GROWTH PERFORMANCE, MORTALITY, AND BLOOD BIOCHEMISTRY OF BROILER CHICKENS SUPPLEMENTED WITH CYPRESS AND JUNIPER ESSENTIAL OILS

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ABSTRACT

The objective of the present study was to investigate the effects of cypress (*Cupressus sempervirens* L.) and juniper (*Juniperus communis* L.) essential oils (EOs), alone and in combination, on the growth performance and blood biochemical parameters of commercial broilers (Arbor Acres Plus) under natural health challenge. A total of 480 one-day-old chicks were randomly allocated following a completely randomized design into five treatment groups (96 per group; 8 replicates of 12 birds each). Birds in the negative control (NC) group were fed a corn-soybean meal diet and provided plain water (total solids of 1085 mg/L) without additives. The positive control (PC) group was fed the same diet supplemented with an antibiotic growth promoter (AGP) and additionally received two antibiotic treatments via drinking water following a colibacillosis outbreak during the trial; this group was therefore not considered a pure AGP control, limiting its suitability as a benchmark for growth performance. The essential oil-treated groups were supplemented with cypress (CEO), juniper (JEO), and a mixture of both (MEO), administered at a concentration of 625 ppm in a pulsed cycle of three days on and three days off, repeated throughout the 42-day trial. Mortality increased during the trial, with significantly higher rates ($P \leq 0.05$) in the PC group. CEO Supplementation was associated with improved feed conversion ratio (FCR) at day 35 and significantly enhanced survivability and European Production Efficiency Factor (EPEF) ($P \leq 0.05$). On day 42, serum biochemical analysis revealed significantly lower total cholesterol and LDL concentrations in the JEO group, whereas the MEO group exhibited the lowest triglyceride and creatinine levels ($P \leq 0.05$). In summary, CEO primarily improved EPEF through enhanced survivability, while JEO and MEO favorably modulated lipid metabolism and renal markers. These findings suggest that cypress and juniper EOs could serve as an effective prophylactic strategy against colibacillosis outbreaks in broilers.

Keywords: Broiler chicken, Cypress, Disease challenge, Essential oil, European Production Efficiency Factor, Juniper.

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INTRODUCTION

For decades, antibiotics have been widely used as growth promoters in poultry diets to enhance productivity, improve feed efficiency, and maintain flock health. This practice has ultimately been reshaped worldwide in response to growing concerns over antimicrobial resistance, which is driven by antibiotic residues in animal-derived food products, as well as the proven link between antimicrobial use in food-producing animals and the emergence of resistant bacteria transmissible to humans (Abate *et al.*, 2025; Theodoridou Oxinou *et al.*, 2025). Consequently, antibiotic growth promoters (AGPs) have been progressively restricted and eventually banned in many regions, beginning with a complete ban in the European Union in 2006 (Beber *et al.*, 2025), with similar restrictions implemented in the United States in 2017 (Gens *et al.*, 2022), followed by China in 2020 (Wen *et al.*, 2022). These regulatory changes have pushed the poultry industry to develop effective non-antibiotic approaches capable of sustaining performance, ensuring food safety, and satisfying increasing consumer expectations for antibiotic-free products.

The withdrawal of AGPs has created a production environment in which broilers are more susceptible to intestinal imbalance, subclinical health challenges, and performance losses, particularly under intensive, high-density rearing conditions (Fernández Miyakawa *et al.*, 2024; Maria Cardinal *et al.*, 2019; Selim *et al.*, 2024). In response to these challenges, phytogenic feed additives (PFAs), commonly termed phytobiotics or botanicals (Windisch *et al.*, 2008), have attracted considerable attention. These additives comprise plant-derived bioactive compounds, including herbs, spices, plant extracts, and essential oils (EOs), and are characterized by a high content of secondary metabolites such as flavonoids, alkaloids, tannins, saponins, and terpenoids (Adetunji *et al.*, 2025; Aminullah *et al.*, 2025). Among PFAs, EOs have received particular interest due to their broad biological activities, including antimicrobial, antioxidant, anti-inflammatory, and immunomodulatory effects (Mirković *et al.*, 2025; Pezantes-Orellana *et al.*, 2024; Varijakzhan *et al.*, 2021).

A growing body of literature confirms that EOs from diverse botanical sources can act as effective growth-promoting additives in broiler diets. Supplementation with EO blends has been shown to improve body weight, feed conversion ratio (Abd El-Hack *et al.*, 2022; Khan *et al.*, 2024), intestinal morphology, and digestive enzyme activity (Johnson *et al.*, 2022; Su *et al.*, 2021), as well as to beneficially alter serum lipid profiles and antioxidant status (Elbaz *et al.*, 2022b; Yarmohammadi Barbarestani *et al.*, 2020), often under both standard and challenge conditions such as necrotic enteritis (Gharaibeh *et al.*, 2021; Huang *et al.*, 2025). Meta-analyses and controlled trials consistently report that EO supplementation can reduce cecal pathogenic bacteria, enhance beneficial microbiota (Akan *et al.*, 2025; Irawan *et al.*, 2021), and improve carcass and organ traits (Islam *et al.*, 2025; Obeidat *et al.*, 2022), thereby providing a functional alternative to AGPs in modern broiler production.

The biological efficacy of EOs is largely attributed to their dominant terpenoid constituents, of which alpha-pinene is one of the most abundant and widely distributed monoterpenes in nature (Bagchi *et al.*, 2020). Alpha-pinene is a major component of turpentine and of EOs derived from coniferous species (Al-Tel *et al.*, 2020). This compound has been reported to possess broad-spectrum antimicrobial activity (Allenspach *et al.*, 2021; Borges *et al.*, 2022), antiviral effects (Başer *et al.*, 2023), and notable anti-inflammatory and antioxidant properties (Allenspach *et al.*, 2021; Rahimi *et al.*, 2023). Given these bioactivities, alpha-pinene represents a promising target for the development of functional feed additives aimed at enhancing gut health, immune resilience, and overall performance in broilers. Among conifer-derived EOs, those obtained from cypress (*Cupressus sempervirens* L.) and juniper (*Juniperus communis* L.) are particularly rich in alpha-pinene (Ložienė *et al.*, 2016; Patgar *et al.*, 2021). Yet, despite their long-standing use in traditional medicine (Galovičová *et al.*, 2023; Tufail *et al.*, 2023), these oils have received limited attention as feed additives in poultry nutrition. So far, the effects of these oils in broiler chickens remain unexplored, as research on EOs in poultry has mostly focused on a relatively limited set of botanical sources, such as oregano, thyme, rosemary, and eucalyptus species.

