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Article

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Effects of in ovo monolaurin and *Ferula badrakema* root essential oil on broiler performance, intestinal health and oxidative status following *E. coli* challenge

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ABSTRACT

This study investigated the effects of in ovo administration of monolaurin and Ferula badrakema root essential oil (FBEO), alone or with an Escherichia coli (E. coli) challenge, on growth performance, intestinal health, oxidative status, blood biochemical traits, and immune response in broiler chickens. A total of 1,750 Ross 308 eggs were incubated; after day-17 candling, 1,050 viable fertile eggs were allocated to seven treatments with five replicates of 30 eggs. Treatment affected hatchability, hatch weight, and early growth, with FBEO at 5 mg/egg showing favorable responses. At 42 days, treatment affected live body weight, carcass, breast and thigh yields, relative spleen weight, and jejunal villus height and surface area ($P < 0.05$). Cecal E. coli counts were also affected. Serum glucose, low-density lipoprotein, triglycerides, and phosphorus, and hepatic superoxide dismutase, malondialdehyde, and total antioxidant capacity differed among treatments, whereas liver enzyme activities and humoral antibody responses were not significantly affected. Taken together, in ovo monolaurin and FBEO influenced selected growth, intestinal, bacterial, and oxidative responses through 42 days, with FBEO at 5 mg/egg showing particularly favorable responses. These findings support further evaluation of both compounds as natural in ovo bioactive compounds.

Treatment affected hatchability, hatch weight, and early growth performance, with FBEO at 5 mg/egg showing the most favorable early responses. At 42 days of age, treatment affected live body weight, carcass yield, breast and thigh yields, relative spleen weight, and jejunal villus height and surface area ($P < 0.05$). Cecal E. coli counts were also affected. Serum glucose, low-density lipoprotein, triglycerides, and phosphorus, as well as hepatic superoxide dismutase, malondialdehyde, and total antioxidant capacity, differed among treatments, whereas liver enzyme activities and humoral antibody responses were not significantly affected.

Taken together, in ovo administration of monolaurin and FBEO influenced growth performance, intestinal morphology, bacterial colonization, and oxidative status through 42 days of age, with FBEO at 5 mg/egg showing particularly favorable responses. These findings support further evaluation of FBEO and monolaurin as potential natural in ovo bioactive compounds for broiler production.

Keywords: Hatchability; Jejunal morphology; Oxidative stress; Phytogenic compounds; Antibacterial activity

Introduction

Hatchery environments play a pivotal role in the onset of early infections in broiler chickens, particularly those caused by Avian Pathogenic Escherichia coli (APEC). Contamination in hatcheries can arise from multiple sources, including poor egg hygiene, the use of floor-collected eggs, and vertical transmission from infected breeder flocks. These factors facilitate the early colonization of chicks' gastrointestinal tracts with pathogenic E. coli strains during the first hours of life, often leading to systemic infections and increased early mortality rates [1]. Moreover, hatcheries serve as potential hubs for cross-contamination among chicks from various sources, with

environmental stressors, such as poor ventilation and high humidity further exacerbating their vulnerability [2].

Escherichia coli infections, especially during the first week of life, are responsible for significant mortality in broiler flocks, with early deaths accounting for up to 50% of total flock mortality [3]. APEC infections typically manifest as systemic diseases such as colibacillosis, characterized by high morbidity and mortality. Common clinical outcomes include yolk sac infection, enteritis, respiratory infections, cellulitis, and septicemia. In acute cases, sudden death and septicemic lesions are observed, while more chronic forms may lead to pericarditis, airsacculitis, and perihepatitis. Addressing these challenges requires comprehensive strategies including improved hygiene, vaccination, and the development of effective non-antibiotic therapeutic alternatives [4].

One promising approach in this context is the in Ovo injection of beneficial substances. In ovo administration is commonly performed during the late stages of incubation, particularly around the time of transfer, with the amnion representing an effective target site for delivery. This approach provides an opportunity to expose developing embryos to vaccines, nutrients, and other bioactive compounds before hatch and has been investigated as a means of influencing subsequent chick health and productivity [5]. The effectiveness of in ovo administration depends on several factors, including the timing and site of injection, injection volume, dose, and the nature of the administered substance [5, 6].

Among candidate compounds for in Ovo use, monolaurin has shown considerable promise due to its broad-spectrum antimicrobial properties. As a monoglyceride of lauric acid naturally found in coconut oil and human breast milk, monolaurin is recognized as safe by the FDA and widely applied in pharmaceuticals, food, and cosmetics [7]. It exhibits antibacterial activity especially against Gram-positive bacteria by disrupting microbial membranes and inhibiting biofilm

formation [8, 9]. It also possesses antiviral effects against enveloped viruses (e.g., herpes simplex virus, SARS-CoV-2), and antifungal properties against Candida albicans [10], making it a versatile candidate for poultry health applications.

Another bioactive compound of interest is the essential oil of Ferula badrakema, a medicinal plant native to Iran. Phytochemical analysis of F. badrakema fruit essential oil has identified β-pinene (45.8%) and α-pinene (10.9%) as its major constituents, along with several other monoterpenes and sesquiterpenoid compounds. The essential oil has also demonstrated antimicrobial activity against selected microorganisms, supporting its potential as a source of bioactive compounds [11].

Extracts and essential oils from species of the genus Ferula contain diverse bioactive constituents, including sesquiterpene coumarins and sulfur-containing compounds. Sesquiterpene coumarins have been associated with several biological activities, including antibacterial and anti-inflammatory effects, while sulfur-containing compounds and other constituents of Ferula essential oils have also been investigated for their antimicrobial properties [12, 13].

Despite the pharmacological potential of F. badrakema, its application in poultry health remains underexplored.

To model field-relevant pathogenic challenges, this study employed the APEC strain EC-43AP (serotype O2) a clinically relevant isolate frequently associated with colibacillosis, pericarditis, and perihepatitis in poultry [1]. Avian pathogenic Escherichia coli (APEC) possesses a diverse repertoire of virulence-associated factors that contribute to bacterial adhesion, invasion, survival, and evasion of host defense mechanisms and is widely used in poultry pathogenesis research due to its established virulence and resistance profile [3].

A preceding dose-range experiment evaluated different in ovo doses of monolaurin and Ferula badrakema root essential oil (FBEO) with respect to hatchability, embryonic survival, early post-

hatch responses, and bacterial recovery. Based on the responses observed in that study, 5 mg/egg was selected for subsequent evaluation of the biological effects of both compounds in the present experiment.

A challenge dose of 10^2 CFU/egg of Escherichia coli was also selected based on the preceding dose-range experiment. Dimethyl sulfoxide (DMSO) was used as the vehicle control for monolaurin and FBEO.

Therefore, the present study was designed to investigate the effects of in ovo administration of FBEO and monolaurin on broiler chickens challenged with E. coli EC-43AP. The evaluated parameters included hatchability, growth performance (body weight gain, feed intake, and feed conversion ratio), immune response (anti-SRBC antibody titers), antioxidant status (superoxide dismutase, glutathione peroxidase, total antioxidant capacity, and malondialdehyde), small intestinal morphology (villus height, crypt depth, and villus surface area), and cecal E. coli population. The study was intended to determine whether administration of these compounds before hatch could influence subsequent growth, physiological responses, intestinal morphology, and bacterial colonization following E. coli challenge.

Methods

Ethical approval

All procedures involving animals were approved by the Animal Ethics Committee of Ferdowsi University of Mashhad (Mashhad, Iran) and were conducted in accordance with institutional guidelines for the care and use of experimental animals and the ARRIVE 2.0 guidelines.

Anesthesia and euthanasia

At 3 and 42 days of age, birds selected for sampling were anesthetized by carbon dioxide (CO_2) inhalation and subsequently euthanized by cervical dislocation.

