

Effects of Encapsulated Fennel and Oregano Essential Oils on Growth Performance, Intestinal Morphology and Gut Microbiota of Japanese Quails

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Research Article

Keywords: Encapsulated fennel essential oil, oregano essential oil, growth performance, intestinal morphology, gut microbiota, Japanese quails

Posted Date: September 20th, 2026

DOI: <https://doi.org/10.21203/rs.3.rs-10474220/v1>

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Abstract

The present study evaluated the effects of dietary encapsulated fennel essential oil (FEO) and oregano essential oil (OEO), administered individually or in combination, on growth performance, carcass traits, intestinal histomorphology, and gut microbiota of Japanese quails. A total of 160 one-day-old quails were allocated to four dietary treatments in a 2×2 factorial design: control, 250 mg/kg FEO, 250 mg/kg OEO, and 250 mg/kg FEO + 250 mg/kg OEO. Dietary FEO significantly reduced feed intake ($P = 0.012$) and improved feed conversion ratio ($P = 0.012$) without affecting body weight gain. OEO had limited effects on growth performance. Carcass yield was not influenced by either essential oil, although FEO reduced relative liver weight. OEO supplementation improved duodenal histomorphology by increasing villus height ($P = 0.014$), villus height-to-crypt depth ratio ($P = 0.002$), and villus area ($P = 0.042$), indicating enhanced absorptive capacity. In contrast, the combined supplementation of FEO and OEO adversely affected jejunal and ileal villus morphology. Dietary FEO increased *Lactobacillus* spp. populations ($P = 0.007$), while FEO and OEO reduced *Escherichia coli* O157 under specific treatment conditions in female birds ($P = 0.002$), indicating beneficial modulation of the intestinal microbiota. However, these microbiological improvements were not consistently associated with enhanced intestinal morphology or growth performance. Overall, encapsulated FEO appears to be a promising phytogetic feed additive for improving feed efficiency, whereas OEO primarily enhanced intestinal morphology. The combined supplementation of FEO and OEO at the evaluated inclusion level cannot be recommended because it failed to provide additive benefits and negatively affected intestinal architecture. Future studies should optimize dosage and combinations to maximize the biological efficacy of encapsulated essential oils.

1. INTRODUCTION

Due to increasing concerns over antimicrobial resistance and regulatory restrictions, the poultry industry has intensified efforts to identify effective alternatives to antibiotic growth promoters (AGPs) (Irmak et al., 2024). Phytogetic feed additives, including herbs and plant-derived compounds such as garlic, oregano, thyme, and cinnamon, have been widely recognized as natural alternatives to AGPs because of their antimicrobial, antioxidant, and gut health-promoting properties (Mohamed and Hassan, 2023). These phytogetic compounds, including essential oils (EOs) and other plant bioactives, have also been reported to improve animal health, immune function, and production performance (Hassan et al., 2023). Among these alternatives, EOs have attracted considerable attention due to their antimicrobial, antioxidant, and digestive-enhancing properties (Han et al., 2017; Windisch et al., 2008). Previous studies have demonstrated that EOs can improve gut health, enhance nutrient utilization, and positively influence growth performance in poultry (Zeng et al., 2015; Arslan et al., 2021; Movahedi et al., 2024). However, the practical application of EOs is often limited by their volatility, instability, and rapid degradation in the gastrointestinal tract. Encapsulation has therefore been proposed as a strategy to protect essential oils during feed processing and gastrointestinal passage, potentially improving their stability and delivery of bioactive compounds (Kishawy et al., 2019; Movahedi et al., 2024). Previous studies using microencapsulated essential oils have reported improvements in feed efficiency, intestinal health, antioxidant status, immune responses, and gut microbiota modulation in poultry (Moharreri et al., 2021, 2022). Nevertheless, the present study evaluated commercially available encapsulated essential oil products and did not aim to investigate or compare the physicochemical performance of the encapsulation system itself. Among various EOs, fennel and oregano have been widely studied because of their rich bioactive composition. Fennel essential oil, primarily composed of anethole, has been associated with antimicrobial activity and modulation of digestive processes, whereas oregano essential oil contains carvacrol and thymol, which are recognized for their potent antimicrobial and gut health-promoting properties (Basmacioglu-Malayoglu et al., 2014; Ghiasvand et al., 2021). These essential oils have also been