Therefore, the present study addresses the knowledge gap concerning alpha-pinene-rich EOs from cypress and juniper by evaluating their individual and combined effects on broiler growth performance and selected serum biochemical markers. It is hypothesized that supplementation with either cypress or juniper EOs will improve broiler performance and favorably modulate serum biochemical markers compared with non-supplemented controls, while concurrent administration of both oils may exert additive or synergistic effects on performance and health-related parameters, thereby representing a promising natural alternative to AGPs and supporting more sustainable poultry production.

MATERIALS AND METHODS

Study location and environment: This study was conducted at the Salem Poultry Group facility in Biskra, Algeria (Latitude: 34°50′13″ N, Longitude: 5°53′09″ E, Height: 101 m). The broiler production site includes 12 houses, each with a capacity of 50,000 caged birds. The houses were equipped with Big Dutchman’s climate controller system, Viper Touch® (software version: 5.3.4 Build 14).

Essential oils: Juniper (*Juniperus communis*) and cypress (*Cupressus sempervirens*) essential oils (EOs) were purchased from local suppliers, who also provided the main components detected by GSMS (Table 1). To prepare the EOs solutions for administration in drinking water, Tween 20 and propylene glycol were used as emulsifying agents to ensure solubility. The juniper (JEO) and cypress (CEO) solutions contained 25% EO, 25% Tween 20, and 50% propylene glycol. Additionally, a mixture of the two oils (MEO) was prepared containing 12.5% of each oil, 25% Tween 20, and 50% propylene glycol.

Table 1: Main compounds of the essential oil of *C. sempervirens* and J. *communis*

Compound	CEO (%)	JEO (%)
α-Pinene	89.38	68.33
β-Pinene	-	1.01

δ-3-Carene	7.07	5.77
nerolidol	-	4.77
β- Myrcene	0.58	2.56
limonene	0.77	1.26
Terpinyl acetate	0.26	1.26
Germacrene D	-	1.07
terpinen-4-ol	0.28	-
Sabinene	0.53	0.47
p-Cymene	-	0.22
γ-Terpinene	-	0.50
terpinolene	-	0.71
β-Caryophyllene	-	0.40
α-Humulene	-	0.47
δ-Cadinene	-	-
β-Phellandrene	0.65	0.47
p-cymene	-	0.50

CEO: Essential oil of *Cupressus sempervirens*.
JEO: Essential oil of *Juniperus communis*.

Broiler chickens, experimental design, and diets: The experiment was conducted on 480 one-day-old, non-sexed Arbor Acres Plus chicks (mean body weight of 49.02 ± 0.91 g), obtained from a commercial hatchery belonging to the same poultry production group. Hatching eggs from a 54-week-old breeding flock were stored for 4-8 days at 16 °C and approximately 75% relative humidity, with automatic turning every hour. Short periods of incubation during egg storage (SPIDES) were not performed. At hatching, chicks received two vaccine injections: TRANSMUNE® for Infectious Bursal Disease (IBD), and NEWFLEND® ND H9 for Newcastle disease virus (NDV) and low-pathogenic avian influenza virus (LPAIV) subtype H9. Additionally, live vaccines were administered by spraying: CEVAC® VITABRON L for NDV and infectious bronchitis virus (IBV), and CEVAC® IBIRD for an IBV variant.

After a 4-hour processing time at the hatchery, chicks were transported in the early afternoon to a broiler house located at the same facility. Upon arrival, chick quality was assessed using the Pasgar© scoring system, resulting in an average score of 9.72, while mean chick length and cloacal temperature were 19.5 ± 0.41 cm and 39.79 ± 0.37 °C, respectively. After quality assessment and individual weighing, the birds were randomly allocated to five treatment groups following a completely randomized design (CRD), with each treatment comprising eight replicate pens of 12 birds each.

The study was conducted under standard commercial farming conditions concurrently with an ongoing production cycle. The negative control (NC) group received a corn–soybean meal diet without antibiotic growth promoters (AGPs) and plain drinking water. The positive control (PC) group followed the standard management program applied in the broiler facility, including continuous in-feed monensin (80 mg/kg) as AGP, and a preventive course of tylosin (100% active substance) on day 8 for 5 days, as part of the farm's established prophylactic protocol. A therapeutic course of tylosin combined with enrofloxacin (200 mg/ml) was also administered to the PC group via drinking water. The Juniper essential oil (JEO), Cypress essential oil (CEO), and mixed essential oil (MEO) group (a mixture of the two EOs), received their respective EO solutions in drinking water at 2.5 ml/L of 25% stock solution, delivering a final concentration of 0.625 ml/L (625 ppm) of pure EO, for 3 days followed by 3 days of plain water. This administration cycle was repeated until the end of the experiment. Since the chicks were initially vaccinated at the hatchery, they only received a booster vaccination against NDV via spray on day 12 using CEVAC® NEW L vaccine. The physicochemical characteristics of drinking water are shown in Table 2.

Table 2: Physicochemical composition of the water sample.

Parameter	Result	Unit	Reference method
Ammonium	0.03	mg/L	SP-indophenol method
Bromide	0.13	mg/L	SP
Calcium	180	mg/L	SP-AFNOR T90-003
Chlorides	195.25	mg/L	Titrimetry-Mohr method
Electrical conductivity	155.5	µS/cm	Conductivity meter-ISO 7888
Hardness (Hydrometric title TH)	97	°F	Titrimetry-EDTA method
Iron	0.04	mg/L	SP-orthophenanthroline method

Fluoride	0.68	mg/L	SP
Magnesium	70	mg/L	SP
Nitrates	30	mg/L	SP-cadmium reduction method
Nitrites	0.00	mg/L	SP-Griess method
pH	7.40	-	pH meter-ISO 10523
Total solids (Dry residue at 105°C)	1085	mg/L	Gravimetric (Rodier *et al.*, 2016)
Sulfates	800	mg/L	SP-barium chloride method
Salinity	0.333	g/L	NA 6360/92[1]
Total alkalinity (TAC)	19.5	°F	Titrimetry
Alkalimetric title (TA)	00	°F	Titrimetry

SP: Spectrophotometry.

[1] Algerian National Standard for water quality (1992 version).

Broilers had ad libitum access to the following diets: starter (1–19 days of age), grower (20–35 days of age), and finisher (36–42 days of age). The feed ingredients and nutrient compositions of the basal diets are presented in Table 3.