Plant material and essential oil extraction

Roots of *Ferula badrakema* Koso-Pol. were collected from the wild in Tandoureh National Park, Dargaz County, Razavi Khorasan Province, northeastern Iran. The plant was identified by Dr. Hamid Moazzeni, Herbarium Research Group, Research Institute of Plant Sciences, Ferdowsi University of Mashhad, Iran, and a voucher specimen was deposited in the Herbarium of Ferdowsi University of Mashhad (FUMH; voucher No. 9818). Permission for plant collection was obtained from the General Department of Natural Resources and Watershed Management of Razavi Khorasan Province (Authorization No. 114/70/1405/01010). Plant collection and related field procedures complied with applicable institutional, national, and international regulations and guidelines, including the IUCN Policy Statement on Research Involving Species at Risk of Extinction and CITES, where applicable.

The roots were subjected to hydrodistillation for 3 h using a Clevenger-type apparatus. The essential oil was dried over anhydrous sodium sulfate, transferred to amber glass vials, and stored at 4 °C until use. The chemical composition of the root essential oil was determined by gas chromatography as described previously [21].

Experimental compounds and bacterial strain

Monolaurin and Ferula badrakema root essential oil (FBEO) were prepared in dimethyl sulfoxide (DMSO). The Escherichia coli strain used for challenge was EC-43AP O2, obtained from the Department of Veterinary Microbiology.

The doses used in the present study were selected based on a separate dose-range and antibacterial evaluation of in ovo-administered monolaurin and FBEO conducted by the same research group and submitted concurrently with the present manuscript. Based on the findings of that study, 5 mg/egg of monolaurin, 5 mg/egg of FBEO, and 10^2 CFU/egg of E. coli were selected for the present 42-day experiment. The dose-range study and the present study involved independent experimental datasets, with the former serving only as the basis for dose selection in the latter.

Experimental design and incubation

A total of 1,750 Ross 308 broiler hatching eggs, with an average weight of 60.90 ± 1.56 g, were obtained from a commercial broiler breeder flock at 25 weeks of age (Saleh Kashmar Agro-Industrial Company, Iran). Eggs were incubated at the Poultry Research Hatchery of the Animal Science Department, Faculty of Agriculture, Ferdowsi University of Mashhad. During incubation, eggs were maintained at approximately 37.6 °C and 65% relative humidity. On day 17 of incubation, eggs were transferred to the hatcher, where the temperature and relative humidity were maintained at 37.1 °C and 75%, respectively. On day 17, eggs were candled to identify infertile eggs and embryos that had died during incubation, and non-viable eggs were excluded from the experiment. From the remaining viable fertile eggs, 1,050 eggs were randomly selected and allocated to seven treatments, with five replicate trays per treatment and 30 eggs per replicate.

The treatments were: (1) non-injected control, (2) DMSO vehicle control, (3) monolaurin at 5 mg/egg, (4) FBEO at 5 mg/egg, (5) E. coli at 10^2 CFU/egg, (6) monolaurin + E. coli, and (7) FBEO + E. coli.

In ovo administration

On day 17 of incubation, the eggshell surface was disinfected with 70% ethanol. Treatments were administered into the amniotic cavity using a sterile needle. The injection volume was 0.05 mL per

egg. The injection site was sealed with hot-melt adhesive, and the eggs were returned to the hatcher. The hatching conditions were 37.1°C and 75% relative humidity.

Hatchability was recorded at day 21, and hatch weight was determined at hatch. Hatchability was analyzed using the replicate as the experimental unit.

Post-hatch management and growth performance

After hatch, chicks were reared under standard commercial conditions. At 3 days of age, one healthy chick from each pen was selected for early sampling. For the growth trial, 18 healthy chicks from each replicate were randomly allocated to floor pens, with the replicate serving as the experimental unit. Individual body weights were recorded at placement, and body weight and feed intake were used to calculate body weight gain, feed intake, and feed conversion ratio during the experimental periods.

The chicks were fed standard corn–soybean diets formulated according to the nutrient requirements of Ross 308 broilers. Starter, grower, and finisher diets were provided from days 1–10, 11–24, and 25–42, respectively. The ingredient composition and calculated nutrient contents of the diets are presented in Table 1.

Cecal E. coli enumeration

At 3 and 42 days of age, cecal samples were collected aseptically from the selected birds. For the 3-day samples, 100 mg of cecal content was mixed with 900 μL sterile phosphate-buffered saline (PBS; pH 7.2 ± 0.2). For the 42-day samples, 1 g of cecal content was mixed with 2 mL PBS. Serial 10-fold dilutions were prepared, and 100 μL of appropriate dilutions was spread on MacConkey agar. Plates were incubated at 37 ± 1°C for 24–48 h. Colonies showing typical morphological

characteristics of E. coli were counted, and plates containing 30–300 colonies were used for enumeration.

Bacterial counts were calculated based on the colony number, dilution factor, and plated volume and were expressed as $\log_{10}$ CFU/g of cecal content. The enumeration procedure was performed according to [23], with minor modifications.

Carcass characteristics

At 42 days of age, one bird per replicate was selected for carcass evaluation. Birds were weighed, euthanized, defeathered, and eviscerated. Carcass weight and carcass yield were recorded. Breast, thigh, liver, heart, gizzard, spleen, and abdominal fat were weighed. Organ and carcass component weights were expressed as percentages of live body weight.

Jejunal histomorphology

At 42 days of age, a 1-cm segment from the middle portion of the jejunum was collected from the same bird used for carcass evaluation. Samples were washed with PBS and fixed in 10% neutral buffered formalin. After routine processing and paraffin embedding, 5-µm sections were prepared and stained with hematoxylin and eosin.

Villus height (VH) was measured from the villus tip to the villus–crypt junction, villus width (VW) at the midpoint of the villus, and crypt depth (CD) from the villus–crypt junction to the crypt base. Ten intact and well-oriented villi were measured per sample, and their mean values were used for statistical analysis. The VH:VW and VH:CD ratios were calculated. Villus surface area was calculated using the equation:

Surface area = $\pi \times$ villus width $\times$ villus height

The units and scaling of the surface-area values were reported according to the measurement system used in the study.

Hepatic oxidative stress parameters

Liver samples were collected at 42 days of age, immediately frozen, and stored until analysis. Approximately 1 g of liver tissue was homogenized in 1.15% KCl buffer (pH 7.4). Homogenates were centrifuged at 5,000 × g for 15 min at 4°C, and the supernatants were used for analysis.

Total superoxide dismutase (SOD) and glutathione peroxidase (GPx) activities were measured using commercial Ransod and Ransel kits, respectively. Total antioxidant capacity (TAC) and malondialdehyde (MDA) concentrations were also determined. SOD and GPx activities and TAC were normalized to total protein concentration and expressed as U/mg protein or the corresponding kit units.

Hepatic MDA was determined using the thiobarbituric acid reactive substances method. Briefly, liver homogenate was reacted with thiobarbituric acid and heated at 95–100°C for 30 min. The MDA–TBA complex was extracted with n-butanol and measured spectrophotometrically at 532 nm. A standard curve prepared from tetraethoxypropane was used for quantification. Hepatic MDA concentrations were expressed as nmol/mg protein.

Serum AST, ALT, MDA, and TAC

Blood samples were collected at 42 days of age, allowed to clot, and centrifuged at 3,000 × g for 10 min. Serum was separated and stored at −20°C until analysis.

Serum AST and ALT activities were determined using IFCC-based commercial kits (Pars Azmun, Iran) with an Alcyon 300 analyzer at 340 nm and expressed as U/L.