reported to improve intestinal morphology and microbial balance in poultry (Majrashi, 2022; Wang et al., 2021). However, responses to combinations of essential oils remain inconsistent, and their efficacy may vary according to dosage, formulation, and animal species (Yang et al., 2015; Ameer et al., 2024; İpçak et al., 2024). The dietary inclusion level of 250 mg/kg used in the present study was selected based on previous studies reporting biological activity of encapsulated essential oils within similar dosage ranges (İpçak et al., 2025). Therefore, the present study aimed to evaluate the biological effects of commercially available encapsulated fennel and oregano essential oils, administered individually or in combination, on growth performance, intestinal morphology, and gut microbiota in Japanese quails. The objective of this study was not to evaluate the encapsulation technology itself, but rather the biological efficacy of commercially available encapsulated products under practical feeding conditions. Accordingly, the study focused on assessing the in vivo biological responses to these commercial phytochemical products rather than the physicochemical characteristics of the encapsulation system.

2. MATERIALS AND METHODS

2.1. Animals, housing, and diets

A total of 160 one-day-old Japanese quails (*Coturnix coturnix japonica*) were randomly assigned to a 2 × 2 factorial arrangement with two dietary factors: fennel essential oil (FEO; 0 or 250 mg/kg diet) and oregano essential oil (OEO; 0 or 250 mg/kg diet). Accordingly, four treatment groups were established: Control (0 mg/kg FEO + 0 mg/kg OEO), FEO (250 mg/kg FEO + 0 mg/kg OEO), OEO (0 mg/kg FEO + 250 mg/kg OEO), and FEO + OEO (250 mg/kg FEO + 250 mg/kg OEO). Each treatment consisted of 10 replicate cages with four birds per cage (two males and two females), resulting in a total of 40 birds per treatment. Birds were randomly allocated to cages to ensure similar initial body weight and an identical sex ratio across all treatments. The cage was considered the experimental unit for growth performance measurements (body weight gain, feed intake, and feed conversion ratio), whereas individual birds served as the experimental units for carcass characteristics, intestinal morphology, and microbiological analyses. Because all treatment groups were maintained under identical stocking density and the same male-to-female ratio, any potential effects associated with mixed-sex housing were equally distributed among treatments. Quails had ad libitum access to feed and water throughout the 37-day experimental period. Diets were formulated as mash at the University's Department of Animal Science according to NRC (1994) recommendations for Japanese quails, and their proximate composition was determined using the Weende method (Horwitz, 2000) (Table 1). Encapsulated essential oils were supplied in ready-to-use form by a commercial manufacturer (Birlikas, İzmir, Türkiye). According to the manufacturer, the essential oils were encapsulated using sodium alginate as the wall material through an ionotropic gelation process. The dietary inclusion level of 250 mg/kg refers to the commercial encapsulated product as supplied by the manufacturer rather than to the amount of pure essential oil. The present study focused on evaluating the biological effects of these commercially available encapsulated products rather than the physicochemical characterization of the encapsulation system. Feed was provided ad libitum and replenished daily, while fresh water was continuously supplied through a nipple-drinker system (two nipples per cage). Birds were reared under a 23L:1D lighting schedule. Ambient temperature was maintained at 33°C during the first week and gradually reduced to 22–24°C thereafter. Relative humidity was maintained between 55% and 60% and monitored daily using a digital thermo-hygrometer (VZN, Türkiye).

Table 1

Ingredients, composition (g/kg), nutrient content (%), and metabolizable energy (kcal/kg) levels of diets.

Item	%
Sunflower oil (8,800 kcal/kg)	2.75
Corn	146.75
Bone meal (27% Ca, 18% CP)	0
Soybean meal (44% CP)	92.5
Dicalcium phosphate	2.5
DL-Methionine	0.5
Limestone (CaCO ₃)	3
L-Lysine	0.5
NaCl	0.87
Vitamin—mineral premix ¹	0.62
Analyzed chemical composition	%
Dry matter	89.6
Crude protein	22.3
Crude ash	5.9
Crude fiber	2.8
Ether extract	2.17
Starch	34.48
Sugar	4.68
Calculated values	
Metabolizable energy (ME poultry), kcal/kg	2905
L-Lysine, %	1.33
Methionine + cystine, %	0.89
Ca, %	0.98
Available P, %	0.58
¹ Vitamin and mineral premix provided per 2.5 kg of diet: vitamin A, 12,000,000 IU; vitamin D3, 1,500,000 IU; vitamin E, 40,000 mg; vitamin K3, 5,000 mg; vitamin B1, 3,000 mg; vitamin B2, 7,000 mg; vitamin B6, 5,000 mg; vitamin B12, 30 mg; CAL-D pantothenate, 10,000 mg; Biotin, 75 mg; Folic acid, 1,000 mg; Niacin amide, 4,000 mg; Choline chloride, 400,000 mg; Mn sulfate, 80,000 mg; Fe (II) sulfate, 60,000 mg; Cu (II) sulfate, 5,000 mg; Zn sulfate, 60,000 mg; Ca iodide, 1,000 mg; Na selenite, 150 mg, and CaCO ₃ 1,135,000 mg.	