Table 3: Ingredient (%) and nutrient composition of the basal diet (as-fed basis).

Item %	**Days**		
	0-19[1]	**20-35[2]**	**36-42[3]**
Ingredients			
Maize	60.70	62.89	64.35
Soybean Meal (48% CP)	34.00	32.00	30.00
Soybean oil	1.50	1.50	2.00
Calcium carbonate	1.00	1.00	1.00
Monocalcium phosphate	1.10	1.10	1.05
Vit-Min Premix[1,2,3]	1.06	0.87	0.99
Dl-Methionine	0.30	0.27	0.28
L-Lysine-HCl	0.26	0.28	0.26
L-Threonine	0.08	0.09	0.07
Total	100.00	100.00	100.00
Analyzed nutrient composition[4]			
Moisture	11.6	11.85	11.92
Crude protein	21.24	20.76	18.44
Ash	5.61	5.44	4.54
Crude fiber	2.54	2.74	2.42
Crude fat	4.34	4.53	4.92
Phosphorus	0.61	0.58	0.56
Calcium	0.86	0.83	0.71
Calculated nutrient composition[5]			
ME (MJ/kg)	12.86	12.92	13.06
Lysine	1.12	1.07	1.01
Methionine	0.32	0.31	0.30
Methionine + cysteine	0.69	0.66	0.64

Vitamin and mineral premix supplied per kg of diet

[1] Starter phase : 833,333 IU of vitamin A; 250,000 IU of vitamin D3; 3,750 mg of vitamin E; 191.67 mg of vitamin K3; 150 mg of thiamine; 450 mg of riboflavin; 3,750 mg of niacin; 1,225 mg of pantothenic acid; 375 mg of pyridoxine; 116.67 mg of folic acid; 2.25 mg of cyanocobalamin; 8.33 mg of biotin; 33,600 mg of choline chloride; 4583 mg of Fe; 1,167 mg of Cu; 4,583 mg of Zn; 5,250 mg of Mn; 167 mg of I; 16.67 mg of Se.

[2] Grower phase: 666,666 IU of vitamin A; 200,000 IU of vitamin D3; 3,000 mg of vitamin E; 153.33 mg of vitamin K3; 120 mg of thiamine; 360 mg of riboflavin; 3,000 mg of niacin; 980 mg of pantothenic acid; 300 mg of pyridoxine; 93.33 mg of folic acid; 1.8 mg of cyanocobalamin; 6.67 mg of biotin; 16,800 mg of choline chloride; 3667 mg of Fe; 933 mg of Cu; 3,667 mg of Zn; 4,200 mg of Mn; 133.33 mg of I; 13.33 mg of Se.

[3] Finisher phase: 623,338 IU of vitamin A; 187,001 IU of vitamin D3; 2,805 mg of vitamin E; 143.37 mg of vitamin K3; 112.2 mg of thiamine; 336.6 mg of riboflavin; 2,805 mg of niacin; 916.31 mg of pantothenic acid; 280.5 mg of pyridoxine; 87.27 mg of folic acid; 1.68 mg of cyanocobalamin; 6.23 mg of biotin; 23,400 mg of choline chloride; 3667 mg of Fe; 933 mg of Cu; 3,667 mg of Zn; 4,200 mg of Mn; 133.33 mg of I; 13.33 mg of Se.

[4] Values were determined using NIR spectroscopy.

[5] Values were calculated from data provided by the INRA CIRAD AFZ Feed Tables (2020 edition, as-fed).

Health challenge and interventions: A disease outbreak occurred during the experimental period, with the first clinical signs, including depression and ruffled feathers, observed at 28 days of age. To investigate the etiology of the outbreak, 10 clinically affected birds, selected across the experimental groups, were subjected to necropsy and submitted to the Veterinary Hospital Center "Le Refuge", Ayoun El Assafir, Batna, Algeria. Gross pathological examination revealed hepatomegaly with fibrinous perihepatitis, lesions consistent with colibacillosis. Liver and heart samples were aseptically collected and cultured on MacConkey agar and blood agar, followed by aerobic incubation at 37 °C for 24–48 h. Bacterial isolates were identified as *Escherichia coli* based on Gram stain, colony morphology and confirmation using API 20E biochemical tests system, after that, serotyping by agglutination has confirmed belonging to Serotype O1, which is highly implicated in avian colibacillosis. Antimicrobial susceptibility testing was performed using the Kirby–Bauer disk diffusion method on Mueller–Hinton agar.

Following laboratory confirmation of *E. coli* infection and demonstration of in vitro susceptibility to enrofloxacin, a therapeutic antibiotic intervention was initiated in the PC group on day 29 and administered for five consecutive days. Enrofloxacin was selected based on the antibiogram results, while tylosin was co-administered to address the potential involvement of concurrent bacterial respiratory pathogens commonly associated with field outbreaks. No therapeutic antibiotic intervention was administered to the NC or EO-treated groups during the outbreak.

Management and rearing of birds: Broiler chickens were placed in a 240 × 160 × 50 cm cage divided into four compartments, each comprising a pen, resulting in a stocking density of approximately 12 birds per square meter. The drinking nipples and automatic feeding systems were disabled, and manual feeders (6 kg) and water reservoir tanks (3 L) were used instead. Feed and water quantities were manually measured and recorded daily to ensure precise intake control.

Lighting was provided using LED tube lamps installed in each cage. White light intensity was adjusted according to the rearing phase, decreasing from 50 lux during the starter period to 20 lux during the finisher phase. The light color was temporarily switched to blue at an intensity of 5 lux during stressful handling procedures, such as vaccination and weighing. Continuous light was given for the first two days (24 h), followed by 23 h of light and 1 h of darkness until day 9. From day 9 onwards, the light cycle was 18 h of light followed by 6 h of continuous darkness.

The house temperature was carefully controlled using an automated climate control system, starting at 33 °C and gradually decreasing to 21 °C. Relative humidity (RH) averaged 48.4 ± 6.23%, and the mean temperature-humidity index (THI) was 72.9 ± 5.04. The THI was calculated according to NRC (1971) using the formula:

$$THI = (1.8 \times T + 32) - [(0.55 - 0.0055 \times RH) \times (1.8 \times T - 26)]$$

where T is dry-bulb temperature (°C) and RH is relative humidity (%).

Adequate ventilation and climatic parameters were maintained throughout the study to ensure optimal conditions for birds. The evolution of temperature, RH and THI over the study period are shown in Figure 1.