Serum MDA was determined by the thiobarbituric acid method. Briefly, 500 μL of serum was mixed with 3 mL of 1% phosphoric acid and 1 mL of 0.675% thiobarbituric acid. The mixture was heated at 100°C for 45 min and extracted with 3 mL n-butanol. After vortexing and centrifugation at 3,000 × g for 10 min, the absorbance of the upper phase was measured at 532 nm. Tetraethoxypropane was used to prepare the standard curve, and MDA concentration was expressed as nmol/mL serum.

Serum TAC was determined using a commercial colorimetric assay according to the manufacturer's instructions. Absorbance was measured at 600 nm using an Alcyon 300 analyzer, and results were expressed according to the unit specified by the assay kit.

Serum biochemical parameters

Serum concentrations of total protein, albumin, glucose, total cholesterol, high-density lipoprotein (HDL), low-density lipoprotein (LDL), triglycerides, uric acid, calcium, and phosphorus were determined using commercial diagnostic kits (Pars Azmun Co., Tehran, Iran) with an Alcyon 300 autoanalyzer (Abbott Laboratories, USA), according to the manufacturers' instructions. HDL-cholesterol was determined using the direct method, whereas LDL-cholesterol was calculated using the Friedewald equation. Results were expressed in the units specified by the respective assay kits.

Antibody Response to Sheep Red Blood Cells

Humoral immune response was evaluated using sheep red blood cells (SRBC) as an antigen. At 28 and 35 days of age, one bird per replicate was intramuscularly injected into the breast muscle with 0.1 mL of a 25% (v/v) SRBC suspension prepared in phosphate-buffered saline (PBS). Blood samples were collected 7 days after each immunization, corresponding to days 35 and 42, respectively.

Serum was separated by centrifugation at 3,000 × g for 10 min and stored at −20°C until analysis. Before analysis, serum samples were heat-inactivated at 56°C for 30 min. Total antibody titers were determined using a direct microhemagglutination assay in 96-well microtiter plates with two-fold serial dilutions. The antibody titer was recorded as the reciprocal of the highest serum dilution showing visible hemagglutination and expressed as $\log_2$ values.

For IgG determination, serum was treated with an equal volume of 1.4% 2-mercaptoethanol prepared in PBS and incubated at 37°C for 30 min before the hemagglutination assay was repeated. IgM titers were calculated from the total antibody and IgG titers according to the method described by [24].

Statistical analysis

Use of AI-assisted technologies

Generative AI tools were used during manuscript preparation for language editing, structural formatting, and consistency checks. All scientific content, data interpretation, and final wording were reviewed and verified by the authors.

The experiment was conducted as a completely randomized design. Statistical analyses were performed using PROC GLM in SAS 9.4. Treatment was included as the fixed effect. The replicate was considered the experimental unit for hatchability, whereas the pen/replicate was considered the experimental unit for post-hatch growth performance and pen-based measurements. For

measurements obtained from one sampled bird per pen, the bird-level value was used as the observation corresponding to its experimental pen.

Treatment means were compared using Tukey's honestly significant difference test. Differences were considered significant at $P \leq 0.05$, whereas $0.05 < P \leq 0.10$ was considered a tendency.

Results

Chemical Composition of Ferula badrakema Root Essential Oil

Gas chromatography–mass spectrometry (GC–MS) analysis identified 33 compounds in Ferula badrakema root essential oil (FBEO), as presented in Table 2. The major constituents were isoshyobunone (11.19%), δ-cadinene (9.19%), shyobunone (6.09%), β-pinene (5.93%), and cis-isolongifolanone (5.92%). Other relatively abundant compounds included epi-α-cadinol (4.85%), α-eudesmol (4.55%), epi-shyobunone (4.23%), myrtenol (3.35%), and n-heneicosane (3.35%).

The total phenolic content and total flavonoid content of FBEO were 35.34 μg GAE/mL and 21.37 μg QE/mL, respectively. The DPPH radical-scavenging activity of the essential oil was 17.21%.

Hatch Weight, Hatchability, and Mortality Rate of Broiler Chicks During Days 1–7

The effects of in ovo administration of monolaurin and F. badrakema root essential oil on hatchability, hatch weight, and mortality during the first 7 days after hatch are presented in Table 3.

Treatment significantly affected hatchability and hatch weight ($P < 0.05$). The FBEO-treated group receiving 5 mg/egg showed the highest numerical values for hatchability and hatch weight, whereas the group challenged with 10^2 CFU/egg Escherichia coli showed the lowest values.

Mortality during the first 7 days after hatch was also affected by treatment ($P < 0.05$). The lowest mortality was observed in the FBEO-treated groups, particularly in birds receiving 5 mg/egg, whereas the highest mortality was observed in the E. coli-challenged group.

Growth Performance of Broiler Chicks During Days 1–7

The effects of in ovo administration of monolaurin and F. badrakema root essential oil on growth performance during the first 7 days after hatch are presented in Table 4.

Treatment significantly affected body weight gain and feed conversion ratio ($P < 0.05$). Chicks receiving FBEO at 5 mg/egg exhibited the highest body weight gain and the lowest feed conversion ratio among the experimental groups, whereas chicks challenged with 10^2 CFU/egg Escherichia coli showed the lowest body weight gain and the highest feed conversion ratio.

Feed intake did not differ significantly among treatments ($P > 0.05$). In the E. coli-challenged groups, co-administration of FBEO or monolaurin resulted in numerical improvements in body weight gain and feed conversion ratio compared with the E. coli-challenged group receiving E. coli alone.

Carcass Characteristics of Broiler Chicks at 3 Days of Age

The effects of in ovo administration of monolaurin and Ferula badrakema root essential oil (FBEO) on carcass characteristics and relative organ weights of broiler chicks at 3 days of age are presented in Table 5.

No significant differences were observed among treatments in live body weight or the relative weights of the carcass, liver, abdominal fat pad, spleen, and bursa of Fabricius ($P > 0.05$).

Intestinal Histomorphology of Broiler Chicks at 3 Days of Age

The effects of in ovo administration of monolaurin and F. badrakema root essential oil (FBEO) on jejunal histomorphological characteristics of broiler chicks at 3 days of age are presented in Table 6.

Treatment significantly affected jejunal histomorphology ($P < 0.05$). The highest villus height and villus width were observed in the monolaurin + E. coli and FBEO + E. coli groups, respectively. These treatments resulted in significantly greater values for the corresponding villus characteristics than those observed in the control and E. coli-challenged groups.

Cecal Escherichia coli Counts and Intestinal Measurements of Broiler Chicks at 3 Days of Age

The effects of in ovo administration of monolaurin and F. badrakema root essential oil (FBEO) on intestinal measurements and cecal E. coli counts of broiler chicks at 3 days of age are presented in Table 7.

Treatment significantly affected jejunum length and cecal E. coli counts ($P < 0.05$). Chicks receiving 5 mg/egg FBEO had the longest jejunum and the lowest cecal E. coli count among the experimental groups. In contrast, the highest bacterial count was observed in the E. coli-challenged group. Duodenum and ileum lengths were not significantly affected by treatment ($P > 0.05$).

Serum Biochemical and Antioxidant Profile of Broiler Chicks at 3 Days of Age

The effects of in ovo administration of monolaurin and Ferula badrakema root essential oil (FBEO) on serum biochemical parameters and antioxidant status of broiler chicks at 3 days of age are presented in Table 8.

Treatment significantly affected serum cholesterol, triglyceride, total antioxidant capacity (TAC), and malondialdehyde (MDA) concentrations ($P < 0.05$). Chicks receiving FBEO had the lowest serum cholesterol, triglyceride, and MDA concentrations and the highest TAC among the experimental groups. In contrast, the E. coli-challenged group exhibited the highest cholesterol, triglyceride, and MDA concentrations and the lowest TAC.

