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Oregano Essential Oil Supplementation Modulates Growth Performance, Digestive Efficiency, Cecal Microbiota, and Immunological Traits of Two Lines of Growing Rabbits

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ABSTRACT

Weaning-associated intestinal and immunometabolic instability in rabbits has intensified interest in oregano essential oil (OEO) as a bioactive regulator of gut integrity, microbial balance, and systemic physiology. This study investigated the dose-dependent effects of OEO supplementation and its interaction with genotype in V-line and Alexandria growing rabbits. A total of 108 weaned rabbits (54 per line; 28 d old) were randomly allocated to six treatments (n = 18/treatment) in a 2 × 3 factorial design comprising two rabbit lines and three OEO levels: 0% (control), 0.0125%, or 0.0250%, administered orally 3 d/week for 35 d. Growth performance, nutrient digestibility, cecal microbiota, serum biochemistry, and humoral immunity were evaluated. Results revealed that OEO supplementation significantly improved final body weight, average daily gain, feed conversion ratio, and performance index and reduced mortality rate (p≤0.003), predominantly through linear dose-dependent responses (p≤0.002). Enhanced digestibility coefficients of crude protein, crude fiber, neutral detergent fiber, and acid detergent fiber (p≤0.008), with linear and quadratic trends detected for most nutrients. OEO reduced cecal pH and *Clostridium perfringens* counts (p≤0.025), whereas *Lactobacillus* abundance exhibited a hormetic response, peaking at 0.0125% OEO, and declining at 0.0250%. Serum total protein, albumin, globulin, HDL, IgG, and IgM increased, whereas cholesterol, triglycerides, LDL, and total lipids decreased (p≤0.022), with predominantly linear responses (p≤0.008). V-line rabbits showed superior digestive and fermentative adaptation, whereas Alexandria rabbits exhibited greater immunometabolic resilience, particularly for IgM response (p<0.001). In conclusion, OEO linked microbial modulation with improved digestive efficiency, metabolic homeostasis, and immune competence, supporting it as a promising nutritional strategy for sustainable rabbit production.

Keywords: cecal ecosystem; humoral immunity; nutrient digestibility; oregano essential oil; rabbit genotypes

INTRODUCTION

The growing demand for animal protein remains a major challenge, particularly in developing countries such as Egypt, where population growth continues to increase pressure on conventional livestock systems (Abdel-Rahman & Ashour, 2023). Rabbit production is an efficient component of sustainable livestock systems (Šufliarský *et al*., 2024). However, rabbit productivity is particularly vulnerable during the weaning and post-weaning periods, when intestinal instability can impair growth and increase mortality (El-Ashram *et al*., 2020). The transition to solid feed, maternal separation, and immature digestive and immune systems induce nutritional and microbial challenges (de Blas *et al*., 2012), leading to intestinal dysbiosis, oxidative stress, impaired barrier function, and reduced productive performance (MIGDAŁ *et al*., 2025). Increased oxidative stress during this stage disrupts intestinal barrier integrity by elevating reactive oxygen species (ROS) production, thereby altering tight junction function and increasing epithelial permeability (Hendawy *et al*., 2020). Simultaneously, dysbiosis within the cecal microenvironment, characterized by the proliferation of pathogenic bacteria, further exacerbates intestinal mucosal injury and triggers localized inflammatory responses (Liang *et al*., 2022). Therefore, maintaining intestinal homeostasis during weaning is a major objective in modern rabbit nutrition.

Historically, antibiotic growth promoters were widely used to improve productive efficiency and control enteric disorders; however, concerns regarding antimicrobial resistance and drug residues have led to increasing restrictions on their use in animal production (Cervantes, 2015). Consequently, interest has shifted toward natural alternatives, particularly phytogenic feed additives such as essential oils (EOs), which exhibit antimicrobial, antioxidant,

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anti-inflammatory, and immunomodulatory activities (Movahedi *et al*., 2024; Ebeid *et al*., 2025). The bioactive phenolic compounds in EOs contribute to free radical scavenging, redox regulation, microbial modulation, and improved digestive processes, supporting their increasing application in monogastric animal nutrition (Zeng *et al*., 2015).

Among phytogenic additives, oregano essential oil (OEO) has emerged as one of the most biologically active and extensively studied (Cui *et al*., 2024). Its major phenolic constituents, carvacrol and thymol, account for most of its antimicrobial and antioxidant activities, while minor compounds such as γ-terpinene and p-cymene further contribute to its bioactivity (Ghazi *et al*., 2015). In rabbits, OEO supplementation has been associated with improved feed efficiency, intestinal morphology, modulation of the gut microbiota, antioxidant status, and immune responses (Ayoub *et al*., 2021; Areda *et al*., 2026). These responses have been linked in previous studies to the biological activities of oregano phenolics, including disruption of bacterial membranes, modulation of intestinal fermentation, and improved nutrient utilization, as well as antimicrobial activity against major enteric pathogens such as *Escherichia coli, Salmonella typhimurium*, and *Staphylococcus aureus* (Li *et al*., 2021; Cui *et al*., 2024). However, the physiological mechanisms underlying OEO action in rabbits remain insufficiently characterized, as previous studies have primarily evaluated individual responses rather than the integrated effects of OEO on digestive function, cecal microbiota, systemic metabolism, and immune homeostasis (Abd El-Aziz *et al*., 2024; Ahmed *et al*., 2024).

Importantly, previous investigations have mainly focused on dietary OEO supplementation without considering the potential role of genetic background in phytogenic responsiveness (Jinling *et al*., 2022; He *et al*., 2023). Moreover, research on OEO administration through drinking water in rabbits remains limited despite its practical advantages under commercial production conditions (Abd El-Aziz *et al*., 2024). To date, the dose-dependent effects of water-supplemented OEO on growth performance, nutrient digestibility, cecal microbiota, serum biochemical traits, and humoral immunity, particularly in relation to genotype × phytogenic interactions, have not been comprehensively investigated. This knowledge gap is important because host genotype may influence microbial colonization, digestive efficiency, immune responses, and metabolic adaptation to phytogenic compounds (Abdelsalam *et al*., 2025; Hendawy *et al*., 2025).

Therefore, the present study was conducted to evaluate the effects of two levels of OEO supplementation in drinking water on productive performance, nutrient digestibility, caecal microbial populations, serum biochemical parameters, and humoral immunity in V-line and Alexandria-line growing rabbits. The study further aimed to clarify the optimal OEO inclusion level and to determine whether rabbit genetic background modulates the physiological efficacy of oregano phytochemicals under post-weaning conditions.

MATERIALS AND METHODS

Ethical Approval and Experimental Site

All procedures involving animals were performed in compliance with the ethical standards of the Animal Care Committee at Alexandria University (Approval No. Alex. Agri. 092507312). Throughout the study, rabbits were handled and managed in accordance with established welfare guidelines. The trial was performed at the nucleus rabbit breeding unit of the Poultry Research Center, Department of Poultry Production, Faculty of Agriculture, Alexandria University, located at 31°20′ N and 30° E in Alexandria.

Experimental Additive

The experimental additive consisted of oregano essential oil (OEO) formulated of 60.2% carvacrol and 4% thymol, combined with 100 mL of propylene glycol and 200 mL of polysorbate 80, with purified water added to reach a final volume of 1 liter (EVAXO International Inc., USA).