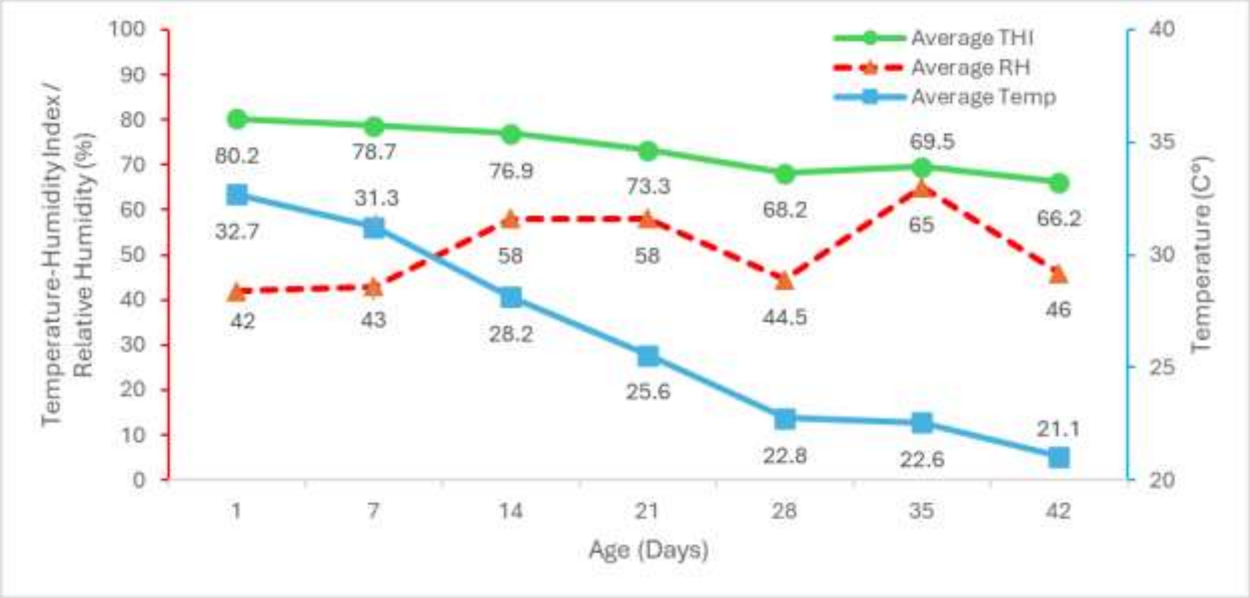

Figure 1: Air temperature, relative humidity and average temperature-humidity index during experimental period.

Traits Evaluated

Growth performance: During the rearing period, body weight (BW) was calculated weekly by weighing all the birds individually. Feed intake (FI) and water intake (WI) were recorded daily. Feed conversion ratio (FCR) and body weight gain (BWG) were then calculated. At the end of the study, the European production efficiency factor (EPEF) was calculated according to the following formula: (BW (kg) × livability (%) / age (days) × FCR) x 100 (Huff *et al.*, 2013).

Collection of blood samples for serum biochemical analyses: On day 42 of the experiment and after 8 h of fasting, blood samples were collected from the left-wing vein of one randomly selected chicken per pen (eight birds per treatment group). Immediately after collection, the blood samples were transported to a private medical laboratory for serum biochemical analyses. The samples were analyzed for various biochemical parameters including blood glucose, uric acid, creatinine, total protein, total cholesterol, HDL and LDL cholesterol, and triglyceride concentrations.

Statistical analysis: Prior to analysis, data collected and calculated during the experiment were tested for normality with the Shapiro–Wilk test and for homogeneity of variances using Levene's test. One-way analysis of variance (ANOVA) was performed to evaluate statistical differences between the treatment groups in a completely randomized design using IBM SPSS software (version 26). The following mathematical model was applied:

$$Y_{ij} = \mu + T_i + e_{ij}$$

In which Y_{ij} is the j-th observation in the i-th treatment group, μ is the overall mean, T_i is the fixed effect of the i-th treatment group, and e_{ij} is the random error. When the ANOVA indicated significant effects, treatment means were compared using Tukey's honestly significant difference (HSD) test. Differences were considered statistically significant at $p \leq 0.05$.

RESULTS

Table 4: Effects of different treatments on body weight during the experiment.

Age	Treatments					SEM	P-value
	NC	**PC**	**CEO**	**JEO**	**MEO**		
07D	213^{a}	203^{b}	205^{ab}	210^{ab}	208^{ab}	1.099	0.022
14D	543	541	525	528	525	3.189	0.212
21D	1061	1068	1035	1041	1042	6.469	0.456
28D	1545	1554	1590	1567	1574	9.485	0.621
35D	2055	2109	2232	2126	2136	23.508	0.200
42D	2531	2630	2702	2652	2609	22.555	0.181

$^{a, b}$ Means within the same row carrying different superscripts are significantly different ($p \leq 0.05$).
NC: Negative control; PC: Positive control; CEO: Cypress essential oil; JEO: Juniper essential oil; MEO: Mixed essential oils; SEM: Standard error of the mean.

The effects of essential oils (EOs) on body weight (BW) are summarized in Table 4. Significant differences in BW between the treatment groups were observed only on day 7 (p = 0.022), with the NC group recording the highest weight. From day 14 onward, BW did not differ significantly among treatments. However, from day 35 onward, EO-supplemented groups exhibited numerically higher BW than the controls, with the CEO group achieving the greatest increase (8.6% vs NC), followed by MEO (3.9%) and JEO (3.5%). This numerical trend persisted to day 42, where final BW was highest in the CEO group (6.8% relative to NC), followed by JEO (4.8%) and MEO (3.1%). Additionally, the PC group also outperformed the NC group by 3.9% and showed final BW values comparable to those of the JEO and MEO groups.

Table 5: Effects of different treatments on cumulative feed conversion ratio during the experiment.

Age	Treatments					SEM	P-value
	NC	**PC**	**CEO**	**JEO**	**MEO**		
07D	0.97	0.96	0.95	0.95	0.97	0.003	0.223
14D	1.06^{b}	1.00^{a}	1.04^{ab}	1.06^{b}	1.07^{b}	0.006	0.0001
21D	1.17^{b}	1.09^{a}	1.15^{b}	1.17^{b}	1.17^{b}	0.007	0.0001

28D	1.37^{b}	1.30^{a}	1.32^{ab}	1.36^{ab}	1.37^{b}	0.008	0.006
35D	1.49^{b}	1.40^{ab}	1.37^{a}	1.43^{ab}	1.45^{ab}	0.011	0.008
42D	1.57	1.49	1.50	1.54	1.57	0.012	0.135

$^{a,\,b}$ Means within the same row carrying different superscripts are significantly different ($p \leq 0.05$).