Serum uric acid concentration was also significantly affected by treatment ($P < 0.05$), with the lowest value observed in the monolaurin-treated group. No significant differences were detected among treatments in serum glucose, total protein, or albumin concentrations ($P > 0.05$).

Growth Performance of Broiler Chickens During Different Rearing Periods

The effects of in ovo administration of monolaurin and Ferula badrakema root essential oil (FBEO) on growth performance during different rearing periods and over the overall experimental period are presented in Table 9.

Treatment significantly affected feed intake during days 1–42 and body weight gain during days 25–42 and 1–42 ($P < 0.05$). Birds receiving FBEO had the highest body weight gain during the finishing period (days 25–42) and over the entire rearing period (days 1–42), whereas the E. coli-challenged group exhibited the lowest values.

Feed conversion ratio was not significantly affected by treatment during any of the evaluated periods ($P > 0.05$).

Carcass Characteristics of Broiler Chickens at 42 Days of Age

The effects of in ovo administration of monolaurin and Ferula badrakema root essential oil (FBEO) on carcass characteristics at 42 days of age are presented in Table 10.

Treatment significantly affected live body weight, carcass yield, breast yield, thigh yield, and relative spleen weight ($P < 0.05$). In contrast, no significant differences were observed among treatments in the relative weights of the liver, abdominal fat pad, heart, or gizzard ($P > 0.05$).

Intestinal Histomorphology of Broiler Chickens at 42 Days of Age

The effects of in ovo administration of monolaurin and Ferula badrakema root essential oil (FBEO) on jejunal histomorphological characteristics of broiler chickens at 42 days of age are presented in Table 11.

Treatment significantly affected villus height and villus surface area ($P = 0.01$), whereas villus width, crypt depth, villus height-to-width ratio, and villus height-to-crypt depth ratio were not significantly affected by treatment ($P > 0.05$).

The highest villus height was observed in the FBEO + E. coli and FBEO groups (1677.7 and 1655.4 μm, respectively), whereas the lowest value was observed in the E. coli-challenged group. Villus surface area was also significantly greater in the monolaurin + E. coli and FBEO + E. coli groups than in the control and E. coli-challenged groups.

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Cecal Escherichia coli Counts and Small Intestinal Segment Lengths of Broiler Chickens at 42 Days of Age

The effects of in ovo administration of monolaurin and Ferula badrakema root essential oil (FBEO) on small intestinal segment lengths and cecal Escherichia coli counts in broiler chickens at 42 days of age are presented in Table 12.

No significant differences were detected among treatments in the lengths of the duodenum, jejunum, or ileum ($P > 0.05$). In contrast, cecal E. coli counts were significantly affected by treatment ($P = 0.02$). The E. coli-challenged group had the highest cecal E. coli count (7.31 $\log_{10}$ CFU/g), which was significantly greater than that observed in the FBEO group (5.77 $\log_{10}$ CFU/g). The FBEO group exhibited the lowest cecal E. coli count among the experimental groups.

Cecal E. coli counts were numerically lower in birds receiving monolaurin alone or monolaurin combined with E. coli than in the E. coli-challenged group; however, these differences were not statistically significant. Similarly, the FBEO + E. coli group showed a lower numerical cecal E. coli count than the E. coli-challenged group. Differences among treatment groups should be interpreted according to the multiple-comparison results presented in Table 12.

Serum Biochemical Profile of Broiler Chickens at 42 Days of Age

The effects of in ovo administration of monolaurin and Ferula badrakema root essential oil (FBEO) on the serum biochemical profile of broiler chickens at 42 days of age are presented in Table 13.

Treatment significantly affected serum low-density lipoprotein (LDL; $P = 0.0001$), glucose ($P = 0.0001$), triglycerides ($P = 0.0005$), and phosphorus ($P = 0.03$). In contrast, no significant treatment effects were observed for total protein, albumin, uric acid, high-density lipoprotein (HDL), total cholesterol, or calcium concentrations ($P > 0.05$).

Serum LDL concentration differed significantly among treatments. The E. coli-challenged group had the lowest LDL concentration (69.88 mg/dL; $P < 0.05$), whereas all other treatment groups had higher LDL concentrations ranging from 89.38 to 92.98 mg/dL and did not differ significantly from one another.

Serum glucose concentration was significantly affected by treatment. The FBEO + E. coli group had the highest glucose concentration (217.60 mg/dL; a), whereas the E. coli-challenged group had the lowest concentration (136.80 mg/dL; d). The control (179.40 mg/dL; bc), DMSO (193.00 mg/dL; ab), monolaurin (184.20 mg/dL; b), and monolaurin + E. coli (153.60 mg/dL; cd) groups showed intermediate values, with statistical differences according to the superscript letters presented in Table 13.

Serum triglyceride concentration was also significantly affected by treatment. The E. coli-challenged group had the highest triglyceride concentration (124.40 mg/dL; a), whereas the FBEO + E. coli group had the lowest concentration (64.80 mg/dL; c). The control group had an intermediate concentration (101.80 mg/dL; ab), while the monolaurin, DMSO, monolaurin + E. coli, and FBEO groups showed intermediate concentrations of 93.00, 85.40, 77.60, and 84.00 mg/dL, respectively. The significance of pairwise differences is indicated by the superscript letters in Table 13.

Serum phosphorus concentration differed significantly among treatments ($P = 0.03$). The monolaurin (7.88 mg/dL; a), monolaurin + E. coli (8.54 mg/dL; a), and E. coli-challenged (7.96

mg/dL; a) groups had higher concentrations than the DMSO group (5.86 mg/dL; b). The control (6.96 mg/dL; ab), FBEO (7.12 mg/dL; ab), and FBEO + E. coli (7.36 mg/dL; ab) groups had intermediate concentrations and did not differ significantly from either group according to the multiple-comparison results.

No significant differences were detected among treatments for serum total protein, albumin, uric acid, HDL, total cholesterol, or calcium concentrations ($P > 0.05$).

Serum Oxidative Stress Parameters of Broiler Chickens at 42 Days of Age

The effects of in ovo administration of monolaurin and Ferula badrakema root essential oil (FBEO) on serum malondialdehyde (MDA) concentration, total antioxidant capacity (TAC), and liver enzyme activities of broiler chickens at 42 days of age are presented in Table 14. Treatment significantly affected serum MDA concentration ($P = 0.007$), whereas no significant treatment effects were observed for serum aspartate aminotransferase (AST), alanine aminotransferase (ALT), or TAC ($P > 0.05$).

Serum MDA concentration differed significantly among treatments. The E. coli-challenged group had the highest MDA concentration (3.60 nmol/mL; a), which was significantly higher than those observed in all other treatment groups (1.80–2.58 nmol/mL; b). Among the groups with the lower MDA concentrations, the FBEO + E. coli group had the lowest numerical value (1.80 nmol/mL), followed by the monolaurin (1.86 nmol/mL), FBEO (1.92 nmol/mL), and monolaurin + E. coli (1.90 nmol/mL) groups. However, these groups did not differ significantly from the control (2.40 nmol/mL) or DMSO (2.58 nmol/mL) groups.

Serum AST activity was not significantly affected by treatment ($P = 0.97$), with values ranging from 300.80 to 326.40 U/L. Similarly, serum ALT activity did not differ significantly among treatments ($P = 0.21$), with values ranging from 23.00 to 27.80 U/L. Although the monolaurin and

FBEO groups showed numerically lower AST and ALT activities than the E. coli-challenged group, these numerical differences were not statistically significant.

Serum TAC was also not significantly affected by treatment (P = 0.34). TAC values ranged from 1.10 to 1.67 nmol/L, with the highest numerical value observed in the FBEO group (1.67 nmol/L) and the lowest in the control group (1.10 nmol/L). No statistically significant differences were detected among the treatment groups.