Rabbits, Experimental Design, Management, and Diets

A total of 108 growing rabbits, comprising 54 V-line and 54 Alexandria rabbits, were enrolled in a 63-day trial. At the start of the experiment, all rabbits underwent a health assessment to confirm their health status and verify that they met the required breed, age, and body weight criteria for inclusion in the study. At 28 days of age, the mean initial body weights were 657.55 g for the V-line rabbits and 668.87 ± 10.414 g for the Alexandria rabbits. Rabbits were randomly allocated to six experimental treatments, with each breed distributed across three groups: a first group received untreated plain tap water without supplementation as a control; a second group received drinking water supplemented with 0.0125% OEO; and a third group received drinking water supplemented with 0.0250% OEO. Each treatment group consisted of 18 rabbits, divided into six replicates of three rabbits per pen.

Water consumption (mL/day) was measured using a graduated container placed in each pen throughout the study. OEO was administered orally for three days per week over a five-week supplementation period in accordance with the manufacturer's recommendations for the commercial product used in this study. This supplementation regimen was adopted to evaluate the responses of the two rabbit lines to OEO under the specified experimental conditions. Pens were cleaned daily by removing fecal droppings. Throughout the experiment, rabbits were housed in single-tier galvanized wire cages (35 × 40 × 50 cm) equipped with feeders and an automatic watering system and were randomly assigned to pens. Pens were the experimental units for the intake and performance studies. All rabbits were maintained under uniform management, hygiene, and controlled environmental conditions, including an ambient temperature of 20–25 °C, relative humidity of 55%–65%, and a lighting schedule of 14–16 h per day. Feed and

fresh water were provided *ad libitum*. All experimental diets were formulated to meet or exceed the nutrient requirements of growing rabbits by integrating the baseline reference of NRC (1977) with modern rabbit nutrition recommendations (De Blas & Mateos, 2020), ensuring balanced digestible energy-to-crude protein ratios (DE: CP) and adequate digestible fiber fractions (neutral detergent fiber, acid detergent fiber, and acid detergent lignin). Ingredients and chemical analysis of the experimental diet are presented in Table 1.

Productive Performance Measurements

At the beginning of the experiment, initial body weights (IBW) were recorded for all rabbits. Body weight (BW), feed intake (FI), and water consumption (WC) were measured weekly. Meanwhile, the mortality rate (MR) of the rabbits, including the number of dead rabbits per experimental group, was observed and recorded daily. The MR was computed as follows (Wang *et al*., 2018): MR (%) = (number of dead rabbits/ rabbits initially assigned to the group) × 100%. Average daily gain (ADG) for each rabbit was subsequently calculated. Individual body weights were obtained prior to the morning feeding. In addition, the feed conversion ratio (FCR) was determined by dividing total FI (g) by body weight gain (g). The performance index (PI) was calculated using the following equation: PI = (final live body weight, kg / FCR) × 100, as described by Abdel-Azeem *et al*. (2009).

Table 1. Ingredient and chemical composition of the experimental diet

Ingredients	kg
Barley	205
Yellow corn	80
Soybean meal	170
Coarse wheat bran	240
Alfalfa hay	210
Berseem hay	70
Limestone	5
Di-calcium phosphate	12
DL-Methionine	1
Sodium chloride	4
Vitamins and minerals premix1	3
Total	1000
Chemical composition (g/kg DM)	
CP[2]	161.5
DE[2], kcal/kg	2510
EE[2]	28.7
CF[2]	141.9
NDF[2]	38.5
ADF[2]	18.3
ADL[2]	6.6
Calcium	9.6
Available phosphorus	4.8
Methionine	4.2
Lysine	10.5
DE: CP	155.4

Note: [1] Vitamin and mineral premix contained per kilogram: 12000 IU vitamin A, 2200 IU vitamin D3, 10 mg vitamin E, 2.0 mg vitamin K, 1.0 mg vitamin B_1, 4.0 mg vitamin B_2, 1.5 mg vitamin B_6, 0.001 mg vitamin B_{12}, 6.7 mg pantothenic acid, 6.67 mg vitamin B_5, 1.07 mg biotin, 1.67 mg folic acid, 400 mg choline chloride, 22.3 mg Zn, 10 mg Mn, 25 mg Fe, 1.67 mg Cu, 0.25 mg I, 0.033 mg Se, 133.4 mg Mg. [2] CP: Crude protein, DE: Digestible energy, EE: Ether extract, CF: Crude fiber, NDF: Neutral detergent fiber, ADF: Acid detergent fiber, ADL: Acid detergent lignin.

Determining Digestion Coefficients and Chemical Analysis

To assess nutrient digestibility coefficients, six rabbits from each treatment (one rabbit per replicate) were randomly selected and placed in individual metabolic cages fitted with devices that separated feces and urine for each animal. Rabbits were maintained for 7 days to adapt to the new conditions, followed by 4 days of collection. Throughout the collection period, FI for each rabbit was accurately determined by deducting the remaining feed from the amount offered. Feces samples were collected daily before morning feeding for 4 days, weighed, mixed, and stored at -20 °C. At the end of the experiment, they were dried for 48 hours at 60 °C and transferred to the Animal Nutrition Laboratory, Animal and Fish Production Department, Faculty of Agriculture, Alexandria University, for proximate analysis. The digestion coefficient of nutrients was calculated using lignin as an internal marker, where the formula described by García *et al*. (1999) was used:

Digestion coefficient (%)=
100 – [(% of lignin in feed / % of lignin in faeces) x (% of nutrient in faeces / % of nutrient in feed)] x 100

Chemical characterization of feed and fecal samples was conducted following the standardized analytical procedures of AOAC (2000), including determination of dry matter (DM; 934.01), ash (942.05), crude protein (CP; 986.06), ether extract (EE; 920.39), and crude fiber (CF; 978.10). Subsequently, nitrogen-free extract (NFE) values were estimated mathematically by difference. In parallel, concentrations of neutral detergent fiber (NDF), acid detergent fiber (ADF), and acid detergent lignin (ADL) were quantified using an Ankom fiber analyzer system (ANKOM model A2001, Macedon, NY, USA) according to the methodology described by Van Soest *et al*. (1991).

Caecal Microbial Count, pH, and Gastrointestinal Measurements

For microbiological analysis, fresh cecal samples were collected at slaughter from one randomly selected rabbit per replicate, yielding a total of six samples for each treatment. The caecal samples were kept on ice and immediately transferred to the laboratory for further processing. Digesta samples (0.1 g) from the cecum were aseptically homogenized in sterile buffered peptone water and serially tenfold diluted (10^{-1}- 10^{-10}). *Lactobacillus* counts were determined on de Man-Rogosa-Sharpe (MRS) agar (Oxoid, Germany), incubated at 37 °C for 48 h under anaerobic conditions (Tarabees *et al*., 2019). Additionally, *Clostridium perfringens* was enumerated using Tryptose Sulfite Cycloserine (TSC) agar supplemented with egg yolk (Oxoid, UK), followed by incubation at 37 °C for 24–48 h

under anaerobic conditions (Hustá *et al*., 2020). Data were presented as $\log_{10}$ CFU per gram of digesta, and the pH of caecal contents was estimated with a digital pH meter (MP511 Benchtop; Apera Instruments). Moreover, the weights of the cecum and intestines were measured and expressed as percentages of pre-slaughter body weight, and the lengths of the intestines and ceca were also recorded.