NC: Negative control; PC: Positive control; CEO: Cypress essential oil; JEO: Juniper essential oil; MEO: Mixed essential oils; SEM: Standard error of the mean.

Feed conversion ratio (FCR) did not differ among treatments at 7 days of age ($p = 0.223$). Subsequently, significant differences were observed, with the PC group exhibiting a significantly lower FCR at 14 and 21 days of age ($p < 0.001$). This pattern persisted until day 28 ($P = 0.006$), with the PC group maintaining the lowest FCR, while EO-supplemented groups showed values comparable to the NC group.

At day 35, the CEO group exhibited a significantly lower FCR compared to the NC group ($P = 0.008$), while the PC group and the other EO-treated groups showed intermediate values. By the end of the trial (day 42), this difference was no longer statistically significant; however, the CEO group maintained a numerically lower FCR (8.1% relative to NC) and remained comparable to the PC group.

Table 6: Effects of different treatments on the zootechnical parameters of broilers.

Item	Treatments					SEM	P-value
	NC	**PC**	**CEO**	**JEO**	**MEO**		
Mort (%)	22.9^{b}	28.1^{b}	10.4^{a}	17.7^{ab}	18.7^{ab}	1.509	0.001
FI (g)	3962	3917	4040	4076	4098	36.434	0.489
WI (ml)	10854	10679	11074	11259	11000	68.782	0.076
ADG (g)	59	61	63	62	61	0.544	0.220
EPEF	292^{b}	296^{b}	379^{a}	331^{ab}	316^{b}	7.347	0.0001

$^{a,\,b}$ Means within the same row carrying different superscripts are significantly different ($p \leq 0.05$).

NC: Negative control; PC: Positive control; CEO: Cypress essential oil; JEO: Juniper essential oil; MEO: Mixed essential oils; SEM: Standard error of the mean; Mort: Mortality; FI: Feed intake; WI: Water intake; ADG: Average daily gain; EPEF: European production efficiency factor.

Significant differences were observed between treatments for mortality ($p = 0.001$) and the European Production Efficiency Factor (EPEF) ($p \leq 0.001$). The CEO group recorded the lowest mortality rate (54.6% lower relative to NC), whereas the PC group showed the highest mortality (22.7% higher relative to NC). Weekly mortality rates are presented in Figure 2, showing a pronounced peak around week 5. Accordingly, the PC group received additional therapeutic interventions during the trial and was therefore not considered a pure AGP control or used as a benchmark for growth performance comparisons.

EO-supplemented groups achieved higher EPEF values than the NC group, with the CEO differing significantly compared to the control groups, exhibiting the greatest increase (29.8% relative to NC), followed by JEO (13.4%) and MEO (8.2%). No significant differences were found between the treatment groups in terms of feed intake (FI) ($p = 0.489$), water intake (WI) ($p = 0.076$), or average daily gain (ADG) ($p = 0.220$). Nevertheless, CEO group exhibited numerically higher ADG than the NC group (6.8%).

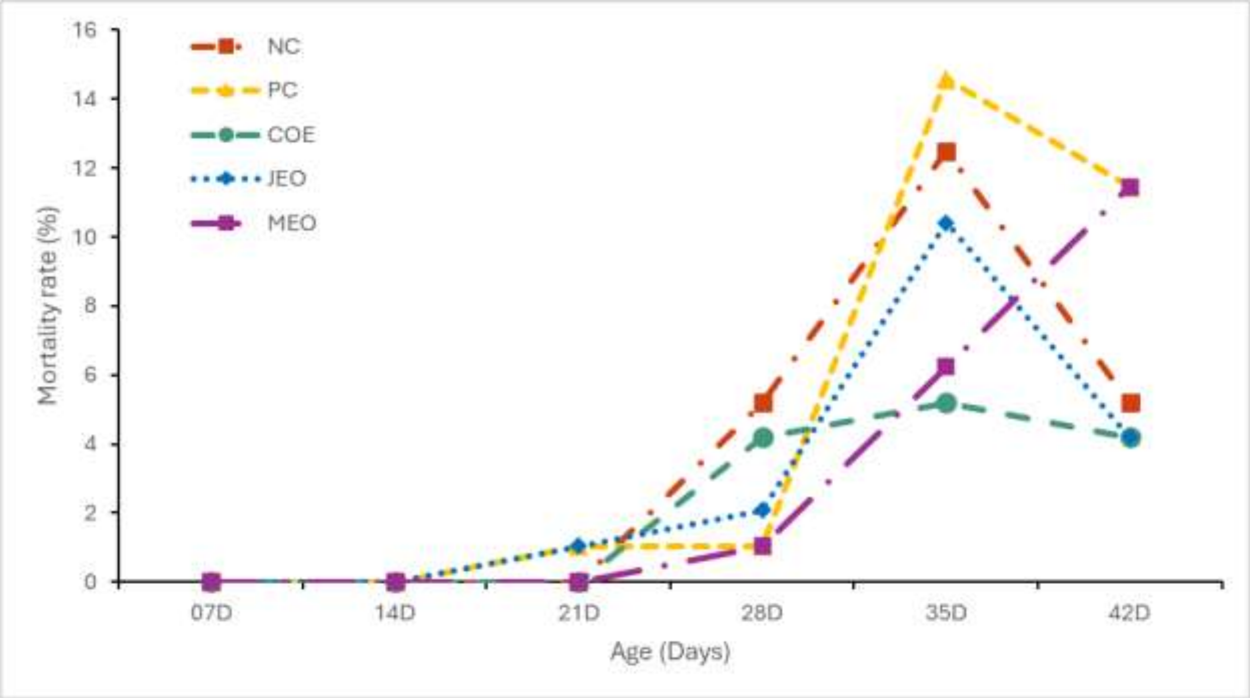

Figure 2: Weekly (non-cumulative) mortality rates during the experimental period.

Table 7: Effects of different treatments on the blood serum parameters of broilers.