Table 1 here.

2.2 Performance measurements and sampling

Body weight and feed intake were recorded weekly on a cage basis, and growth performance parameters were calculated accordingly. At the end of the experiment, 10 birds per group (selected based on average body weight) were slaughtered for carcass and organ measurements. For microbiological and histological analyses, intestinal samples were collected aseptically. Sections of the small intestine were fixed for histological examination, while jejunal and ileal contents were collected for microbial analysis.

2.3 Histomorphological analyses

For histological analyses, intestinal samples (duodenum, jejunum, and ileum) were collected aseptically. Tissue samples were fixed in 10% neutral buffered formalin, dehydrated through a graded ethanol series, cleared in xylene, and embedded in paraffin. Sections (4–5 µm) were prepared and stained with hematoxylin–eosin for microscopic examination. Histomorphometric measurements, including villus height (VH), villus width (VW), and crypt depth (CD), were performed using a light microscope. For each sample, at least 10 well-oriented villi and their associated crypts were selected and measured. The villus height-to-crypt depth ratio (VH/CD) was calculated accordingly.

2.4. Microbiological analysis

Jejunal and ileal contents were stored at – 20°C, while ileocecal samples were preserved in thioglycolate broth (Sigma-Aldrich, UK) until analysis. Samples were vortexed, and aliquots (0.01 mL) were inoculated onto 5% sheep blood Brucella agar (Oxoid, UK). Plates were incubated under anaerobic conditions (80% N₂, 10% H₂, 10% CO₂) at 35 ± 2°C for 72 h using an Anoxomat system (Mart, Netherlands). Bacterial colonies were enumerated and expressed as colony-forming units (CFU/g). Identification at the genus and species level was performed using MALDI-TOF MS (Bruker, Germany). For analysis, colonies were applied to a steel target plate, treated with 70% formic acid, and overlaid with a matrix solution (α-cyano-4-hydroxycinnamic acid). Spectra were acquired in linear positive mode and analyzed using MALDI Biotyper software. Bacterial identification was based on database comparison, with score values ≥ 2.0 indicating species-level identification and ≥ 1.7 indicating genus-level identification. *E. coli* standard was used for calibration, and matrix solution served as a negative control.

2.5 Statistical Analysis

Statistical analyses were performed using SPSS software (version 22.0; SPSS Inc., Chicago, IL, USA). Data were initially tested for normality using the Shapiro–Wilk test and for homogeneity of variances using Levene's test. The experiment was arranged as a 2 × 2 factorial design, with two dietary factors: FEO supplementation (0 or 250 mg/kg) and OEO supplementation (0 or 250 mg/kg). Data were analyzed using a two-way analysis of variance (ANOVA) within the General Linear Model (GLM) procedure to evaluate the main effects of FEO, the main effects of OEO, and their interaction (FEO × OEO). For growth performance parameters (body weight gain, feed intake, and feed conversion ratio), the cage was considered the experimental unit. For carcass traits, intestinal morphology, and microbiological analyses, individual birds were considered the experimental units. For microbiological data, sex was included as an additional fixed factor in the model to account for potential sex-related variation. When significant main effects or interactions were detected, means were separated using Tukey's honestly significant difference (HSD) test. Results are presented as mean ± standard error of the mean (SEM), and statistical significance was declared at $P < 0.05$.