Hepatic Oxidative Stress Parameters of Broiler Chickens at 42 Days of Age

The effects of in ovo administration of monolaurin and Ferula badrakema root essential oil (FBEO) on hepatic oxidative stress parameters at 42 days of age are presented in Table 15. Treatment significantly affected SOD activity (P = 0.01), MDA concentration (P = 0.04), and TAC (P = 0.02), whereas GPx activity was not affected (P = 0.99).

Hepatic SOD activity was lowest in the monolaurin + E. coli group (25.94 U/mg protein) and was significantly lower than in the DMSO, FBEO, FBEO + E. coli, and E. coli groups. Control and monolaurin groups showed intermediate values. Hepatic MDA concentration was highest in the DMSO (0.74 nmol/mg protein) and E. coli (0.72 nmol/mg protein) groups and lowest in the FBEO (0.41 nmol/mg protein) and monolaurin + E. coli (0.44 nmol/mg protein) groups. Hepatic TAC was higher in the monolaurin, FBEO, and FBEO + E. coli groups (1.93, 2.01, and 1.98 nmol/mg protein, respectively) than in the DMSO and E. coli groups (1.50 and 1.51 nmol/mg protein, respectively). Hepatic GPx activity ranged from 20.37 to 21.78 U/mg protein and did not differ among treatments.

Antibody Titer Against Sheep Red Blood Cells (SRBC)

The effects of in ovo administration of monolaurin and Ferula badrakema root essential oil (FBEO) on antibody responses against sheep red blood cells (SRBC) at 35 and 42 days of age are presented

in Table 16. Treatment had no significant effect on total antibody, IgG, or IgM titers at either 35 or 42 days of age ($P > 0.05$). At 35 days, total antibody, IgG, and IgM titers ranged from 1.80 to 2.80, 5.00 to 6.60, and 1.00 to 1.40 $\log_2$, respectively. At 42 days, the corresponding ranges were 2.40 to 4.00, 0.60 to 1.80, and 2.00 to 4.00 $\log_2$, respectively.

Discussion

The phytochemical profile of Ferula badrakema root essential oil (FBEO) was characterized by a predominance of oxygenated sesquiterpenes and monoterpenes, with isoshyobunone, δ-cadinene, shyobunone, β-pinene, and cis-isolongifolanone as the major constituents. This composition differs from that reported for the fruit oil of F. badrakema, highlighting the influence of plant organ on essential-oil composition. The presence of oxygenated terpenes, together with the detected phenolic and flavonoid compounds, may contribute to the antimicrobial and antioxidant properties of FBEO. These findings are consistent with our previous study, in which dietary supplementation with F. badrakema root powder improved antioxidant status, intestinal morphology, and cecal E. coli populations in broiler chickens [14].

The antioxidant potential of FBEO may also have contributed to these responses, as antioxidant protection is important for embryo viability during incubation and the early post-hatch period [17]. The reduced survival observed following E. coli challenge suggests that bacterial exposure adversely affected hatch and early chick viability, whereas the lower mortality in FBEO-treated chicks may indicate a protective effect of the essential oil. Similar beneficial responses to natural in ovo supplements have been reported for Nigella sativa extract, which improved selected hatching and post-hatch traits in broilers [17]. the present findings suggest that FBEO, particularly at 5 mg/egg, has potential as a natural in ovo supplement for improving hatchability and early chick

viability. However, further studies are needed to determine whether these effects are directly related to the antioxidant or antimicrobial properties of its bioactive constituents.

The present study showed that in ovo administration of Ferula badrakema root essential oil (FBEO) and monolaurin supported early growth performance under Escherichia coli challenge. The strongest response was observed with 5 mg/egg FBEO, which improved body weight gain and feed conversion during the first week after hatch. In contrast, E. coli challenge impaired growth and feed efficiency, indicating an adverse effect of bacterial stress during the early post-hatch period.

These findings are also consistent with our previous study, in which dietary supplementation with F. badrakema root powder improved antioxidant status and selected indicators of intestinal health in broilers, including reduced hepatic lipid peroxidation and increased jejunal villus height [14]. Monolaurin may also contribute to intestinal development and productive performance through its antimicrobial and gut-modulating properties. Previous studies have reported that dietary glycerol monolaurate can improve feed efficiency and intestinal morphological characteristics in broilers [19]. The present results suggest that early exposure to FBEO and monolaurin may influence chick responses to bacterial challenge, although the mechanisms underlying these effects require further investigation.

Both monolaurin and FBEO improved selected jejunal morphological traits at 3 days of age, with greater villus height and width particularly evident under E. coli challenge. These changes may indicate better preservation of intestinal structure during bacterial stress. Rapid intestinal development occurs around hatch, and changes in villus structure can increase the surface available for nutrient absorption [15].

The response observed in the present study is consistent with previous in ovo studies. [20] reported that in ovo delivery of Bacillus subtilis improved selected intestinal morphological traits, while

[21] showed that in ovo administration of B. subtilis and raffinose affected intestinal morphology and gut microbial characteristics.

FBEO and monolaurin reduced cecal E. coli counts, with the strongest response observed for 5 mg/egg FBEO. This finding supports a direct role of these treatments in controlling intestinal bacterial pressure. The antimicrobial activity of essential oils is generally associated with their ability to interact with bacterial membranes and alter membrane function, although the activity depends strongly on their chemical composition.

The present finding is particularly relevant because the essential oil used in this study differed chemically from the fruit oil previously described for F. badrakema. Our previous feeding study also showed that F. badrakema root powder reduced cecal E. coli and improved selected intestinal traits, providing independent support for the potential of this plant as a gut-active phytogenic additive [14].

Monolaurin may provide a complementary antimicrobial effect. Previous broiler studies have reported beneficial effects of α-monolaurin on intestinal morphology and feed conversion, particularly when antibiotic growth promoters were absent or reduced [18, 21]. The simultaneous reduction in E. coli and improvement in jejunal morphology therefore suggest that both compounds may support gut health through related but not necessarily identical pathways.

The lack of a significant FCR response in the overall production period should also be considered. The increase in body weight was accompanied by increased feed intake, indicating that the growth response was not necessarily associated with a clear improvement in feed efficiency. Therefore, the main production benefit of FBEO in the present study appears to be related to greater body weight rather than a consistent reduction in FCR.

At 42 days, treatment affected serum glucose, triglycerides, LDL, and phosphorus, whereas total protein, albumin, uric acid, HDL, cholesterol, and calcium were not significantly affected. These findings suggest that the metabolic effects of the treatments were selective rather than indicative of a general disturbance in nutrient or mineral metabolism.

The changes in glucose and triglycerides may be related to the interaction between intestinal health, nutrient absorption, and the metabolic response to bacterial challenge. However, because glucose and lipid metabolism were not directly examined, these responses should not be attributed to specific hepatic pathways without further evidence. The previous dietary F. badrakema study is relevant here because root supplementation reduced serum cholesterol and triglycerides and improved antioxidant status in broilers [14].

The main serum oxidative response was observed for MDA. E. coli challenge increased serum MDA, whereas FBEO and monolaurin maintained lower values. This pattern suggests that bacterial challenge increased lipid peroxidation and that the treatments reduced oxidative damage. The antioxidant capacity of the embryo and newly hatched chick is important during this period because antioxidant reserves change rapidly around hatch [17].

The unchanged AST and ALT activities indicate that neither FBEO nor monolaurin caused detectable hepatic injury. The lack of a significant change in serum TAC also suggests that the lower MDA concentration was not necessarily accompanied by an increase in total circulating antioxidant capacity.

The hepatic results further support an effect of the treatments on oxidative balance. Treatment affected hepatic SOD, MDA, and TAC, whereas GPx was unchanged. The lower MDA observed in the FBEO and monolaurin + E. coli groups indicates reduced hepatic lipid peroxidation, particularly under bacterial challenge.