Item	Treatments					SEM	P-value
	NC	**PC**	**CEO**	**JEO**	**MEO**		
Glu (g/l)	1.90	1.91	1.90	1.97	1.87	0.031	0.899
TG (g/l)	0.29^{a}	0.39^{b}	0.36^{ab}	0.39^{b}	0.26^{a}	0.013	0.0001
CHOL (g/l)	1.31^{b}	1.13^{ab}	1.09^{ab}	1.05^{a}	1.30^{b}	0.030	0.008
HDL (g/l)	0.88^{b}	0.77^{ab}	0.72^{a}	0.74^{ab}	0.86^{ab}	0.018	0.012
LDL (g/l)	0.34^{b}	0.32^{ab}	0.33^{ab}	0.24^{a}	0.34^{b}	0.011	0.020
Crea (mg/l)	0.87^{b}	0.75^{ab}	0.97^{b}	0.76^{ab}	0.61^{a}	0.030	0.0001
UA (mg/l)	21.5^{a}	26.2^{ab}	30.1^{b}	29.2^{b}	27.4^{ab}	0.862	0.009
TP (g/l)	47.0	43.5	44.9	44.4	43.2	0.801	0.616

$^{a,\ b}$ Means within the same row carrying different superscripts are significantly different ($p \leq 0.05$).

NC: Negative control; PC: Positive control; CEO: Cypress essential oil; JEO: Juniper essential oil; MEO: Mixed essential oils; SEM: Standard error of the mean; Glu: Glucose; TG: Triglycerides; CHOL: Cholesterol; HDL: High-density lipoprotein; LDL: Low-density lipoprotein; Crea: Creatinine; UA: Uric acid; TP: Total protein.

Glycaemia levels were similar across all treatment groups, with no significant differences observed ($p = 0.899$), indicating that the inclusion of EOs did not significantly impact blood glucose levels compared to the control groups.

Triglyceride concentrations varied significantly between the groups ($p \leq 0.001$), with the MEO group having the lowest value. Total cholesterol levels also differed ($p = 0.008$) with the JEO group had the lowest value, significantly lower than those of the NC and MEO groups, whereas the PC and CEO groups showed intermediate values. Dietary supplementation with CEO significantly reduced HDL concentrations ($p = 0.012$) compared to the other treatment groups. Significant differences were also observed in LDL levels ($p = 0.020$), with the JEO group recording the lowest value, significantly lower than those in the NC and MEO groups.

Serum creatinine concentrations varied significantly among the treatment groups ($p \leq 0.001$), with the MEO group having the lowest value. Uric acid levels also varied significantly ($p = 0.009$); the NC group had the lowest concentration, significantly lower than those in the CEO and JEO groups.

No significant differences were observed in serum total protein levels between the treatment groups ($p = 0.616$), indicating that the EOs did not significantly affect the total protein compared to the control groups.

DISCUSSION

Alpha-pinene, a major monoterpene, is widely found in the essential oils (EOs) of various plant species, particularly in conifers of the *Pinaceae* family (Bakó *et al.*, 2024; Dudek *et al.*, 2025), as well as in some herbs of the *Lamiaceae* family (Spréa *et al.*, 2024). It is known for its distinctive pine aroma and its diverse biological activities have been extensively studied (Silva *et al.*, 2012; Park *et al.*, 2021). Numerous studies have explored the effects of EOs containing alpha-pinene in broiler chickens, highlighting their potential as natural growth promoters (Hairui *et al.*, 2024; Hesabi Nameghi *et al.*, 2019; Tekce *et al.*, 2020; Yesilbag *et al.*, 2011). Nevertheless, this study is the first to investigate the EOs of juniper (*Juniperus communis*) and cypress (*Cupressus sempervirens*), both of which are rich in alpha-pinene (Zheljazkov *et al.*, 2021; Medini *et al.*, 2025).

The current study's trial was conducted under routine commercial field conditions in an intensive broiler production facility, following standard industry management practices. During the late grower phase, a natural outbreak of colibacillosis occurred, causing clinical signs and characteristic post-mortem lesions. As a result, the study was effectively transformed from a standard growth performance evaluation into a disease challenge model, providing a realistic context to assess the response of EO-supplemented groups under bacterial stress. The positive control (PC) group, which received an in-feed antibiotic growth promoter (AGP) along with preventive and therapeutic antibiotic courses, does not represent a pure AGP control and should not be considered a valid benchmark for growth performance under these conditions.

Overall, the core growth parameters showed non-uniform temporal patterns, with statistically significant differences appearing sporadically, rather than persisting throughout the experimental period. Such transient responses are consistent with the widely reported variability in the efficacy of EOs, which tend to exert more pronounced benefits under challenging conditions, such as environmental heat stress or increased pathogenic pressure (Placha *et al.*, 2019; Abdelli *et al.*, 2021; Hosseinzadeh *et al.*, 2023). The spontaneous infection in the current study may have interacted with EOs supplementation to drive the observed transient improvements in performance and health parameters in the treated groups. For instance, although differences were not statistically significant, numerical improvements were observed in the cypress EO (CEO) group, with body weight (BW) approximately 7% higher than the negative control (NC) group on day 42, and 9% higher on day 35. Similar responses under disease challenge conditions have been reported by Elbaz *et al.* (2024) who observed improvements in BW and body weight gain (BWG) under coccidiosis challenge conditions following supplementation with a mixture of oregano EO and probiotics. Similarly, broiler chickens facing an intestinal challenge involving *Eimeria spp*. and *Clostridium perfringens* showed improvements in feed intake (FI), BWG, and feed conversion ratio (FCR) when supplemented with a protected blend of organic acids and EOs (Stefanello *et al.*, 2020). Although immune parameters, anti-inflammatory markers, and gut health metrics were not measured in the present study, the numerical and transient improvements may be attributed, at least in part, to the immunomodulatory effects exerted by the EO and their bioactive components (Ibrahim *et al.*, 2022; Zaazaa *et al.*, 2022). EO supplementation has been shown to attenuate inflammatory responses, support intestinal barrier integrity, and enhance overall immune competence (Movahedi *et al.*, 2024; Oni *et al.*, 2025; Sampath *et al.*, 2025), thereby facilitating compensatory growth and performance recovery in challenged broiler chickens (Zhang *et al.*, 2023; Mountzouris *et al.*, 2024). However, under *E. coli* challenge conditions, average daily gain (ADG) and FI remained unaffected across all treatments potentially due to nutrient repartitioning toward immune function (Remus *et al.*, 2014). This phenomenon likely limited the potential of the EOs to enhance FI and BWG in the present study, resulting in the final BW of the best-performing group (CEO) fell short of the breed's performance objectives (Aviagen, 2022) by approximately 9.4%. Similarly, Gordillo Jaramillo *et al.* (2021) found no significant effects of oregano and citrus EOs on FI or BWG in broiler chickens orally challenged with a coccidiosis vaccine at 25 times the recommended dose. In line with these findings, under non-challenged conditions, broilers fed diets supplemented with rosemary, thyme and oregano powder showed no significant changes in FI or BWG (Khatun *et al.*, 2025), nor did those receiving thyme and oregano EOs, alone or combined (Zaazaa *et al.*, 2022). Furthermore, several studies have indicated limited EO impacts on growth: for example, Gumus and Gelen (2023) found no effect of thyme and rosemary EOs on BW, BWG or FCR; Noruzi *et al.* (2022) reported no significant changes in BW following dietary supplementation with a blend of nine EOs; and dietary inclusion of different levels of cinnamon EO in Arbor Acres commercial male broilers did not affect performance parameters, including BW and BWG (Yang *et al.*, 2019). In contrast, certain EO formulations have demonstrated benefits, such as 400 mg/kg fenugreek EO enhancing FI and ADG (Fawaz *et al.*, 2025), or a blend of EOs containing 200 mg/kg cinnamaldehyde, 200 mg/kg carvacrol, and 100 mg/kg thymol significantly improving these metrics (Malhi *et al.*, 2025).