3. RESULTS AND DISCUSSION

3.1 Growth performance

As shown in Table 2, dietary supplementation with encapsulated fennel essential oil (FEO) had no significant effect on body weight gain (BWG; $P = 0.182$), but significantly reduced feed intake (FI; $P = 0.012$) and improved feed conversion ratio (FCR; $P = 0.012$). Similarly, oregano essential oil (OEO) supplementation significantly affected feed intake ($P = 0.020$), whereas no significant effects were observed on BWG ($P = 0.100$) or FCR ($P = 0.535$). Furthermore, significant FEO $\times$ OEO interactions were detected for FI ($P = 0.004$) and FCR ($P = 0.012$), but not for BWG ($P = 0.240$). Among the treatment groups, quails receiving 250 mg/kg FEO without OEO exhibited the lowest feed intake and the most favorable FCR, whereas the combined supplementation of FEO and OEO did not provide additional improvements compared with FEO alone.

Table 2				
Main Effects of Dietary Encapsulated Fennel Essential Oil (FEO), Oregano Essential Oil (OEO), and Their Interaction on the Growth Performance of Broiler Quails				
FEO (mg/kg)	OEO (mg/kg)	BWG (g)	FI (g)	FCR
0	0	223.8 ± 18.47	938.4 ^a ± 21.47	4.21 ^a ± 0.285
0	250	242.6 ± 22.40	931.6 ^a ± 31.01	3.87 ^{ab} ± 0.374
250	0	240.5 ± 17.91	876.7 ^b ± 54.49	3.66 ^b ± 0.299
250	250	243.7 ± 23.30	935.9 ^a ± 16.43	3.87 ^{ab} ± 0.364
P-value				
FEO		0.182	0.012	0.012
OEO		0.100	0.020	0.535
FEO $\times$ OEO		0.240	0.004	0.012
BWG: Body weight gain; FI: Feed intake; FCR: Feed conversion ratio.				
Different superscript letters (a–b) within the same row indicate statistically significant differences among treatment groups ($P < 0.05$), as determined by Tukey’s HSD test.				

Table 2 here.

The improvement in feed conversion ratio observed with FEO supplementation is consistent with previous studies reporting enhanced feed efficiency following dietary essential oil supplementation in poultry (Zeng et al., 2015; Windisch et al., 2008). These beneficial effects have generally been attributed to improved digestive enzyme activity, enhanced nutrient utilization, and modulation of intestinal microbiota, resulting in more efficient feed utilization. In addition, nanoencapsulation has been shown to improve the stability, bioavailability, and controlled release of essential oils, thereby enhancing their biological efficacy compared with conventional forms (Adil et al., 2024). This may explain the improved feed efficiency observed in the present study, particularly in birds receiving FEO alone. Although FEO significantly improved feed efficiency, this effect was not accompanied by a significant increase in body weight gain. Similar findings have been reported previously, where essential oil supplementation enhanced feed efficiency without markedly affecting growth performance, possibly due to differences in dosage, formulation, bird species, or experimental conditions (Hashemipour et al., 2016). Likewise, OEO supplementation reduced feed intake but did not significantly improve FCR or BWG, suggesting that its influence on growth performance was limited under the conditions of the present study. Interestingly, significant interactions between FEO and OEO were observed for feed intake and feed conversion ratio. The lowest feed intake and the best FCR were achieved in quails supplemented with FEO alone, whereas the combination of FEO and OEO resulted in intermediate values, indicating

that the beneficial effect of FEO on feed efficiency was not enhanced by the simultaneous inclusion of OEO. This interaction suggests that combining the two essential oils may modify their individual physiological effects rather than producing additive or synergistic responses. Overall, these findings indicate that encapsulated FEO can improve feed efficiency in broiler quails without increasing body weight gain. Although OEO influenced feed intake, its effects on overall growth performance were limited. The significant interaction between FEO and OEO further highlights that the response to combined phytogetic additives may depend on their specific composition and mode of action. Therefore, additional studies are warranted to optimize the dosage and combinations of encapsulated essential oils to maximize both feed efficiency and growth performance.

3.2 Carcass traits and internal organ weights

As presented in Table 3, dietary supplementation with encapsulated fennel essential oil (FEO), oregano essential oil (OEO), or their combination did not significantly influence carcass yield ($P > 0.05$). Likewise, no significant effects were observed on the relative weights of the proventriculus, heart, or small intestine ($P > 0.05$), indicating that dietary essential oil supplementation had limited effects on overall carcass characteristics and most internal organs under the conditions of the present study.