These findings are consistent with our previous dietary study, in which F. badrakema root powder improved antioxidant status by reducing MDA and increasing SOD activity [14]. Together, the serum and hepatic results suggest that FBEO and monolaurin may help limit oxidative damage, although the exact antioxidant pathways remain to be established.

Neither FBEO nor monolaurin significantly affected total antibody, IgG, or IgM titers against SRBC at 35 or 42 days of age. Therefore, under the conditions of the present study, these treatments did not produce a measurable effect on SRBC-specific humoral immunity.

The absence of an antibody response does not necessarily indicate a lack of biological activity. In ovo administration may have stronger effects on early intestinal development, microbial establishment, and antioxidant status than on later systemic antibody production. Previous studies have shown that in ovo bioactive compounds can influence intestinal and physiological responses without necessarily producing consistent changes in all immune variables [17, 20].

Conclusion

Taken together, in ovo administration of monolaurin and FBEO influenced selected growth, intestinal morphological, bacterial, biochemical, and hepatic antioxidant responses through 42 days of age. FBEO at 5 mg/egg showed particularly favorable responses in hatchability, early growth, selected jejunal traits, and cecal E. coli counts. The treatments did not significantly alter SRBC-specific antibody responses or serum AST and ALT activities. Because microbiological measurements were limited to E. coli counts and no direct measurements of microbiota, barrier

function, or inflammatory pathways were performed, the underlying mechanisms remain to be established.

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Author Contributions

H.K. conceived and conducted the study, performed the experimental work, analyzed the data, and drafted the manuscript. A.H. contributed to study design, supervision, interpretation of the findings, and critical revision of the manuscript. M.A.B. contributed to microbiological analyses, interpretation of microbiological findings, and critical revision of the manuscript. All authors reviewed and approved the final manuscript.

Data Availability

The data supporting the findings of this study are available from the corresponding author upon reasonable request.

Additional Information

Competing interests

The authors declare no competing interests.

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Table 1. Ingredients and nutrient composition of the experimental diets (as-fed basis).

Ingredients (%)	1-10 d	11-24 d	25-42 d
Corn	35.63	29.84	25.63
Wheat	10.00	20.00	30.00
Soybean meal (44% CP)	41.67	37.22	30.88
Soybean oil	6.73	7.49	8.29
Limestone	1.34	1.2	1.19
Dicalcium phosphate	1.50	1.3	1.11
Common salt	0.3	0.29	0.29
Mineral premix [a]	0.25	0.25	0.25
Vitamin premix [b]	0.25	0.25	0.25
DL-methionine	0.4	0.35	0.32
L-lysine HCl	0.26	0.17	0.17
L-threonine	0.07	0.04	0.02
Sodium bicarbonate	0.10	0.10	0.10

Cellulose	1.50	1.50	1.50
Calculated chemical composition			
Metabolizable energy (kcal/kg)	3000	3100	3200
Crude protein (%)	23.0	21.50	19.50
Calcium (%)	0.96	0.87	0.79
Available phosphorus (%)	0.48	0.435	0.39
Lysine (%)	1.44	1.29	1.16
Methionine (%)	0.61	0.58	0.54
Met + Cys (%)	1.08	0.99	0.91
Threonine (%)	0.97	0.88	0.78

[a] Provided per kilogram of diet: Vitamin A, 12500 IU; vitamin D_3, 5000 IU; vitamin E, 80 IU; vitamin K_3, 3.2 mg; vitamin B_{12}, 0.02 mg; thiamin, 3.2 mg; riboflavin, 8.6 mg; niacin, 62.5 mg; folic acid, 2.2 mg; biotin, 0.25 mg; pyridoxine, 4.9 mg; pantothenic acid, 18.5 mg; antioxidant, 2.5 mg.

[b] Provided per kilogram of diet: Zn, 110 mg; Mn, 120 mg; Se, 0.30 mg; I, 1.25 mg; Cu, 16 mg; Fe, 20.2 mg.

Table 2. Chemical composition of *Ferula badrakema* root essential oil identified by gas chromatography.

Number	Retention time (min)	Compound	Area (%)	Kovats index (KI)
1	32.74	Isoshyobunone	11.199	1516
2	32.98	δ-Cadinene	.196	1521
3	31.80	Shyobunone	.095	1494
4	10.40	β-Pinene	.93	984

5	36.45	cis-Isolongifolanone	5.924	1616
6	37.71	epi-α-Cadinol	.854	1645
7	38.22	α-Eudesmol	.554	1660
8	33.26	epi-Shyobunone	.233	1527
9	19.54	Myrtenol	.353	1203
10	52.32	n-Heneicosane	.35	2100

Table 3. Effects of in ovo administration of monolaurin and *Ferula badrakema* root essential oil, with *Escherichia coli* challenge, on hatchability and hatch weight of broiler chicks.

Treatment	Hatch weight (g)	Hatchability (%)	Mortality rate (1-7 d; %)
Control	39.92^{bc}	83.33^{ab}	25.56^{bc}
DMSO	39.55^{bc}	78.90^{b}	30.00^{b}
Monolaurin	40.22^{ab}	86.67^{ab}	21.11^{bc}
Monolaurin + *E. coli*	39.86^{bc}	78.90^{b}	28.89^{b}
FBEO	41.08^{a}	90.00^{a}	17.78^{c}
FBEO + *E. coli*	39.77^{bc}	81.11^{ab}	27.78^{b}
E. coli	38.89^{c}	62.22_{c}	43.33^{a}
SEM	0.16	1.72	1.67
P-Value	0.01	<0.0001	<0.0001

$^{a-c}$ Means with different lowercase letters within a column differ significantly ($P < 0.05$).

Table 4: Effects of in ovo administration of monolaurin and *Ferula badrakema* root essential oil, with *Escherichia coli* challenge, on growth performance of broiler chicks during 1–7 d.

Treatment	Daily feed intake (g/bird/day)	Daily weight gain (g/bird/day)	Feed conversion ratio
Control	19.01	18.11^{a}	1.05
DMSO	19.24	18.13^{a}	1.07

Monolaurin	19.86	18.32[a]	1.08
Monolaurin + *E. coli*	19.46	18.12[a]	1.07
FBEO	19.91	18.44[a]	1.08
FBEO + *E. coli*	19.88	18.30[a]	1.09
E. coli	18.22	15.13[b]	1.24
SEM	0.29	0.22	0.02
P-Value	0.94	0.0001	0.17

[a-c] Means with different lowercase letters within a column differ significantly ($P < 0.05$).

Table 5: Effects of in ovo administration of monolaurin and *Ferula badrakema* root essential oil on live weight, relative organ weights, and intestinal segment lengths of broiler chicks at 3 d.

Treatment	Live weight (g)	Heart (%)	Liver (%)	Gizzard (%)	Proventriculus (%)	Spleen (%)	Small intestine (%)
Control	71.90	0.89	3.52	7.97	1.19	0.05	10.14
DMSO	73.39	0.91	4.21	7.89	1.15	0.06	10.52
Monolaurin	78.66	0.92	4.28	8.28	1.17	0.06	11.25
Monolaurin + *E. coli*	77.21	0.91	4.03	8.06	1.27	0.04	10.32
FBEO	80.51	0.92	4.30	8.12	1.37	0.05	11.02
FBEO + *E. coli*	74.61	0.91	4.40	8.04	1.21	0.05	10.78
E. coli	68.11	0.90	4.18	7.84	1.25	0.05	10.19
SEM	1.66	0.02	0.12	0.14	0.04	0.002	0.23
P-Value	0.49	1.00	0.54	0.99	0.87	0.23	10.19

[a-c] Means with different lowercase letters within a column differ significantly ($P < 0.05$).