In terms of FCR, the CEO group showed lower values compared to the control groups on day 35, indicating improved feed efficiency at the end of the grower period. This improvement may be related to the beneficial effects of EOs on gut health and inflammatory status (Adil *et al.*, 2025). In this regard, Pham *et al.* (2023) reported that dietary supplementation with a coated EO and organic acids improved FCR in broilers challenged with *E. coli*, an effect associated with reduced intestinal damage, lower bacterial load, and modulation of inflammatory responses. Such mechanisms may partially explain the improved feed efficiency observed, particularly under the natural challenge condition of the present study. The overall trend of improved FCR with cypress EO aligns with previous studies conducted under standard rearing conditions, in which supplementation with lavender, thyme, and clove EOs resulted in reduced FCR (Yarmohammadi Barbarestani *et al.*, 2020; Amouei *et al.*, 2021; Elbaz *et al.*, 2022a).

Previous studies have shown that phytogenic supplements administered in drinking water can influence both water intake (WI) and productive responses in broilers. For example, Ashour *et al.* (2025) reported a decrease in WI following water supplementation with Echinacea extracts, yet this was accompanied by improvements in physiological and productive parameters. In contrast, the present study found no significant differences in WI between treatments, indicating that EO supplementation did not adversely affect water palatability. This stability in water consumption highlights a key advantage of administering EOs via drinking water over conventional in-feed supplementation (Rocha *et al.*, 2024), particularly under health-challenge conditions in which FI may be compromised while water consumption remains relatively stable, thereby ensuring sustained delivery of EO bioactive compounds.

The significant difference in BW on day 7, with the NC group exhibiting the highest values, indicates that neither the EOs nor the AGP provided a growth advantage during the initial starter phase. Although EOs can affect water intake in young chicks due to their strong aroma, no such effects were observed in this study. Instead, the superior performance of the NC group likely reflects the absence of unnecessary interventions in a well-managed, low-stress environment, where birds perform near their genetic potential; therefore, growth promoters tend to have less favorable effects (Attia *et al.*, 2016; Attia *et al.*, 2017; Pourmahmoud *et al.*, 2013). Furthermore, early AGP use can suppress beneficial gut bacteria and induce dysbiosis (Abbas *et al.*, 2024), while the EO dose employed, though beneficial later, may have been relatively high for immature chicks, potentially transiently impairing organ function or nutrient use (Aguilar *et al.*, 2013).

Mortality rates increased across all experimental groups, peaking in the fifth week after the colibacillosis outbreak. Control groups experienced higher mortality, whereas EO-supplemented groups, especially the CEO group, showed significantly lower rates. These findings align with necrotic enteritis challenge studies, in which EO blends resulted in zero mortality compared to 10% in untreated groups, accompanied by a significant reduction in intestinal lesion scores and modulation of the immune response toward an anti-inflammatory pathway (Gharaibeh *et al.*, 2021). As noted previously, the absence of physiological assessments limits direct causal links; nevertheless, the protective effects of the studied EOs are consistent with their well-documented antioxidant, antibacterial, and anti-inflammatory properties (Patgar *et al.*, 2021; Albrecht *et al.*, 2022; Tufail *et al.*, 2023; Maral *et al.*, 2024; Mirković *et al.*, 2025), which are mainly linked to their major active components, including alpha-pinene (Borges *et al.*, 2022; Bomfim De Barros *et al.*, 2023; Hoosen *et al.*, 2026). In vitro studies of cypress and juniper EOs have demonstrated notable antimicrobial activity against poultry-relevant pathogens. For example, cypress EO exhibited antibiofilm activity against *Salmonella enterica* isolated from chicken samples (Galovičová *et al.*, 2023), while juniper EO inhibited the growth of *Campylobacter jejuni* (Klančnik *et al.*, 2018), and extracts from *Juniperus* species showed broad-spectrum activity against both *Staphylococcus aureus* and *E. coli* (Darwish *et al.*, 2020). Additionally, EO derived from *Sideritis* species, in which alpha-pinene was identified as the major component, demonstrated antimicrobial activity similar to vancomycin against methicillin-resistant *Staphylococcus aureus* (MRSA) and other multidrug-resistant bacteria (Kose *et al.*, 2010).

However, alpha-pinene itself showed moderate to low antimicrobial action against *E. coli* and multi-resistant *E. coli* (Dorman *et al.*, 2000; Leite-Sampaio *et al.*, 2022), suggesting that the observed reductions in bacterial burden and fibrin deposition in EO-treated groups are likely associated with synergistic interactions among EO constituents. Such synergistic interactions have been documented for combinations such as peppermint and thyme or citrus EOs, resulting in enhanced activity against *E. coli* (Angane *et al.*, 2024; Ellouze *et al.*, 2024).