Table 3
Main Effects of Dietary Encapsulated Fennel Essential Oil (FEO), Oregano Essential Oil (OEO), and their Interaction on carcass traits and internal organ weights in Broiler Quails

FEO (mg/kg)	OEO (mg/kg)	Carcass yield (%)	Proventriculus (% BW)	Heart (% BW)	Gizzard (% BW)	Liver (% BW)	Small intestine (% BW)
0	0	59.6 ± 2.97	0.38 ± 0.15	1.19 ± 1.50	2.67 ± 0.42	2.94 ± 0.93	2.97 ± 0.65
0	250	59.9 ± 3.52	0.32 ± 0.09	0.69 ± 0.12	2.53 ± 0.20	2.69 ± 0.53	3.20 ± 0.60
250	0	58.8 ± 3.40	0.32 ± 0.12	0.58 ± 0.16	2.14 ± 0.25	2.40 ± 0.76	3.27 ± 0.59
250	250	60.3 ± 4.06	0.35 ± 0.04	0.62 ± 0.24	2.61 ± 0.43	2.30 ± 0.54	3.25 ± 0.57
<i>P-value</i>							
FEO		0.304	0.627	0.165	0.051	0.048	0.372
OEO		0.197	0.772	0.343	0.148	0.447	0.593
FEO × OEO		0.168	0.243	0.268	0.008	0.747	0.537
SW: Slaughter weight, Organ weights expressed relative to slaughter weight (g/kg SW)							
Different superscript letters (a–b) within the same row indicate statistically significant differences among treatment groups ($P < 0.05$), as determined by Tukey's HSD test.							

FEO supplementation significantly affected relative liver weight ($P = 0.048$), with birds receiving FEO showing lower liver weights than those without supplementation. The liver plays a central role in lipid metabolism, detoxification, and nutrient metabolism; therefore, changes in liver weight may reflect alterations in metabolic activity induced by bioactive compounds present in fennel essential oil, particularly anethole (Hong et al., 2012). Similar reductions in liver weight following phytogetic feed additive supplementation have been associated with improved metabolic

efficiency and reduced lipid deposition, although the underlying mechanisms remain to be fully elucidated. Although the main effect of FEO on gizzard weight was only marginally significant ($P = 0.051$), a significant interaction between FEO and OEO was observed ($P = 0.008$), indicating that the response of gizzard development depended on the combined inclusion of the two essential oils. Birds receiving FEO alone exhibited the lowest relative gizzard weight, whereas the addition of OEO appeared to attenuate this reduction. Such interactions may reflect differences in digestive physiology or feed processing activity resulting from the combined actions of phytochemical compounds. Previous studies have suggested that essential oils may influence gastrointestinal morphology by altering digestive enzyme secretion, gut motility, and microbial populations (Jamroz et al., 2005). Overall, the absence of significant effects on carcass yield suggests that encapsulated FEO and OEO did not adversely affect carcass development in broiler quails. The reduction in relative liver weight associated with FEO supplementation and the significant interaction observed for gizzard weight indicate that these phytochemical additives may influence specific digestive and metabolic organs without substantially altering overall carcass traits. Further studies investigating liver metabolism and gastrointestinal morphology would help clarify the physiological mechanisms underlying these responses.

Table 3 here.

3.3 Intestinal histomorphology

As shown in Table 4, dietary supplementation with oregano essential oil (OEO) significantly improved duodenal morphology. OEO supplementation increased villus height (VH; $P = 0.014$), villus height-to-crypt depth ratio (VH/CD; $P = 0.002$), and villus area (VA; $P = 0.042$), while significantly reducing villus width (VW; $P = 0.012$). These findings indicate an improvement in absorptive surface architecture and suggest enhanced intestinal functionality in the duodenum. In contrast, significant FEO $\times$ OEO interactions were observed in the jejunum for villus height ($P = 0.012$) and villus width ($P = 0.024$). Birds receiving the combined FEO and OEO treatment exhibited markedly lower villus height and width than the other treatment groups, indicating that simultaneous supplementation adversely affected jejunal mucosal development. Similar interaction effects were also observed in the ileum, where the combined treatment significantly reduced villus height ($P = 0.040$) and villus area ($P = 0.022$). In addition, FEO supplementation significantly influenced ileal villus width ($P = 0.022$) and villus area ($P = 0.001$), whereas OEO significantly affected ileal villus area ($P = 0.036$). However, crypt depth and the VH/CD ratio in the ileum were not significantly altered by either the main effects or their interaction ($P > 0.05$). The beneficial effects of OEO on duodenal morphology are consistent with previous reports demonstrating that oregano essential oil enhances intestinal development through its antimicrobial, antioxidant, and anti-inflammatory properties (Xu et al., 2003; He et al., 2017; Wang et al., 2021). Increased villus height and villus area generally indicate an expanded absorptive surface, thereby improving nutrient digestion and absorption (Zeng et al., 2015; Movahedi et al., 2024). The significant increase in the VH/CD ratio further supports improved intestinal maturation and epithelial function in birds supplemented with OEO. Conversely, the significant interaction between FEO and OEO in the jejunum and ileum suggests that the simultaneous administration of these essential oils did not produce additive or synergistic effects. Instead, the combined treatment was associated with reduced villus dimensions, indicating impaired intestinal architecture. Similar inconsistent responses have been reported when different phytochemical additives are combined, with outcomes depending on their chemical composition, inclusion levels, and potential interactions among bioactive compounds (Basmacioglu-Malayoglu et al., 2014; Ameer et al., 2024). Because the combined treatment also represented the highest total essential oil inclusion level (500 mg/kg), it remains unclear whether these adverse morphological responses resulted from antagonistic interactions between fennel and oregano essential oils or from the increased total dosage. Overall, these findings demonstrate that oregano essential oil exerted beneficial effects on duodenal intestinal morphology, whereas the combination of encapsulated fennel and oregano essential oils adversely affected jejunal and ileal villus development. Since no significant changes were observed in crypt depth, the negative