Blood Collection and Serum Biochemical Parameters

At the conclusion of the trial, six rabbits per treatment group (one per replicate) were randomly selected. Approximately 3 mL of blood was collected from the marginal ear vein of each rabbit between 08:00 and 09:00 h using non-heparinized tubes. The samples were centrifuged at 4,000 × g for 15 min, and the obtained serum was stored at −20 °C until biochemical analyses were performed using commercial diagnostic kits (Stanbio Laboratory, Boerne, TX, USA) according to the manufacturer's instructions. Serum was analyzed for total protein, albumin, total lipids, triglycerides, total cholesterol, high-density lipoprotein (HDL), and low-density lipoprotein (LDL). Immunoglobulin G (IgG) and immunoglobulin M (IgM) concentrations were determined by ELISA using commercial kits (Bethyl Laboratories, Montgomery, TX, USA). Serum globulin levels were calculated as the difference between total protein and albumin, since fibrinogen contributes negligibly to total protein.

Statistical Analysis

Data were statistically analyzed using the GLM procedure of SAS software (version 9.4; SAS Institute Inc., Cary, NC, USA) following a factorial analysis of variance. The statistical model used was:

$$Y_{ijk} = \mu + B_i + T_j + (B \times T)\,ij + e_{ijk}$$

where Y_{ijk} is an observed value, μ is the overall mean, B_i is the fixed effect of i^{th} breed, T_j is the fixed effect of j^{th} OEO treatment, $(B \times T)_{ij}$ represents the interaction between breed and treatment, and e_{ijk} is the random experimental error. A multivariate linear model was employed, with rabbit breed (Alexandria and V-line) as the first factor and OEO supplementation (0, 0.0125, and 0.0250 % as oral doses) as the second factor, assuming normally distributed residuals. Linear and quadratic orthogonal contrasts were applied to evaluate the dose–response effects of increasing OEO levels and to compare the control group with the OEO-supplemented treatments. Least squares means (LSM) and standard errors were estimated, and Duncan's multiple range test was used to determine significant differences among means. Statistical significance was declared at $p<0.05$.

RESULTS

Growth-Related Measurements

Growth-related measurements are summarized in Table 2, highlighting variations attributable to genetic background, graded OEO inclusion, and their combined influence. Differences between rabbit lines were evident for average feed intake (AFI) and MR ($p<0.001$), with higher values recorded in the V-line compared to the Alexandria line. Alterations associated with OEO inclusion were detected for final BW, AFI, ADG, WC, MR, and PI ($p\leq0.003$). Compared with the non-supplemented group, both supplemented groups demonstrated increased ADG and PI, along with reduced FCR, WC, and MR. The highest values for growth and efficiency indices were observed at 0.0250% inclusion, whereas the lowest values were observed in the control treatment.

In contrast, peak values for FCR, WC, and MR were recorded in the absence of supplementation. Dose-dependent responses followed a significant linear pattern ($p\leq0.002$), with additional curvature detected for FCR and MR. Combined effects were evident for AFI and MR, where the highest AFI occurred in V-line rabbits at 0.0250% OEO, while the lowest was observed in Alexandria-line rabbits at the same level. MR reached its maximum in untreated V-line rabbits and its minimum in Alexandria-line rabbits receiving 0.0250% OEO.

Serum Biochemical and Immunological Indices

Biochemical and immunological indices are detailed in Table 3, encompassing protein fractions, lipid constituents, and immunoglobulin concentrations. Line-related variation was apparent for total protein, globulin, total lipid, triglycerides, LDL, and IgM ($p\leq0.036$), with consistently higher values recorded in the Alexandria line relative to the V-line. OEO inclusion modulated all measured variables ($p\leq0.022$). Compared with the unsupplemented condition, progressive inclusion of OEO was associated with elevated levels of protein fractions, HDL, IgG, and IgM, alongside reductions in total lipids, cholesterol, triglycerides, and LDL. Peak concentrations for protein-related and immunological traits were observed at 0.0250% inclusion, whereas the lowest levels were observed in the control group. Conversely, lipid-associated parameters were maximal in the absence of supplementation and minimal at the highest inclusion level. Linear dose-responses predominated ($p\leq0.008$), while a quadratic component was identified for globulin only. A differential response between lines was observed for IgM ($p = 0.027$), where the maximum value occurred in Alexandria-line rabbits with 0.0250% OEO, and the minimum in V-line rabbits without supplementation.

Caecal Microbial Populations and Gastrointestinal Characteristics

Table 4 shows the microbial populations and gastrointestinal characteristics of growing rabbits as affected by OEO supplementation and rabbit line. Variation attributable to genetic origin was limited to intestinal length ($p<0.001$), with greater values recorded in the V-line. The inclusion of OEO induced significant shifts in microbial counts and anatomical measurements ($p\leq0.025$). Compared with the control condition, supplemented groups exhibited increased caecal and intestinal proportions and lengths, accompanied by

Table 2. Growth performance traits of two growing rabbit lines supplemented with oregano essential oil (OEO) in drinking water

Treatments	Growth performance							
	Initial BW[1] (g)	Final BW[1] (g)	FCR[1]	ADG[1] (g/d)	AFI[1] (g/d)	WC[1] (mL/day)	Mortality (%)	PI[1]
Breeds								
V-line	657.55	1752.60	2.76	31.14	84.45[a]	185.62	11.11[a]	65.25
Alex-line	668.87	1701.54	2.86	29.46	81.42[b]	188.35	3.70[b]	62.58
SEM	10.414	21.965	0.069	0.680	0.395	11.701	0.884	2.132
P-value	0.446	0.078	0.307	0.085	<0.001	0.549	<0.001	0.361
OEO Supplementation								
0 %	663.97	1626.45[b]	3.13[a]	27.38[b]	83.34	241.39[a]	11.11[a]	54.51[b]
0.0125%	662.64	1762.35[a]	2.68[b]	31.28[a]	82.61	186.49[b]	5.55[b]	67.21[a]
0.0250%	663.38	1778.38[a]	2.66[b]	31.86[a]	82.66	138.65[c]	5.55[b]	69.31[a]
SEM	12.997	25.160	0.080	0.786	0.540	3.450	1.193	2.397
P-value	0.997	<0.001	0.001	0.003	0.519	<0.001	<0.001	<0.001
Linear effect	0.966	<0.001	0.001	0.002	0.422	<0.001	<0.001	<0.001
Quadratic effect	0.953	0.054	0.035	0.095	0.401	0.456	<0.001	0.076
Interaction								
V-line 0 %	646.76	1644.29	2.98	28.35	83.14[b]	242.45	16.66[a]	57.16
V-line 0.0125%	665.83	1810.88	2.64	32.55	84.76[ab]	189.29	5.55[c]	70.14
V-line 0.0250%	659.68	1782.50	2.70	32.08	85.26[a]	136.48	11.11[b]	67.63
Alex-line 0 %	681.17	1611.76	3.26	26.59	83.51[ab]	240.54	5.55[c]	52.18
Alex-line 0.0125%	659.44	1713.82	2.72	30.00	80.45[c]	183.69	5.55[c]	64.28
Alex-line 0.0250%	666.67	1774.72	2.62	31.66	80.36[c]	140.83	0.00[d]	70.80
SEM	7.499	15.855	0.051	0.491	0.320	8.118	0.892	1.523
P-value	0.538	0.419	0.254	0.616	0.001	0.655	<0.001	0.344

Note: Means within the same column, with different superscripts, are significantly different (p<0.05). SEM, standard error of the mean. [1] ADG, average daily gain; BW, body weight; AFI, average feed intake; FCR, feed conversion ratio; WC, water consumption; PI, performance index.