Beyond direct pathogen inhibition, EOs can modulate host immunity and intestinal microbiota, thereby enhancing systemic immune responses (Ge *et al.*, 2024). For instance, dietary supplementation with a blend of peppermint and clove EOs enhanced digestive enzyme activity and improved immune organ development (Reda *et al.*, 2025). Additionally, supplementation with basil, thyme, and sage EOs restored *Lactobacillus* populations and suppressed enteric pathogens (Vlaicu *et al.*, 2023). Similarly, clove EO has been shown to boost intestinal IgA and *Lactobacillus* counts and reduced *E. coli* populations (Elbaz *et al.*, 2022a), while oregano EO has been reported to improve gut morphology (villus height and crypt depth) and support lymphocyte proliferation and phagocytic activity (El-Sayed *et al.*, 2024). Collectively, these immunomodulatory and microbiota-stabilizing actions may help explain the enhanced resilience observed in the EO-supplemented groups.

The higher mortality in the PC group highlights a limitation in mechanistic interpretation inherent to the study design. The PC group likely included birds with more severe initial disease presentations; however, because disease severity was not objectively assessed, and the protocol involved administration of therapeutic antibiotics during a disease episode, in addition to baseline AGP supplementation, multiple confounding variables are introduced. Furthermore, the timing and dosage of the therapeutic intervention may have been insufficient or delayed to fully mitigate the disease impact, complicating the determination of individual factors to the observed mortality.

The European Production Efficiency Factor (EPEF), which combines several zootechnical parameters, showed a highly significant improvement in the CEO group. This supports the notion that EOs can enhance the overall production efficiency, as reported by İpçak *et al.* (2024), who found that the addition of fennel seeds EO at different doses significantly improved both FCR and EPEF. Another study indicated that broiler chickens fed diets supplemented with EOs from basil, thyme, and sage showed enhanced EPEF and European Broiler Index (EBI) (Vlaicu *et al.*, 2023). In contrast, Ghiasvand *et al.* (2021) found that fennel EO reduced BWG during the finisher period and lowered the EPEF. In the present experiment, the improvement in EPEF observed in the CEO group was primarily driven by the significantly lower mortality rate, as differences in BW and FCR were only numerical. Thus, the higher EPEF recorded in the CEO group can be directly attributed to improved survivability rather than to changes in growth performance parameters.

The results of the present study showed no significant differences in blood glucose levels between the treatment groups, suggesting that EOs did not interfere with carbohydrate metabolism. This is consistent with previous reports by Santos *et al.* (2019), who similarly reported no significant differences in glycemia, total cholesterol, or total protein levels between the control groups and those receiving diets supplemented with lemongrass EO. The JEO group showed significantly lower cholesterol and LDL levels, and MEO significantly reduced triglycerides levels, in contrast to the study of Khan *et al.* (2024), who found that adding different levels of a blend of EOs containing eucalyptus and citrus to broiler diets did not significantly affect cholesterol, triglycerides, LDL, or HDL levels. The plasma lipid-lowering effects observed with JEO and MEO are consistent with previous findings; for example, dietary supplementation with a commercial blend of EOs has shown similar effects (Kahiel *et al.*, 2025). Similarly, Chowdhury *et al.* (2018) reported lower cholesterol concentrations in diets supplemented with cinnamon and clove EO compared to the control and AGP groups. Furthermore, a linear reduction in cholesterol levels was observed in laying hens as dietary inclusion of clove bud powder increased (Rahman Alizadeh *et al.*, 2017). This effect could be attributed to the lipid-lowering activity of specific bioactive compounds, particularly those found in EOs, which may interfere with cholesterol synthesis or enhance lipid metabolism (Ahmad Firdaus B *et al.*, 2020; Zhu *et al.*, 2023). In addition, it has been reported that the 3-hydroxy-3-methylglutaryl-CoA reductase (HMG-CoA) is the rate-limiting enzyme involved in cholesterol synthesis (Baskaran *et al.*, 2015), thus, it was suggested that the decrease in cholesterol content could be due to the inhibiting effects of geraniol and carvacrol on HMG-CoA reductase (Almalki *et al.*, 2024).

In the current study, no significant differences were observed between treatments in total protein levels, in contrast to the studies of Hosseinzadeh *et al.* (2023), who reported an increase in total protein in broilers fed 200 mg/kg of Indian borage EO, while uric acid, glucose, and triglycerides concentrations remained unchanged. Furthermore, supplementation with different levels of eucalyptus EO did not significantly affect glucose, total protein, cholesterol, triglyceride, and HDL levels (Mohebodini *et al.*, 2021). Significant differences were observed in blood creatinine levels, with the MEO group showing the lowest values. This is consistent with the findings of Abo Ghanima *et al.* (2021), who reported that dietary supplementation with tea tree EO significantly reduced creatinine and uric acid concentrations. The lower creatinine levels observed in the MEO group may indicate enhanced renal function and reduced muscle catabolism, as elevated creatinine concentrations are typically associated with significant muscle damage (Ritchie *et al.*, 1994).

Although the precise modes of action of EOs remain incompletely defined, the present findings indicate that their beneficial effects are more pronounced under health-challenging conditions. In this study, the improved performance of the CEO group, particularly in survivability and overall production efficiency, appears to reflect enhanced physiological resilience rather than a sustained improvements in growth performance. Meanwhile, juniper and the combined EO treatments mainly contributed to maintaining stable performance relative to the control groups.

A key limitation of the present study is that mechanistic effects of cypress and juniper EOs were not directly assessed. Parameters such as gut morphology, microbiota composition, anti-inflammatory function, and immune markers were not measured; thus, the suggested benefits remain hypothetical and based on previous literature. Another limitation concerns the design of the PC group, which received additional antibiotic treatments in combination with the in-feed AGP. Consequently, this group does not represent a strict AGP-only control, and comparisons of growth performance involving this group should be viewed in light of the potential confounding effects of the additional therapeutics. Furthermore, weekly growth parameters (BW and FCR) were analyzed independently at each time point, using repeated-measures model could have provided a more robust analysis and allowed assessment of treatment and time interactions.

Conclusion: Within the constraints of the current study, the tested EOs exerted their primary benefits by improving resilience and survivability under conditions of compromised flock health. The EOs favorably modulated lipid profiles and creatinine concentrations, while CEO in particular significantly reduced mortality and improved EPEF.

The observed variability in responses may be related to pulsed administration regimen or dosage optimization, highlighting the need to explore different concentrations and continuous supplementation. Further limitations include the absence of direct assessment of nutrient digestibility, gut microbiota, and immune responses which limit physiological interpretation of the observed effects. Future studies should therefore focus on alpha-pinene-rich EOs, with particular emphasis on gut health and immune function, to better define their role in poultry nutrition.

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