responses appear to be primarily associated with alterations in villus architecture rather than epithelial turnover. Further dose–response studies employing equivalent total inclusion levels are required to distinguish between dose-dependent effects and potential interactions between the two essential oils.

Table 4

Main Effects of Dietary Encapsulated Fennel Essential Oil (FEO), Oregano Essential Oil (OEO), and their Interaction on histomorphology of small intestine in Broiler Quails

Intestinal segment	FEO (mg/kg)	OEO (mg/kg)	VH (μm)	VW (μm)	CD (μm)	VH/CD	VA (mm^2)
Duodenum	0	0	532.0 $\pm$ 40.54	135.7 $\pm$ 9.22	38.1 $\pm$ 1.44	14.9 $\pm$ 1.02	2.30 $\pm$ 0.19
	250	0	581.2 $\pm$ 42.12	87.2 $\pm$ 8.30	33.9 $\pm$ 1.06	16.5 $\pm$ 1.44	1.59 $\pm$ 0.12
	0	250	721.1 $\pm$ 46.13	125.9 $\pm$ 8.74	31.3 $\pm$ 1.66	23.0 $\pm$ 1.66	2.85 $\pm$ 0.11
	250	250	419.0 $\pm$ 39.62	77.8 $\pm$ 10.14	31.5 $\pm$ 1.88	13.6 $\pm$ 1.02	1.09 $\pm$ 0.21
P-value							
FEO			0.066	0.233	0.412	0.056	0.056
OEO			0.014	0.012	0.244	0.002	0.042
FEO $\times$ OEO			0.137	0.120	0.333	0.071	0.121
Jejunum	0	0	463.9 $\pm$ 21.80	93.9 $\pm$ 6.04	41.2 $\pm$ 1.02	11.79 $\pm$ 1.16	1.36 $\pm$ 0.12
	250	0	446.1 $\pm$ 19.64	93.8 $\pm$ 6.12	33.3 $\pm$ 1.66	12.78 $\pm$ 1.08	1.36 $\pm$ 0.20
	0	250	510.2 $\pm$ 22.48	108.0 $\pm$ 6.24	33.6 $\pm$ 1.08	18.35 $\pm$ 1.25	1.69 $\pm$ 0.09
	250	250	309.1 $\pm$ 21.12	53.9 $\pm$ 6.10	31.1 $\pm$ 1.50	10.16 $\pm$ 1.02	1.15 $\pm$ 0.10
P-value							
FEO			0.334	0.448	0.155	0.223	0.165
OEO			0.127	0.232	0.748	0.322	0.242
FEO $\times$ OEO			0.012	0.024	0.365	0.128	0.334
Ileum	0	0	352.2 $\pm$ 26.33	76.7 $\pm$ 7.33	29.8 $\pm$ 2.99	12.18 $\pm$ 0.60	0.55 $\pm$ 0.19
	250	0	381.2 $\pm$ 24.44	124.9 $\pm$ 6.12	31.5 $\pm$ 2.01	13.33 $\pm$ 0.52	1.49 $\pm$ 0.13
	0	250	401.2 $\pm$ 29.2	85.8 $\pm$ 8.21	29.6 $\pm$ 2.18	13.35 $\pm$ 0.44	1.07 $\pm$ 0.21
	250	250	170.1 $\pm$ 24.10	58.5 $\pm$ 7.36	13.4 $\pm$ 2.04	12.88 $\pm$ 0.67	0.31 $\pm$ 0.14
P-value							
FEO			0.512	0.022	0.633	0.122	0.001

VH = Villus height; VW = Villus width; CD = Crypt depth; VA = Villus surface area. All measurements are in micrometers (μm) except VA (mm^2).