Table 3. Serum protein profile, lipid profile, and immunoglobulins of two growing rabbit lines supplemented with oregano essential oil (OEO) in drinking water

Treatments	Protein profile			Lipid profile					Immunoglobulin	
	Total protein, g/dl	Albumin, g/dl	Globulin, g/dl	Total Lipids mg/ dl	Cholesterol, mg/ dl	Triglyceride, mg/ dl	HDL[1], mg/ dl	LDL[1], mg/ dl	IgG[1], mg/ dl	IgM[1], mg/ dl
Breeds										
V-line	5.22[b]	3.76	1.50[b]	432.22[b]	55.22	62.42[b]	28.00	15.78[b]	11.33	4.42[b]
Alex-line	5.73a	3.81	1.92[a]	524.33[a]	57.54	78.78[a]	26.78	18.22[a]	11.94	6.00[a]
SEM	0.099	0.047	0.054	16.421	1.993	3.830	1.224	1.061	0.322	0.254
P-value	<0.001	0.325	<0.001	<0.001	0.291	0.005	0.440	0.036	0.055	<0.001
OEO Supplementation										
0 %	5.10[c]	3.66[b]	1.49[b]	529.67[a]	63.05[a]	83.63[a]	24.17[b]	20.33[a]	10.51[b]	4.38[c]
0.0125%	5.55[b]	3.77[b]	1.78[a]	479.33[b]	54.20[b]	66.67[b]	28.17[a]	16.83[b]	11.93[a]	5.05[b]
0.0250%	5.77[a]	3.92[a]	1.85[a]	425.83[c]	51.89[b]	61.50[b]	29.83[a]	13.83[c]	12.47[a]	6.20[a]
SEM	0.112	0.043	0.085	21.527	1.591	4.415	1.226	0.996	0.283	0.339
P-value	<0.001	0.002	<0.001	0.001	0.001	0.005	0.022	0.005	0.001	<0.001
Linear effect	<0.001	0.006	<0.001	<0.001	0.005	0.002	0.008	0.001	<0.001	<0.001
Quadratic effect	0.202	0.655	0.023	0.922	0.165	0.164	0.487	0.829	0.177	0.225
Interaction										
V-line 0 %	4.80	3.64	1.26	485.33	61.20	78.67	24.67	18.33	9.98	3.29[d]
V-line 0.0125%	5.37	3.78	1.59	453.00	53.47	55.63	29.33	15.33	11.97	4.22[c]
V-line 0.0250%	5.50	3.85	1.65	358.33	51.00	52.97	30.00	13.67	12.03	5.75[b]
Alex-line 0 %	5.41	3.68	1.72	574.00	64.90	88.60	23.67	22.33	11.03	5.48[b]
Alex-line 0.0125%	5.73	3.75	1.97	505.67	54.93	77.70	27.00	18.33	11.90	5.88[b]
Alex-line 0.0250%	6.04	3.99	2.05	493.33	52.78	70.03	29.67	14.00	12.90	6.64[a]
SEM	0.086	0.033	0.057	15.067	1.400	3.153	0.859	0.790	0.233	0.247
P-value	0.426	0.433	0.745	0.109	0.898	0.446	0.866	0.376	0.289	0.027

Note: Means within the same column with different superscripts are significantly different (p<0.05). SEM, standard error of the mean. [1] HDL, high-density lipoprotein; LDL, low-density lipoprotein; IgG, immunoglobulin-G; IgM, immunoglobulin-M.

Table 4. Cecal microbial count and gastrointestinal measurements of two growing rabbit lines supplemented with oregano essential oil (OEO) in drinking water

Treatments	Variables measured						
	Lactobacillus (log10 CFU/g)	*Clostridium perfringens* (log10 CFU/g)	Caecal pH	Caecum (%)	Intestine (%)	Caecum length (cm)	Intestine length (cm)
Breeds							
V-line	7.95	4.72	6.41	6.14	6.22	43.17	410.42^{a}
Alex-line	7.94	4.73	6.33	6.07	6.27	42.32	391.33^{b}
SEM	0.236	0.064	0.093	0.125	0.113	1.188	5.456
P-value	0.704	0.791	0.545	0.265	0.697	0.385	<0.001
OEO Supplementation							
0 %	8.10^{b}	5.00^{a}	6.61^{a}	5.50^{b}	5.84b	37.63^{b}	379.75^{c}
0.0125%	8.81^{a}	4.65^{b}	6.17^{c}	6.44^{a}	6.44^{a}	44.92^{a}	403.75^{b}
0.0250%	6.92^{c}	4.53^{c}	6.33^{ab}	6.39^{a}	6.45^{a}	45.69^{a}	419.13^{a}
SEM	0.026	0.018	0.103	0.064	0.102	0.843	5.368
P-value	0.001	0.001	0.025	<0.001	0.002	<0.001	<0.001
Linear effect	0.001	0.001	0.074	<0.001	0.002	<0.001	<0.001
Quadratic effect	0.001	0.007	0.029	<0.001	0.021	0.003	0.313
Interaction							
V-line 0 %	8.09	5.03	6.81	5.63^{c}	5.74	38.25	392.50^{b}
V-line 0.0125%	8.82	4.62	6.14	6.55^{a}	6.60	46.50	422.50^{a}
V-line 0.0250%	6.95	4.52	6.28	6.26^{b}	6.32	44.75	416.25^{a}
Alex-line 0 %	8.12	4.98	6.41	5.37^{d}	5.94	37.00	367.00^{c}
Alex-line 0.0125%	8.80	4.67	6.20	6.33^{ab}	6.29	43.33	385.00^{b}
Alex-line 0.0250%	6.90	4.54	6.39	6.53^{a}	6.57	46.63	422.00^{a}
SEM	0.163	0.044	0.070	0.088	0.078	0.830	4.275
P-value	0.641	0.424	0.196	0.001	0.103	0.116	0.005

Note: Means within the same column with different superscripts are significantly different (p<0.05). SEM, standard error of the mean.

reduced *Clostridium perfringens* counts and lower caecal pH. The highest values for gastrointestinal dimensions were consistently associated with the 0.0250% level, while the lowest values were observed in the non-supplemented group. *Lactobacillus* counts peaked at 0.0125% and declined to 0.0250%, whereas Clostridium perfringens reached its highest level in the control group and its lowest in the treated group. Linear trends predominated for most variables, with additional quadratic patterns detected for several traits. Interactive influences were evident for caecum proportion and intestinal length, where the maximum caecum proportion was recorded in Alexandria-line rabbits at 0.0250% OEO and the minimum in the same line without supplementation. The longest intestines were observed at 0.0250% in both lines, while the shortest were found in untreated Alexandria-line rabbits.

Nutrient Digestibility

Digestibility coefficients for major nutrients are reported in Table 5. Genetic differences were evident for NFE, NDF, and ADF (p≤0.049), with higher coefficients in the V-line and lower values in the Alexandria-line. Dietary inclusion of OEO significantly affected most digestibility parameters (p≤0.008), except for NFE. Regarding the control, supplemented groups showed enhanced digestibility of DM, OM, CP, EE, CF, NDF, and ADF. The highest coefficients were generally associated with inclusion levels of 0.0125% or 0.0250%, whereas the lowest values were consistently observed in the absence of supplementation. Both linear and quadratic responses were detected for the most affected variables. Significant interaction effects were observed for DM, OM, CF, NDF, and ADF. For these traits, the highest coefficients were observed in V-line rabbits with 0.0125% OEO, while the lowest values were observed in Alexandria-line rabbits under the control treatment.

