resulted in a significantly higher feed conversion ratio (P<0.01). However, Haselmeyer et al. (2015), and Sabir et al. (2023), did not observe differences in feed conversion ratio with essential oil additions in broiler diet. Essential oil supplements can enhance performance by improving antioxidant and digestive enzymes, nutrient digestibility, and intestinal health, leading to improved growth rates and feed efficiency (Gopi et al., 2014). Essential oils also contribute to enhancing the feed conversion ratio by promoting weight gain while maintaining a constant feed intake. Furthermore, the beneficial effects of essential oils on gut microflora and their bactericidal properties may be attributed to the increased broiler weight and improved FCR. This is because essential oils contain acids that disrupt bacterial cell membranes and macromolecules, affecting energy metabolism and nutrient transport (ELnaggar & EL-Maaty, 2017).

In this study, the addition of different levels of a blend of essential oils to the diet of the experimental birds did not have an impact on the serum concentrations of glucose, total protein, albumin, uric acid, triglycerides, and cholesterol. These results indicate that blood chemistry remained stable, demonstrating the safety of the essential oil blend for bird consumption. These findings are consistent with previous studies by Pathak et al. (2016), Abd El-Hack et al. (2024), and Alimohammadi et al. (2024), which suggest that the rapid metabolism and excretion of essential oils from the body contribute to these results. In contrast, Abo Ghanima et al. (2020) found that groups supplemented with rosemary and cinnamon essential oils had significantly lower blood cholesterol levels compared to the control group (P<0.05 or 0.01). Similarly, Saied et al. (2022), Hussein et al. (2023), Abd El-Hack et al. (2024), and Rio et al. (2024), reported decreased levels of total cholesterol, triglycerides, and low-density lipoproteins in birds supplemented with essential oils compared to control groups. It has been proposed that essential oils may reduce serum cholesterol concentrations by inhibiting hepatic 3-hydroxy-3-methylglutaryl coenzyme A reductase activity, a key enzyme in cholesterol synthesis (Case et al., 1995). However, in the current study, no significant differences were observed in cholesterol levels between the treatments. This lack of significance may be attributed to the specific essential oil components used in this study, which may not effectively inhibit hepatic 3-hydroxy-3-methylglutaryl coenzyme A reductase or may be rapidly degraded in the liver.

In this study, there were no significant differences in antibody titer levels for IBDV or NDV among the treatment groups on day 28 and day 35. Kondhare et al. (2024) similarly found no statistically significant differences in haemagglutination inhibition titers against NDV at different ages with varying concentrations of cinnamon aldehyde essential oil. However, previous studies have shown increased antibody titers against IBD and ND vaccines when essential oils were added to broiler chicken diets. Hong et al. (2012) reported higher antibody titers against ND vaccine with essential oils from citrus peel, oregano, and anise. Saleh et al. (2014) demonstrated that thyme oil and ginger oil supplementation enhanced antibody titers and phagocytic activity against ND and IBD viruses. Doneria et al. (2022) showed improved serum HI titers against NCDV with cinnamon oil supplementation. Dey et al. (2023) found that a combination of cinnamon, clove, and ajwain oils increased NDV antibody titers. Parveen et al. (2020) also observed higher ND vaccine antibody titers in essential oil supplemented groups compared to control groups.

In this study, there were no significant differences (P>0.05) in carcass traits between the groups. Previous research has shown that supplementing essential oils, either alone or in blends, does not affect carcass traits in broiler chickens. Deeb et al. (2024) found no significant differences in carcass characteristics when thyme leaf powder was added to broiler diets. Similarly, Ghazanfari et al. (2024) reported no significant effects on carcass traits when using peppermint essential oil in broiler diets. Hassan et al. (2024) studied the effects of ginger, thyme, and their nano-composts on broiler diets and found that while some traits like abdominal fat and Bursa of Fabricius weight were affected, most carcass characteristics remained unchanged. Other studies have shown varying results, with some reporting significant changes in specific traits with certain supplements, while others found no significant effects on carcass characteristics (Adam et al., 2020; Petričević et al., 2021; Qaid et al., 2021; Diogo et al., 2022; Saied et al., 2022 ; Sabir et al., 2023). Overall, the impact of essential oils and plant extracts on carcass traits in broiler chickens remains inconclusive. The presence of phenolic compounds and antioxidants in essential oils reduces harmful bacteria in birds' digestive tracts and increases amino acid intake, improving carcass quality. Essential oils also stimulate pancreatic secretions, enhancing digestion and nutrient absorption, leading to better carcass features (Ragaa et al., 2016). Improved protein and amino acid digestion contribute to enhanced muscle development, further enhancing carcass quality (Hossain & Nargis, 2016).