Different superscript letters (a–b) within the same row indicate statistically significant differences among treatment groups ($P < 0.05$), as determined by Tukey's HSD test.

Intestinal segment	FEO (mg/kg)	OEO (mg/kg)	VH (μm)	VW (μm)	CD (μm)	VH/CD	VA (mm ²)
OEO			0.066	0.224	0.242	0.240	0.036
FEO × OEO			0.040	0.462	0.460	0.444	0.022
VH = Villus height; VW = Villus width; CD = Crypt depth; VA = Villus surface area. All measurements are in micrometers (μm) except VA (mm ²).							
Different superscript letters (a–b) within the same row indicate statistically significant differences among treatment groups ($P < 0.05$), as determined by Tukey's HSD test.							

Table 4 here.

3.4 Microbiota composition

The intestinal microbiota plays a pivotal role in nutrient utilization, immune function, and intestinal homeostasis in poultry and is strongly influenced by dietary composition (Ma et al., 2024). As presented in Table 5, dietary supplementation with encapsulated essential oils modulated the intestinal microbiota in a sex-dependent manner. In male broiler quails, FEO supplementation significantly increased the population of *Lactobacillus* spp. ($P = 0.007$), whereas no significant effects were observed for *Clostridium perfringens* or *Escherichia coli* O157:H7 as main effects. However, a significant FEO × OEO interaction was detected for *E. coli* O157:H7 counts ($P = 0.030$), indicating that the combined supplementation altered the abundance of this bacterium compared with the individual treatments. In female broiler quails, FEO supplementation significantly reduced *E. coli* O157:H7 counts ($P = 0.002$) while increasing *Lactobacillus* spp. populations ($P = 0.045$). OEO supplementation also significantly decreased *E. coli* O157:H7 counts ($P = 0.045$). Furthermore, a significant interaction between FEO and OEO was observed for *Lactobacillus* spp. ($P = 0.034$), suggesting that the response of beneficial intestinal bacteria depended on the combined inclusion of both essential oils. In contrast, *Clostridium perfringens* populations were not significantly affected by either essential oil or their interaction in either sex ($P > 0.05$). The increase in *Lactobacillus* spp. observed following FEO supplementation agrees with previous studies demonstrating that phytogetic feed additives promote the proliferation of beneficial bacteria while improving intestinal microbial balance (Ghiasvand et al., 2021; Movahedi et al., 2024). Beneficial bacteria such as *Lactobacillus* spp. contribute to intestinal health through competitive exclusion of pathogenic microorganisms, production of antimicrobial metabolites, and maintenance of epithelial barrier integrity. The reductions in *E. coli* O157:H7 populations observed in female birds receiving FEO or OEO and the significant interaction detected in males are likely associated with the well-documented antimicrobial activities of phenolic compounds present in essential oils, including carvacrol, thymol, and anethole (Marques et al., 2015). Similar reductions in *Escherichia coli* together with increases in *Lactobacillus* populations have been reported in broiler chickens supplemented with oregano and other phytogetic essential oils (Vlaicu et al., 2023). Likewise, plant-derived phytogetic compounds have been shown to beneficially modify intestinal microbial communities while improving antioxidant status and immune responses (Liu et al., 2023). Interestingly, the microbiological responses differed between male and female birds, suggesting that sex may influence the interaction between dietary phytogetic additives and intestinal microbial ecology. Although the mechanisms responsible for these differences were not investigated in the present study, hormonal regulation, immune function, or subtle physiological differences between sexes may contribute to the observed variation and warrant further investigation. Overall, these findings demonstrate that encapsulated fennel essential oil positively modulated beneficial intestinal microbiota by increasing *Lactobacillus* spp., while both FEO and OEO contributed to reducing *E. coli* O157:H7 populations under specific conditions. Nevertheless, the absence of significant changes in *Clostridium perfringens* indicates that the antimicrobial activity of these essential oils may be selective rather than broad spectrum. Because the present