DISCUSSION

Weaning is a critical stage in rabbit production because the immature digestive and immune systems predispose young rabbits to intestinal dysbiosis, impaired growth, and increased mortality (Benlemlih *et al*., 2020). OEO, rich in the bioactive phenolics thymol and carvacrol, has attracted considerable attention as a phytogenic additive owing to its antimicrobial, antioxidant, anti-inflammatory, and digestive-stimulatory properties (Li *et al*., 2021; Cui *et al*., 2024). In the present study, OEO supplementation through drinking water improved growth performance, nutrient digestibility, cecal microbial populations, metabolic profile, and immune responses; however, the magnitude of these effects varied between the V-line and Alexandria rabbit lines. Because information on OEO supplementation in rabbits remains limited, the present findings were interpreted in the context of previous studies involving oregano essential oil and other phytogenic additives in rabbits and monogastric species.

The progressive improvements in final BW, ADG, FCR, and PI with increasing OEO supplementation indicate enhanced nutrient utilization rather than a simple increase in feed intake. Similar improvements in BW, ADG, and feed intake at moderate OEO supplementation levels have been reported in

Table 5. Digestion coefficients of nutrients in two growing rabbit lines supplemented with oregano essential oil (OEO) in drinking water

Treatments	Digestion coefficients							
	DM[1] (%)	OM[1] (%)	CP[1] (%)	EE[1] (%)	CF[1] (%)	NFE[1] (%)	NDF[1] (%)	ADF[1] (%)
Breeds								
V-line	60.16	58.11	73.15	72.78	36.77	67.34^{a}	50.15^{a}	31.42^{a}
Alex-line	60.02	57.22	73.03	71.20	35.92	65.55^{b}	48.64^{b}	29.50^{b}
SEM	0.483	0.485	0.506	1.397	1.536	0.608	0.580	0.617
P-value	0.794	0.127	0.816	0.236	0.611	0.049	0.010	0.004
OEO Supplementation								
0 %	58.49^{b}	56.28^{b}	70.83^{b}	65.93^{b}	32.05^{b}	65.94	47.25^{b}	28.42^{b}
0.0125%	61.07^{a}	58.71^{a}	74.45^{a}	74.55^{a}	38.71^{a}	67.08	50.41^{a}	31.44^{a}
0.0250%	60.72^{a}	58.00^{a}	73.99^{a}	75.33^{a}	38.84^{a}	66.30	50.54^{a}	31.51^{a}
SEM	0.480	0.514	0.405	1.271	1.628	0.779	0.546	0.644
P-value	0.008	0.004	0.001	0.001	0.005	0.560	0.001	0.004
Linear effect	0.002	0.019	0.001	0.001	0.005	0.739	0.001	0.004
Quadratic effect	0.015	0.014	0.004	0.022	0.091	0.310	0.015	0.032
Interaction								
V-line 0 %	58.95^{bc}	56.49^{bc}	70.70	64.95	34.69^{ab}	66.19	47.82^{c}	29.50^{cd}
V-line 0.0125%	61.75^{a}	60.21^{a}	74.96	76.23	40.12^{a}	68.83	52.21^{a}	33.58^{a}
V-line 0.0250%	59.78^{abc}	57.63^{bc}	73.78	77.18	35.91^{ab}	66.98	50.43^{ab}	31.16^{bc}
Alex-line 0 %	58.03^{c}	56.08^{c}	70.97	67.10	29.41^{b}	65.70	46.67^{c}	27.35^{d}
Alex-line 0.0125%	60.39^{ab}	57.21^{bc}	73.94	72.55	37.29^{a}	65.33	48.60^{bc}	29.30^{cd}
Alex-line 0.0250%	61.65^{a}	58.37^{ab}	74.20	73.49	41.28^{a}	65.63	50.64^{a}	31.87^{ab}
SEM	0.337	0.349	0.354	1.029	1.078	0.452	0.427	0.464
P-value	0.041	0.034	0.410	0.182	0.049	0.362	0.026	0.009

Note: Means within the same column with different superscripts are significantly different ($p<0.05$). SEM: standard error of the mean. [1] DM, Dry matter; OM, Organic matter; CP, Crude protein; EE, Ether extract; CF, Crude fiber; NDF, Neutral detergent fiber; ADF, Acid detergent fiber; NFE, Nitrogen-free extract.

broilers (Yang *et al*., 2025). The linear improvement in productive performance up to 0.0250% OEO suggests that its antimicrobial, digestive-enhancing, and immunomodulatory properties collectively improved nutrient availability and utilization (Abdel-Wareth *et al*., 2022; He *et al*., 2023). These responses are particularly relevant in rabbits, where hindgut fermentation plays a pivotal role in nutrient recovery and energy supply (Gidenne, 2015). Consistent with the present findings, dietary OEO improved growth performance, reduced diarrhea incidence, and enhanced intestinal barrier integrity in growing rabbits (Li *et al*., 2021), while OEO combined with antimicrobial peptides further improved growth performance and intestinal health in meat rabbits (Jinling *et al*., 2022).

The improvement in productive performance was closely associated with enhanced digestive efficiency and gastrointestinal stability. Oregano phenolics improve digestive function, intestinal absorptive capacity, and microbial balance while reducing inflammatory stress, thereby enhancing nutrient utilization (Abdel-Wareth *et al*., 2022; He *et al*., 2023). This interpretation is supported by the reduced mortality rate, particularly the absence of mortality in Alexandria-line rabbits receiving 0.0250% OEO, indicating improved intestinal health and physiological resilience. Similar improvements in feed efficiency and growth performance following OEO supplementation have been reported in poultry, attributed to enhanced nutrient digestibility, modulation of the intestinal microbiota, and reduced oxidative stress (Feng *et al*., 2021; Yang *et al*., 2025). The differential responses between rabbit lines further suggest genotype-dependent adaptation to phytogenic supplementation (Hendawy *et al*., 2025). Consistent with this observation, Abdelsalam *et al*. (2025) reported greater feed intake in V-line than Alexandria-line rabbits following probiotic supplementation, indicating line-dependent responses to functional feed additives.

The improved nutrient digestibility observed in OEO-supplemented rabbits provides a physiological basis for the enhanced productive performance. The increased digestibility of DM, OM, CP, EE, CF, NDF, and ADF indicates that OEO enhanced digestive efficiency and microbial fermentation (Abdel-Wareth *et al*., 2022). Similar improvements in nutrient utilization have been reported in rabbits supplemented with oregano-based additives, including enhanced digestibility of DM, EE, CF, and CP (Omer *et al*., 2013; Ahmed *et al*., 2024). Likewise, supplementation of drinking water with OEO improved fiber digestibility, particularly CF and NDF digestibility, in V-line rabbits (Sayedahmed *et al*., 2026). Because the cecum is the primary site of microbial fermentation of structural carbohydrates in rabbits (Saleh *et al*., 2025), the improved NDF and ADF digestibility observed in the present study suggests enhanced hindgut fermentation and more efficient utilization of fibrous substrates (Gidenne, 2015), thereby contributing to improved growth performance and feed efficiency. Digestibility responses also differed between rabbit lines, with the V-line exhibiting greater NDF and ADF digestibility, indicating that the physiological response to OEO was influenced by genetic background (Abdelsalam *et al*., 2025). In contrast, the Alexandria line exhibited a stronger immunological response, particularly

higher IgM concentrations, suggesting that OEO may differentially modulate digestive and immune functions according to genotype. Similar genotype-dependent responses to phytogenic supplementation have previously been reported in rabbits (Abd El-Aziz *et al*., 2022).