The caeca are considered the major site of microbial fermentation in the avian gastrointestinal tract (Levy et al., 2015). Therefore, caecal microflora (*Escherichia coli*, *Salmonella*, and *Lactobacillus*) of broilers were determined in our study. Gut microbiota plays an important role for animal health, performance, and product safety. Decreased numbers of pathogenic bacteria in the gut may improve the ability of epithelial cells to regenerate villus and thus enhance intestinal absorptive capacity (Zeng et al., 2015). In this study, the caecal *E. coli* and *Salmonella* counts decreased in groups supplemented with a blend of essential oils and antibiotics compared to the control group. Furthermore, *Lactobacillus* sp counts significantly increased in groups supplemented with the essential oil blend compared to the control group, which is consistent with previous research. Vlaicu et al. (2023) observed lower *E. coli* levels and higher *Lactobacillus* counts in broilers fed diets containing 0.05% sage, thyme, or basil compared to the control group. Champati et al. (2024) demonstrated that supplementing broiler diets with formic acid and thymol led to increased *Lactobacillus* populations and decreased *Coliform* levels. Similar findings were reported by Chowdhury et al. (2018), Parveen et al. (2020), Aly et al. (2023), Zheng et al. (2023), and Dong et al. (2024). The essential oil blend treatment has a selective antimicrobial effect, targeting harmful microbes while leaving beneficial bacteria like *Lactobacillus* unaffected (Tiihonen et al., 2010). Controlling *Salmonella* contamination in poultry meat is crucial for food safety and reducing the risk of human salmonellosis. Lowering *Salmonella* levels in the intestines and on carcasses can enhance food safety standards in the poultry industry. Therefore, reducing the number of pathogenic bacteria in the intestines is a significant step for poultry producers and consumers alike.

In this study, the duodenal villi height significantly increased in groups supplemented with EO200, EO100, and AGP compared to the CON group. The ileal villi height was significantly higher in the EO200 group compared to CON, EO100, and AGP groups, with no significant changes in the jejunum. There were no significant effects on crypt depth or the villi height to crypt depth ratio in any intestinal segment. The beneficial effects of essential oil blend on intestinal morphology might be due to regulation and balance of intestinal microflora (Zeng et al., 2015). Furthermore, the anti-inflammatory and antioxidant properties of essential oils may contribute to better intestinal morphology (Gao et al., 2019). This study supports previous research on the beneficial effects of essential oils on broiler gut morphology. Doneria et al. (2022) supplemented broiler diets with different concentrations of cinnamon essential oil, showing increased villi height in the duodenum and jejunum with higher oil amounts. Pakiza and Mahbuba, (2020) found improved ileum villus height with clove and cinnamon powder in meals. Moharreri et al. (2022) reported increased villus height with essential oil-loaded microcapsules. Qaid et al. (2021) found enhanced duodenal villus height and goblet cell density with cinnamon supplements. Li et al. (2023) showed improved jejunum and ileum morphology with a thymol and carvacrol blend. Khan et al. (2024) demonstrated improved crypt depth and villus height with an essential oil blend. Liu et al. (2024) reported increased ileal villus height, VH/CD ratio, goblet cells, and crypt depth with specific compounds. Chowdhury et al. (2018) found enhanced jejunum villi length with AGP and various essential oils. Deminicis et al. (2024) showed enhanced gut morphometry with specific essential oils in quail diets. In contrast, Dey et al. (2023) found no significant changes in villi height or crypt depth with different essential oil concentrations in the diet.

Birds supplemented with EOB200 and EOB100 showed improved feed conversion ratios (FCR) compared to the control and AGP groups. Additionally, EOB200 and EOB100 supplementation resulted in reduced levels of *E. coli* and *Salmonella* sp in the caeca compared to other groups. *Lactobacillus* sp levels were significantly higher in birds receiving EOB200 and EOB100 compared to those in the control and antibiotic groups. These results suggest that essential oil blends could be effective alternatives to antibiotics in poultry farming, especially for broilers on an antibiotic-free diet, promoting overall bird health. This study highlights the potential of essential oil blends as a promising option for enhancing gut health in poultry without the use of antibiotics.

Conflict of interests: We wish to confirm that there are no known conflicts of interest associated with this publication, and there has been no significant financial support for this work that could have influenced its outcome.

Author's contribution: SM: Involved in the investigation, data generation, preparing original draft; AS: Involved in the investigation, data generation; GPM: Formulated diet; BR: Methodology editing; JM: Supervised ELISA; SM: Interpretation of gut morphology; IS: Supervised the gut microbiota; AR: Supervised the broiler management; SS: Engaged in conceptualization, supervision and final editing.

Data availability statement: Raw data of this study are available from the corresponding author.

Ethical statement: The study protocol was approved by Intuitional Animal Ethics Committee of West Bengal University of Animal and Fishery Sciences, (Ethics Approval Number: 763/GO/Re-S/ReRc-L/03/CCSEA/75/2024-25) Kolkata, India.

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