Table 6: Effects of in ovo administration of monolaurin and *Ferula badrakema* root essential oil on jejunal morphology of broiler chicks at 3 d.

Treatment	Villus height (µm)	Villus width (µm)	Crypt depth (µm)	Villus height to width ratio (µm/ µm)	Villus height to crypt depth ratio (µm/ µm)	Villus surface area ($\mu m^2 \times 10^3$)
Control	371.10[c]	95.32[c]	89.30[c]	4.05	4.31	0.11[c]
DMSO	458.65[bc]	101.03[c]	84.13[c]	4.64	5.59	0.15[c]
Monolaurin	508.05[bc]	107.73[bc]	98.05[bc]	4.78	5.38	0.17[c]
Monolaurin + *E. coli*	692.32[a]	142.06[a]	161.65[a]	4.89	4.49	0.31[ab]
FBEO	603.72[ab]	137.42[ab]	121.74[b]	4.51	5.08	0.2[bc]
FBEO + *E. coli*	778.18[a]	148.31[a]	126.06[b]	5.01	5.81	0.38[a]
E. coli	352.11[c]	80.00[c]	76.09[c]	4.72	4.77	0.09[c]
SEM	30.82	5.56	5.80	0.19	0.23	0.02
P-Value	0.0001	0.0003	<0.0001	0.91	0.57	0.0001

[a-c] Means with different lowercase letters within a column differ significantly ($P < 0.05$).

Table 7: Effects of in ovo administration of monolaurin and *Ferula badrakema* root essential oil on intestinal segment lengths and cecal *Escherichia coli* counts in broiler chicks at 3 d.

Treatment	Duodenum (cm)	Jejunum (cm)	Ileum (cm)	Escherichia coli (log_{10} CFU g^{-1})
Control	12.50	31.14[bc]	19.14	1.74[b]
DMSO	12.76	30.90[bc]	19.10	1.76[ab]

Monolaurin	12.92	32.86[ab]	21.22	1.70[cd]
Monolaurin + *E. coli*	13.42	32.38[abc]	20.04	1.74[b]
FBEO	13.42	35.20[a]	22.00	1.67[d]
FBEO + *E. coli*	13.00	33.52[ab]	21.72	1.72[b]
E. coli	11.58	20.29[c]	18.30	1.78[a]
SEM	0.22	0.50	0.43	0.007
P-Value	0.30	0.02	0.12	<0.0001

[a-c] Means with different lowercase letters within a column differ significantly (P < 0.05).

Table 8 Effects of in ovo administration of monolaurin and *Ferula badrakema* root essential oil on serum biochemical parameters of broiler chicks at 3 d.

Treatment	Glucose (mg dL^{-1})	Cholesterol (mg dL^{-1})	Triglycerides (mg dL^{-1})	Total Protein (g dL^{-1})	Albumin (g dL^{-1})	Uric Acid (mg dL^{-1})	Total Antioxidant Capacity (nmol/L)	Malondialdehyde level in blood plasma (nmol/mL)
Control	173.60	282.80[ab]	154.60[a]	3.36	1.96	3.10[bc]	1.29[bc]	3.30[a]
DMSO	166.80	312.60[a]	112.60[bc]	3.08	1.92	3.35[b]	1.22[c]	3.00[a]
Monolaurin	154.60	256.40[bc]	107.61[bc]	3.52	2.00	2.58[c]	1.66[a]	2.18[bc]
Monolaurin + *E. coli*	157.40	264.00[b]	117.00[abc]	3.38	2.00	2.66[c]	1.43[b]	2.68[ab]
FBEO	159.00	215.20[c]	97.20[c]	3.54	2.10	2.59[c]	1.76[a]	1.84[c]
FBEO + *E. coli*	158.00	218.00[c]	109.41[bc]	3.50	1.94	2.79[bc]	1.63[a]	2.04[bc]
E. coli	160.40	295.60[ab]	141.80[ab]	3.32	2.00	4.20[a]	1.22[c]	3.12[bc]
SEM	2.32	7.51	5.54	0.05	0.02	0.19	0.04	0.12
P-Value	0.34	<0.0001	0.04	0.33	0.38	<0.0001	<0.0001	<0.0005

[a-c] Means with different lowercase letters within a column differ significantly (P < 0.05). Glu: Glucose, Chl: cholesterol, TG: Triglycerides, TP: Total protein, Alb: Albumin, UA: Uric acid, TAC: Total antioxidant capacity, MDA: Malondialdehyde

Table 9: Effects of in ovo administration of monolaurin and *Ferula badrakema* root essential oil on growth performance of broiler chickens at different ages.

Treatment	Feed intake (g/bird/day)				Body weight gain (g/bird/day)				Feed conversion ratio			
	1-10 days	11-24 days	25-42 days	1-42 days	1-10 days	11-24 days	25-42 days	1-42 days	1-10 days	11-24 days	25-42 days	1-42 days
Control	19.88	67.30	155.16	94.32[a]	19.75	45.52	74.02[ab]	53.83[a]	1.01	1.48	2.10	1.74
DMSO	19.36	67.13	155.26	93.82[a]	19.62	44.87	73.73[ab]	53.94[a]	0.99	1.52	2.12	1.75
Monolaurin	19.97	67.15	157.32	94.81[a]	20.50	46.70	80.02[a]	55.33[a]	0.98	1.44	1.97	1.71
Monolaurin + *E. coli*	19.78	66.74	156.30	93.88[a]	20.47	45.59	77.86[a]	54.73[a]	0.97	1.46	2.01	1.72
FBEO	20.16	69.66	159.00	95.54[a]	20.56	48.11	80.95[a]	55.75[a]	0.97	1.45	1.96	1.71
FBEO + *E. coli*	19.66	68.27	158.11	94.21[a]	20.10	47.41	76.77[a]	55.49[a]	0.98	1.45	2.06	1.69
E. coli	18.96	66.59	149.13	89.38[b]	19.40	44.17	66.88[b]	49.58[b]	0.98	1.49	2.32	1.80
SEM	0.18	0.54	1.01	0.53	0.19	0.42	1.26	0.40	0.01	0.02	0.04	0.01
P-Value	0.64	0.79	0.17	0.04	0.57	0.14	0.04	0.0001	0.99	0.85	0.30	0.15

a-c Means with different lowercase letters within a column differ significantly (P < 0.05).

Table 10. Effects of in ovo administration of monolaurin and *Ferula badrakema* root essential oil on carcass traits and internal organs (% of live body weight) of broiler chickens at 42 d.

Treatment	Carcass	Breast	Thigh	Liver	Abdominal fat	Heart	Gizzard	Spleen
Control	60.0[b]	24.40[ab]	18.90[b]	2.68	1.08	0.46	2.04	0.10[ab]
DMSO	59.0[b]	21.93[b]	18.63[b]	2.67	1.04	0.43	2.12	0.09[ab]
Monolaurin	64.0[a]	26.29[a]	19.46[ab]	2.69	1.61	0.47	2.29	0.12[a]
Monolaurin + E. coli	67.0[a]	27.07[a]	19.53[ab]	2.68	1.48	0.46	2.38	0.12[a]
FBEO	65.0[a]	25.19[a]	21.01[a]	2.66	1.53	0.41	2.18	0.13[a]
FBEO + E. coli	66.0[a]	25.96[a]	19.78[ab]	2.69	1.06	0.52	2.25	0.12[a]
E. coli	59.0[b]	21.50[b]	18.28[c]	2.70	1.20	0.45	2.64	0.08[b]
SEM	0.007	0.43	0.34	0.03	0.07	0.01	0.06	0.004
P-Value	<0.0001	0.02	0.0004	1.00	0.14	0.23	0.18	0.03

[a-c] Means with different lowercase letters within a column differ significantly ($P < 0.05$).