Modulation of cecal microbial populations appears to be a key mechanism underlying the beneficial effects of OEO on nutrient digestibility, intestinal health, and physiological status (Ahmed *et al*., 2024). The marked reduction in *Clostridium perfringens* demonstrates the selective antimicrobial activity of oregano phenolics, particularly carvacrol, which disrupts bacterial membrane integrity and impairs bacterial survival (Li *et al*., 2021; Visan & Negut, 2024). Consequently, reduced *C. perfringens* colonization likely mitigated toxin-mediated epithelial damage and intestinal inflammation, thereby preserving barrier integrity, improving nutrient absorption, and reducing the metabolic cost of immune activation (Feng *et al*., 2021; van der Sluis *et al*., 2024). This interpretation is further supported by the progressive reduction in WC, indicating improved intestinal barrier function. Concurrently, the increased *Lactobacillus* population at moderate OEO supplementation suggests the establishment of a more favorable cecal microbial ecosystem (Yang *et al*., 2025). Beneficial bacteria promote short-chain fatty acid production, which supports mucosal integrity and intestinal homeostasis (Li *et al*., 2021). Likewise, the lower cecal pH observed in supplemented rabbits is consistent with enhanced microbial fermentation and suppression of opportunistic pathogens (Cui *et al*., 2024). Collectively, these microbial changes likely enhanced hindgut fermentation and fiber degradation, thereby improving nutrient digestibility and productive performance.

Interestingly, *Lactobacillus* abundance was highest at 0.0125% OEO but declined at 0.0250%, suggesting a concentration-dependent microbial response to oregano phenolic compounds. The present pattern suggests that moderate OEO supplementation may differentially affect bacterial populations, whereas higher concentrations may also suppress beneficial commensal populations (Yang *et al*., 2025). Despite the lower *Lactobacillus* count at 0.0250% OEO, growth performance continued to improve, suggesting that the observed productive responses were not necessarily dependent on changes in the abundance of a single bacterial genus and may have been influenced by broader effects of OEO on intestinal function and nutrient utilization. These findings are consistent with Ruan *et al*. (2021), who reported increased *Lactobacillus* abundance and reduced pathogenic bacteria following OEO supplementation, and with Kirkpinar *et al.* (2011), who observed decreased *Clostridium* counts in oregano oil-supplemented animals. Nevertheless, the decline in *Lactobacillus* abundance at the higher OEO inclusion level highlights the importance of considering potential effects on beneficial commensal bacteria when evaluating higher OEO concentrations.

The improvements in serum biochemical and immunological parameters further support the modulation of the gut–metabolic–immune axis by OEO supplementation (Visan & Negut, 2024). Enhanced CP digestibility likely increased amino acid availability for hepatic protein synthesis, thereby contributing to the higher serum total protein and albumin concentrations observed in the supplemented groups (Ahmed *et al*., 2024). Because albumin synthesis depends on adequate amino acid supply and hepatic metabolic activity, the increased albumin concentration also reflects improved liver synthetic function and metabolic efficiency (Farag *et al*., 2023). Likewise, the increases in globulin, IgG, and IgM indicate enhanced humoral immunity, which may be attributed to reduced pathogenic bacteria and improved intestinal integrity following OEO supplementation (Feng *et al*., 2021; Li *et al*., 2021). Under these conditions, the lower inflammatory burden likely reduced the metabolic demand associated with immune activation, allowing greater nutrient allocation toward immunoglobulin synthesis (Hassan *et al*., 2023). In addition, the antioxidant properties of thymol and carvacrol protect immune cells from oxidative damage, thereby maintaining lymphocyte function and supporting antibody production (Beghelli *et al*., 2016). These mechanisms are supported by previous findings showing that OEO supplementation reduced oxidative stress and inflammatory biomarkers and improved the physiological status of rabbits (Hassan *et al*., 2023; Sayedahmed *et al*., 2026). The greater increase in IgM concentration observed in Alexandria-line rabbits, particularly at 0.0250% OEO, together with similar findings reported in rabbits supplemented with OEO or probiotics, indicates that the immunological response to phytogenic supplementation may vary among rabbit genotypes (Abdelsalam *et al*., 2025; Sayedahmed *et al*., 2026).

The improvement in serum lipid profile further confirms the integrated metabolic effects of OEO supplementation. The reductions in total lipids, cholesterol, triglycerides, and LDL, together with increased HDL concentrations, indicate that oregano phytochemicals modulated lipid metabolism by inhibiting hepatic lipogenesis, reducing intestinal cholesterol absorption, and protecting against lipid peroxidation (Abdel-Wareth *et al*., 2022; Sayedahmed *et al*., 2026). Enhanced hindgut fermentation may also have contributed to these responses, as propionate produced by microbial fermentation of fiber can inhibit hepatic HMG-CoA reductase activity, thereby suppressing cholesterol synthesis (Gidenne, 2015). Additionally, carvacrol and thymol may reduce micellar cholesterol solubility and enhance bile acid excretion, collectively decreasing circulating lipid concentrations (Omer *et al*., 2013; Abd El-Aziz *et al*., 2024). Similar hypolipidemic effects have been reported in rabbits receiving oregano-derived phytogenics and other essential oil formulations (Areda *et al*., 2026).

CONCLUSION

OEO supplementation improved growth performance, nutrient digestibility, cecal microbial balance, metabolic profile, and humoral immunity in growing rabbits. These responses were associated with reduced pathogenic bacterial load, enhanced digestive efficiency, and improved intestinal physiological status.

The magnitude of these effects varied among rabbit lines, with the V-line showing greater digestive and fermentative responsiveness, whereas the Alexandria line exhibited stronger immunological resilience. Collectively, the present findings demonstrate that OEO can serve as an effective nutritional strategy to support intestinal health, metabolic efficiency, and productive performance in rabbit production systems.

CONFLICTS OF INTEREST

The authors declare no conflicts of interest.

DECLARATION OF GENERATIVE AI AND AI-ASSISTED TECHNOLOGIES IN THE WRITING PROCESS

During the preparation of this manuscript, the authors used Google Gemini v2.5 and Grammarly v1.2 in some sentences to improve the readability and language quality. After using these tools, the authors carefully reviewed and edited the content as needed and take full responsibility for the publication.