microbiological evaluation relied on conventional culture-based methods, the overall microbial diversity and functional composition of the intestinal ecosystem could not be fully characterized. Therefore, future studies should combine high-throughput 16S rRNA amplicon sequencing with quantitative PCR analyses of intestinal barrier- and inflammation-related genes to provide a more comprehensive understanding of the mechanisms through which encapsulated essential oils influence gut health. It should be emphasized that the present study was designed to evaluate the biological efficacy of commercially available encapsulated essential oil products rather than the physicochemical performance of the encapsulation system itself. In addition, detailed physicochemical characterization of the encapsulated products, including encapsulation efficiency, particle size distribution, and release kinetics, would help establish clearer relationships between encapsulation properties and their biological efficacy in poultry.

Table 5

Main Effects of Dietary Encapsulated Fennel Essential Oil (FEO), Oregano Essential Oil (OEO), and their Interaction on microflora composition of small intestine in Broiler Quails

FEO (mg/kg)	OEO (mg/kg)	<i>Clostridium perfringens</i>	<i>E. coli</i> O157:H7	<i>Lactobacillus</i> spp.
Male				
0	0	1.92 ± 0.938	5.92 ± 1.151	4.79 ± 0.820
0	250	1.55 ± 0.515	5.39 ± 0.682	4.87 ± 0.956
250	0	1.12 ± 0.268	4.72 ± 1.041	5.69 ± 0.448
250	250	1.34 ± 0.526	4.60 ± 0.774	6.03 ± 0.695
P-value				
FEO		0.081	0.634	0.007
OEO		0.782	0.454	0.544
FEO × OEO		0.300	0.030	0.720
Female				
0	0	1.63 ± 1.003	6.3 ± 0.762	5.21 ± 0.246
0	250	1.37 ± 0.520	5.21 ± 09.523	5.52 ± 0.943
250	0	1.64 ± 0.615	4.77 ± 0.553	6.52 ± 0.352
250	250	1.34 ± 0.526	4.66 ± 0.765	5.48 ± 0.778
P-value				
FEO		0.967	0.002	0.045
OEO		0.379	0.045	0.231
FEO × OEO		0.942	0.088	0.034
Microbial counts are expressed as log10 colony-forming units per gram (CFU/g) of intestinal content.				
Different superscript letters (a–b) within the same row indicate statistically significant differences among treatment groups (P < 0.05), as determined by Tukey's HSD test.				

Table 5 here.

3. CONCLUSION

Dietary supplementation with encapsulated fennel essential oil (FEO) and oregano essential oil (OEO) differentially affected growth performance, intestinal morphology, and gut microbiota in broiler quails. FEO improved feed efficiency by reducing feed intake and feed conversion ratio without affecting body weight gain, whereas OEO primarily enhanced duodenal morphology by increasing villus height, villus height-to-crypt depth ratio, and villus area. Although both essential oils beneficially modulated the intestinal microbiota by increasing *Lactobacillus* spp. and reducing *Escherichia coli* O157 under specific conditions, these effects were not consistently reflected in growth performance or intestinal morphology. The combined supplementation of FEO and OEO failed to provide additive benefits and adversely affected jejunal and ileal villus architecture. Overall, encapsulated FEO appears to be a more promising phytogenic feed additive than its combination with OEO under the conditions of the present study. Further studies should evaluate dose–response relationships and optimized combinations to maximize the biological efficacy of encapsulated essential oils.

Declarations

Acknowledgments

The authors gratefully acknowledge the financial support provided by the Scientific Research Projects Coordination Office of Dicle University (DÜBAP) under Project No. Ziraat.22.012 (2022). The authors also thank the Department of Animal Science, Faculty of Agriculture, Dicle University, for providing the facilities required to conduct this study. The authors express their sincere gratitude to Prof. Dr. Simten Yeşilmen Alp for performing the microbiota analyses, Prof. Dr. İsa Coşkun for conducting the intestinal histology analyses.

STATEMENT OF ANIMAL RIGHTS

All animal procedures were conducted in accordance with the ethical standards for the care and use of laboratory animals. The experimental protocol was reviewed and approved by the Ethics Committee of Dicle University (DÜHADEK) (Approval No. 2024/13; Date: 06 May 2024).

DATA AVAILABILITY

The data sets generated and/or analyzed during the current study are available from the corresponding author on reasonable request.

CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest.

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