Table 11: Effects of in ovo administration of monolaurin and *Ferula badrakema* root essential oil on jejunal morphology of broiler chickens at 42 days of age.

Treatment	Villus height (µm)	Villus width (µm)	Crypt depth (µm)	Villus height to width ratio (µm /µm)	Villus height to crypt depth ratio (µm/ µm)	Villus surface area ($µm^2 \times 10^3$)
Control	1383.7[bc]	258.13	197.95	5.67	7.32	1.13[b]
DMSO	1404.8[bc]	234.59	207.47	6.05	6.96	1.04[b]
Monolaurin	1609.3[ab]	261.23	250.97	6.42	6.70	1.32[ab]
Monolaurin + *E. coli*	1575.5[abc]	335.87	239.09	4.94	7.06	1.65[a]
FBEO	1655.4[a]	241.30	225.29	7.87	7.73	1.22[ab]
FBEO + *E. coli*	1677.7[a]	326.22	235.19	5.53	7.59	1.68[a]
E. coli	1362.6[c]	209.70	203.06	7.17	6.95	0.92[b]
SEM	33.81	14.11	9.83	0.35	0.28	0.07
P-Value	0.01	0.13	0.75	0.31	0.97	0.01

[a-c] Means with different lowercase letters within a column differ significantly ($P < 0.05$).

Table 12: Effects of in ovo administration of monolaurin and *Ferula badrakema* root essential oil on intestinal segment lengths and cecal *Escherichia coli* counts in broiler chickens at 42 d.

Treatment	Duodenum (cm)	Jejunum (cm)	Ileum (cm)	*Escherichia coli* (log_{10} CFU g^{-1})
Control	31.60	87.00	64.20	6.27^{bc}
DMSO	31.40	86.40	63.40	6.77^{ab}
Monolaurin	32.40	89.20	67.00	6.02^{bc}
Monolaurin + *E. coli*	32.20	87.60	67.40	6.25^{bc}
FBEO	32.00	90.20	68.00	5.77^{c}
FBEO + *E. coli*	32.40	87.80	65.60	6.12^{bc}
E. coli	31.20	85.40	62.40	7.31^{a}
SEM	0.27	0.63	0.87	0.13
P-Value	0.48	0.49	0.54	0.02

[a-c] Means with different lowercase letters within a column differ significantly ($P < 0.05$).

Table 13: Effects of in ovo administration of monolaurin and *Ferula badrakema* root essential oil on serum biochemical parameters of bro 42 d.

Treatment	Total Protein (g dL^{-1})	Albumin (g dL^{-1})	Uric Acid (mg dL^{-1})	HDL	LDL	Glucose (mg dL^{-1})	Cholesterol (mg dL^{-1})	Triglycerides (mg dL^{-1})	Calcium (mg dL^{-1})
Control	2.86	1.26	3.86	51.80	90.07^{a}	179.40^{bc}	133.20	101.80^{ab}	8.76
DMSO	2.94	1.32	3.93	52.00	92.85^{a}	193.00^{ab}	129.60	85.40^{bc}	7.76
Monolaurin	3.20	1.40	4.79	54.20	92.98^{a}	184.20^{b}	110.20	93.00^{b}	7.82
Monolaurin + *E. coli*	2.98	1.30	4.38	52.80	89.38^{a}	153.60^{cd}	105.40	77.60^{bc}	9.60
FBEO	3.36	1.52	4.82	55.20	92.80^{a}	202.00^{ab}	115.00	84.00^{bc}	8.46
FBEO + *E. coli*	3.28	1.36	4.48	52.23	90.85^{a}	217.60^{a}	117.65	64.80^{c}	9.70
E. coli	2.82	1.20	4.78	50.21	69.88^{b}	136.80^{d}	129.20	124.40^{a}	9.05
SEM	0.08	0.03	0.15	0.75	1.68	5.48	3.54	4.06	0.29
P-Value	0.35	0.34	0.42	0.69	0.0001	0.0001	0.24	0.0005	0.41

[a-c] Means with different lowercase letters within a column differ significantly ($P < 0.05$), High-Density Lipoprotein (mg dL^{-1}), Low-Density Lipop

Table 14 Effects of in ovo administration of monolaurin and *Ferula badrakema* root essential oil on serum malondialdehyde concentration, antioxidant status, and liver enzyme activities of broiler chickens at 42 d.

Treatment	AST (U L^{-1})	ALT (U L^{-1})	MDA (nmol mL^{-1})	TAC (nmol/L)
Control	318.00	24.80	2.40[b]	1.10
DMSO	326.40	25.40	2.58[b]	1.23
Monolaurin	300.80	23.20	1.86[b]	1.60
Monolaurin + *E. coli*	315.75	24.00	1.90[b]	1.24
FBEO	306.20	23.80	1.92[b]	1.67
FBEO + *E. coli*	310.60	23.00	1.80[b]	1.57
E. coli	325.40	27.80	3.60[a]	1.21
SEM	7.23	0.53	0.15	0.08
P-Value	0.97	0.21	0.007	0.34

[a-b] Means with different lowercase letters within a column differ significantly ($P < 0.05$). AST: Aspartate Aminotransferase, ALT: Alanine Aminotransferase, MDA: Malondialdehyde, TAC: Total Antioxidant Capacity

Table 15: Effects of in ovo administration of monolaurin and *Ferula badrakema* root essential oil on hepatic malondialdehyde concentration, antioxidant status, and liver enzyme activities of broiler chickens at 42 d.

Treatment	SOD (U/mg protein)	GPx (U/mg protein)	MDA (nmol/mg protein)	TAC (nmol/mg protein)
Control	32.82[ab]	20.37	0.68[ab]	1.72[ab]
DMSO	33.54[a]	20.64	0.74[a]	1.50[b]
Monolaurin	32.83[ab]	20.38	0.49[ab]	1.93[a]
Monolaurin + *E. coli*	25.94[b]	20.50	0.44[b]	1.76[ab]
FBEO	34.06[a]	21.78	0.41[b]	2.01[a]
FBEO + *E. coli*	37.07[a]	21.03	0.65[ab]	1.98[a]
E. coli	39.94[a]	20.77	0.72[a]	1.51[b]
SEM	1.06	0.64	0.04	0.053
P-Value	0.01	0.99	0.04	0.02

a-c Means with different lowercase letters within a column differ significantly ($P < 0.05$). SOD: Superoxide Dismutase, GPx: Glutathione Peroxidase MDA: Malondialdehyde, TAC: Total Antioxidant Capacity

Table 16: Effects of in ovo administration of monolaurin and *Ferula badrakema* root essential oil on humoral immune response (SRBC antibody titer) of broiler chickens at 35 and 42 d.

Treatment	35 days of age			42 days of age		
	Total antibody	IgG	IgM	Total antibody	IgG	IgM
Control	1.80	5.00	1.20	2.40	0.60	2.20
DMSO	2.40	5.80	1.40	2.40	1.80	3.00
Monolaurin	2.40	5.20	1.00	2.80	1.40	3.00
Monolaurin + *E. coli*	1.80	6.20	1.00	4.00	0.80	2.20
FBEO	2.80	6.60	1.00	2.60	1.60	4.00
FBEO + *E. coli*	2.20	5.20	1.00	2.60	1.20	2.60
E. coli	2.20	5.00	1.00	2.40	1.20	2.00
SEM	0.16	0.26	0.05	0.22	0.16	0.27
P-Value	0.68	0.54	0.15	0.45	0.43	0.51

IgG: Immunoglobulin G, IgM: Immunoglobulin M, Antibody titers are expressed as $\log_2$ values,Total antibody ($\log_2$ titer), IgG ($\log_2$ titer), IgM ($\log_2$ titer)