REFERENCES

Abd El-Aziz, A., Elfadadny, A., Abo Ghanima, M., Cavallini, D., Fusaro, I., Giammarco, M., Buonaiuto, G., & El-Sabrout, K. (2024). Nutritional value of oregano-based products and its effect on rabbit performance and health. Animals, 14(20),3021. https://doi.org/10.3390/ani14203021

Abd El-Aziz, A. H., Abo Ghanima, M. M., Alsanie, W. F., Gaber, A., Alsenosy, A. E.-W., Easa, A. A., Moawed, S. A., Raza, S. H. A., Elfadadny, A., Yossef, H. A., Ghoneem, W. M., Shukry, M., Hendawy, A. O., & Mahrose, K. (2022). Fructooligosaccharide supplementation boosts growth performance, antioxidant status, and cecal microbiota differently in two rabbit breeds. Animals, 12(12), 1528. https://doi.org/10.3390/ani12121528

Abdel-Azeem, F., Hashem, N. A., Badawi, Y. K. E. H., & Farid, A. (2009). Comparative study between probiotic (Bioplus 2B) and antibiotic (Lincofeed) on the performance of growing rabbits. Egyptian Journal of Rabbit Science, 19(1), 7-22. https://research.asu.edu.eg/handle/123456789/230951

Abdel-Rahman, S., & Ashour, G. (2023). Current situation and challenges for lifting up rabbit production in Egypt into industrial level. Egyptian Journal of Rabbit Science, 33(2), 63–83. https://doi.org/10.21608/ejrs.2023.303655

Abdel-Wareth, A. A. A., Kehraus, S., & Südekum, K. H. (2022). Evaluation of oregano leaves and plant bioactive lipid compounds as feed additives for growing rabbits: Effects on performance, nutrient digestibility, serum metabolic profile and carcass traits. Animal Feed Science and Technology, 284,115208. https://doi.org/10.1016/j.anifeedsci.2022.115208

Abdelsalam, M., Fathi, M., El-Raffa, A., El-Latif, G. A., Abou-Emera, O., El-Fatah, M. A., & Rayan, G. (2025). Influence of probiotic supplementation and rabbit line on growth performance, carcass yield, blood biochemistry and immune response under hot weather. Animal Bioscience, 38(9), 2033–2042. https://doi.org/10.5713/ab.24.0904

Ahmed, F. A., El- Sayaad, G. A., Elsayed, T., EL Nagar, A., & Abdelhadi, S. H. (2024). Impacts of the dietary supplementation of oregano leaves extract combined with its bioactive lipid components on nutrients digestibility, growth and carcass traits in rabbits. Annals of Agricultural Science, Moshtohor, 62(3), 67–78. https://doi.org/10.21608/assjm.2024.312816.1310

AOAC. (2000). Official methods of analysis (17th ed.). Association of Official Analytical Chemists.

Areda, H. A., Hassan, M. A. E., Embaby, E. M., Megahed, A., Hamed, S. E., & El-Said, E. A. (2026). The protective influence of orange essential oil nano-emulsion on growth performance, antioxidant activity, immune status, inflammatory response, and hepato-renal function in heat-stressed growing rabbits. Tropical Animal Health and Production, 58(4), 1–15. https://doi.org/10.1007/s11250-026-05005-w

Ayoub, M., Ahmed, H., Noseir, M., Shaaban, S., & Abou Shehata, M. (2021). Potentiality of oregano essential oils as a growth modulator, immune enhancer and natural antioxidative in mite infested Newzealand white rabbits. Damanhour Journal of Veterinary Sciences, 7(1), 1–7. https://doi.org/10.21608/djvs.2021.80425.1040

Beghelli, D., Alunno, A., Cardinali, R., Bistoni, O., Caterbi, S., De Cosmo, A., & Gerli, R. (2016). Effects of oregano (*Origanum vulgare L.*) and rosemary (*Rosmarinus officinalis L.)* aqueous extracts on *in vitro* rabbit immune responses. MOJ Immunology, 4(4), 1-6. https://doi.org/10.15406/moji.2016.04.00136

Benlemlih, M., Barchan, A., Aarab, A., Bakkali, M., Arakrak, A., & Laglaoui, A. (2020). Effect of dietary dried fennel and oregano and thyme supplementation on zootechnical parameters of growing rabbit. World's Veterinary Journal, 10(3), 332–337. https://doi.org/10.36380/scil.2020.wvj43

Cervantes, H. M. (2015). Antibiotic-free poultry production: Is it sustainable? Journal of Applied Poultry Research, 24(1), 91–97. https://doi.org/10.3382/japr/pfv006

Cui, H., Zhang, C., Su, K., Fan, T., Chen, L., Yang, Z., Zhang, M., Li, J., Zhang, Y., & Liu, J. (2024). Oregano essential oil in livestock and veterinary medicine. Animals, 14(11), 1–13. https://doi.org/10.3390/ani14111532

de Blas, C., & Mateos, G. (2020). Feed formulation. In de Blas, C., Wiseman, J. (Eds.), Nutrition of the rabbit (3rd ed.). CABI. https://doi.org/10.1079/9781789241273.0000

de Blas, J. C., Chamorro, S., García-Alonso, J., García-Rebollar, P., García-Ruiz, A. I., Gómez-Conde, M. S., Menoyo, D., Nicodemus, N., Romero, C., & Carabaño, R. (2012). Nutritional digestive disturbances in weaner rabbits. Animal Feed Science and Technology, 173(1–2), 102–110. https://doi.org/10.1016/j.anifeedsci.2011.12.016

Ebeid, T. A., Saleh, A. A., El-Ratel, I. T., Alkhalaf, A. N., & Mousa, E. F. (2025). The beneficial effects of probiotics on rabbit's productivity and health - a review. Annals of Animal Science, 26(1), 189-199. https://doi.org/10.2478/aoas-2025-0043

El-Ashram, S., Aboelhadid, S. M., Abdel-Kafy, E.-S. M., Hashem, S. A., Mahrous, L. N., Farghly, E. M., & Kamel, A. A. (2020). Investigation of pre- and post-weaning mortalities in rabbits bred in Egypt, with reference to parasitic and bacterial causes. Animals, 10(3), 537. https://doi.org/10.3390/ani10030537

Farag, M. R., Gharib, H. S. A., El-Naggar, K., Hendam, B. M., Ahmad, E. A. M., Alagawany, M., & El-Ghazali, H. M. (2023). *Origanum majorana* essential oil ameliorated the behavioral, biochemical, physiological and performance perturbations induced by aflatoxin B1 in growing rabbits. Annals of Animal Science, 23(4), 1201–1210. https://doi.org/10.2478/aoas-2023-0035

Feng, J., Lu, M., Wang, J., Zhang, H., Qiu, K., Qi, G., & Wu, S. (2021). Dietary oregano essential oil supplementation improves intestinal functions and alters gut microbiota in late-phase laying hens. Journal of Animal Science and Biotechnology, 12(1), 1–15. https://doi.org/10.1186/s40104-021-00600-3

García, J., Carabaño, R., & de Blas, J. C. (1999). Effect of fiber source on cell wall digestibility and rate of passage in rabbits. Journal of Animal Science, 77(4), 898. https://doi.org/10.2527/1999.774898x

Ghazi, S., Amjadian, T., & Norouzi, S. (2015). Single and combined

effects of vitamin C and oregano essential oil in diet, on growth performance, and blood parameters of broiler chicks reared under heat stress condition. International Journal of Biometeorology, 59(8), 1019–1024. https://doi.org/10.1007/s00484-014-0915-4

Gidenne, T. (2015). Dietary fibres in the nutrition of the growing rabbit and recommendations to preserve digestive health: a review. Animal, 9(2), 227–242. https://doi.org/10.1017/S1751731114002729

Hassan, M. A., Abo-Elmaaty, A. M. A., Zaglool, A. W., Mohamed, S. A. M., Abou-Zeid, S. M., Farag, M. R., Alagawany, M., Di Cerbo, A., Azzam, M. M., Alhotan, R., & EL-Hady, E. (2023). *Origanum vulgare* essential oil modulates the AFB1-induced oxidative damages, nephropathy, and altered inflammatory responses in growing rabbits. Toxins, 15(1), 1–12. https://doi.org/10.3390/toxins15010069

He, J., Su, X., Guo, S., Shi, H., Guo, C., Li, J., Lv, J., Yu, M., & Huang, M. (2023). Effects of compound essential oil and oregano oil on production performance, immunity and antioxidant capacity of meat rabbits. Italian Journal of Animal Science, 22(1), 934–941. https://doi.org/10.1080/1828051X.2023.2252454

Hendawy, A. O., Amrabit, A. A., Shahba, H. A., Hassan, S. S., Hassan, M., & Habashy, W. S. (2025). Breed-specific responses to peppermint oil supplementation: effects on growth, antioxidant status, and carcass traits in growing rabbits. Advances in Animal and Veterinary Sciences, 13(11), 2424–2434. https://doi.org/10.17582/journal.aavs/2025/13.11.2424.2434

Hendawy, A. O., Khattab, M. S., Sugimura, S., & Sato, K. (2020). Effects of 5-aminolevulinic acid as a supplement on animal performance, iron status, and immune response in farm animals: a review. Animals, 10(8), 1352. https://doi.org/10.3390/ani10081352

Hustá, M., Ducatelle, R., Haesebrouck, F., Van Immerseel, F., & Goossens, E. (2020). A comparative study on the use of selective media for the enumeration of Clostridium perfringens in poultry faeces. Anaerobe, 63, 102205. https://doi.org/10.1016/j.anaerobe.2020.102205

Jinling, N., Yongxu, L., & Fuchang, L. (2022). The effects of combined supplementation of oregano oil and antimicrobial peptides in diet on growth performance and intestinal barrier in meat rabbit. Journal of Animal Nutrition, 34(1), 555–562. https://doi.org/10.3969/j.issn.1006-267x.2022.01.051

Kirkpinar, F., Ünlü, H. B., & Özdemir, G. (2011). Effects of oregano and garlic essential oils on performance, carcase, organ and blood characteristics and intestinal microflora of broilers. Livestock Science, 137(1–3), 219–225. https://doi.org/10.1016/j.livsci.2010.11.010

Li, C., Niu, J., Liu, Y., Li, F., & Liu, L. (2021). The effects of oregano essential oil on production performance and intestinal barrier function in growing Hyla rabbits. Italian Journal of Animal Science, 20(1), 2165–2173. https://doi.org/10.1080/1828051X.2021.2005471

Liang, Z. L., Chen, F., Park, S., Balasubramanian, B., & Liu, W. C. (2022). Impacts of heat stress on rabbit immune function, endocrine, blood biochemical changes, antioxidant capacity and production performance, and the potential mitigation strategies of nutritional intervention. Frontiers in Veterinary Science, 9, 906084. 1–15. https://doi.org/10.3389/fvets.2022.906084

Migdał, A., Kania-Gierdziewicz, J., Pałka, S., Kmiecik, M., & Migdał, Ł. (2025). Effect of early weaning on behaviour, blood immunological parameters and performance in rabbits. Medycyna Weterynaryjna, 81(6), 278–283. https://doi.org/10.21521/mw.7005

Movahedi, F., Nirmal, N., Wang, P., Jin, H., Grøndahl, L., & Li, L. (2024). Recent advances in essential oils and their nanoformulations for poultry feed. Journal of Animal Science and Biotechnology, 15(1), 1–18. https://doi.org/10.1186/s40104-024-01067-8

NRC. (1977). Nutrient requirements of rabbits: Second Revised Edition. National Research Council, Nutrient Requirements of Domestic Animals, Washington, DC. National Academies Press.

Omer, H. A. A., EL-Nomeary, Y. A. A., EL-Kady, R. I., Badr, A. M. M., Ali, F. A. F., Ahmed, S. M., ELAllawy, H. M. H., & Ibrahim, Sh. A. M. (2013). Improving the utilization of rabbit diets containing vegetable oil by using fennel (*Foeniculum vulgare*) and oregano (*Origanum vulgare L*) as feed additives. Life Science Journal, 10(1), 2625–2636.

Ruan, D., Fan, Q., Fouad, A. M., Sun, Y., Huang, S., Wu, A., Lin, C., Kuang, Z., Zhang, C., & Jiang, S. (2021). Effects of dietary oregano essential oil supplementation on growth performance, intestinal antioxidative capacity, immunity, and intestinal microbiota in yellow-feathered chickens. Journal of Animal Science, 99(2), 1-11. https://doi.org/10.1093/jas/skab033

Saleh, A. A., Moustafa, A. M., Ali, M. F., Soliman, M. M., Omar, S. M., & Shukry, M. (2025). Synergistic effect of dietary supplementation of oregano essential oil and curcumin on growth performance, digestibility, blood parameters and antioxidant status in growing rabbits. Egyptian Journal of Veterinary Sciences, 1(3), 1–10. https://doi.org/10.21608/ejvs.2025.415066.3054

Sayedahmed, M. M., Safwat, A. M., Madkour, M., & Seddik, H. E. A. M. (2026). An integrated in vitro antioxidant/in vivo metabolomics approach unravels the synergistic effects between oregano essential oil and vitamin C as a nutritional strategy for alleviating heat stress in rabbits. Journal of Animal Science, 104(3), 1–13. https://doi.org/10.1093/jas/skag083

Šufliarský, P., Volek, Z., Ebeid, T. A., & Zita, L. (2024). Dietary inclusion of white lupine seeds and their byproducts in rabbits can contribute to EU agricultural sustainability: a review. World Rabbit Science, 32(3), 145–160. https://doi.org/10.4995/wrs.2024.22017

Tarabees, R., Gafar, K. M., EL-Sayed, M. S., Shehata, A. A., & Ahmed, M. (2019). Effects of dietary supplementation of probiotic mix and prebiotic on growth performance, cecal microbiota composition, and protection against *Escherichia coli* O78 in broiler chickens. Probiotics and Antimicrobial Proteins, 11(3), 981–989. https://doi.org/10.1007/s12602-018-9459-y

van der Sluis, M., van Zeeland, Y. R. A., & de Greef, K. H. (2024). Digestive problems in rabbit production: moving in the wrong direction? Frontiers in Veterinary Science, 11, 1354651. https://doi.org/10.3389/fvets.2024.1354651

Van Soest, P. J., Robertson, J. B., & Lewis, B. A. (1991). Methods for dietary fiber, neutral detergent fiber, and nonstarch polysaccharides in relation to animal nutrition. Journal of Dairy Science, 74(10), 3583–3597. https://doi.org/10.3168/jds.S0022-0302(91)78551-2

Visan, A. I., & Negut, I. (2024). Coatings based on essential oils for combating antibiotic resistance. Antibiotics, 13(7), 625. https://doi.org/10.3390/antibiotics13070625

Wang, W., Luo, Y., Liu, B., Liang, D., Jiang, J., Luo, D., & Chen, J. (2018). Effect and mechanism of recombinant human granulocyte colony-stimulating factor on diarrhea caused by leukopenia in New Zealand rabbits. International Journal of Clinical and Experimental Medicine, 11(10), 10958–10965.

Yang, J., Li, C., Jiang, S., Fu, Y., Zhou, G., Gao, Y., Yang, W., & Li, Y. (2025). Dietary thymol–carvacrol cocrystal supplementation improves growth performance, antioxidant status, and intestinal health in broiler chickens. Antioxidants, 14(11), 1–23. https://doi.org/10.3390/antiox14111323

Zeng, Z., Zhang, S., Wang, H., & Piao, X. (2015). Essential oil and aromatic plants as feed additives in non-ruminant nutrition: a review. Journal of Animal Science and Biotechnology, 6(1), 7. https://doi.org/10.1186/s40104-015-0004-5