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Research Article

Breed-Specific Responses to Peppermint Oil Supplementation: Effects on Growth, Antioxidant Status, and Carcass Traits in Growing Rabbits

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Abstract | This study assessed the effects of peppermint oil (*Mentha piperita L.*) supplementation on growth performance, blood biochemical profile, gut microbial count, and carcass characteristics in V-line and Alexandria rabbits using a 3 × 2 factorial arrangements (three supplementation levels × two rabbit strains). Ninety-six rabbits (35 days old) were randomly allocated to six treatment combinations. The control group received no supplement, while the other two groups were orally administered peppermint oil at 0.25 or 0.5 ml/kg body weight (BW), twice weekly for five weeks. Peppermint oil supplementation significantly increased high-density lipoprotein (HDL), total antioxidant capacity (TAC), and superoxide dismutase (SOD) levels, while reducing cholesterol, low-density lipoprotein (LDL), and malondialdehyde (MDA). Compared with V-line rabbits, Alexandria rabbits significantly exhibited lower triglycerides, very low-density lipoprotein (VLDL), globulin, and MDA levels. Peppermint oil exerted a dose-dependent antimicrobial effect, reducing total bacterial count, *Escherichia coli*, and *Clostridium* spp., with greater reductions in Alexandria rabbits. Feed intake was significantly higher in rabbits receiving 0.25 ml/kg BW peppermint oil. A significant strain effect on live BW (V-line heavier). Supplementation significantly influenced internal organ development, increasing the weights of the pancreas, spleen, and intestine. Alexandria rabbits also had heavier pancreas, kidneys, abdominal fat, intestine, and cecum compared with V-line rabbits. Peppermint oil supplementation improved blood parameters, lipid profile, antioxidant status, and gut microbial balance in growing rabbits, with breed-specific responses. Alexandria rabbits responded best at 0.25 ml/kg BW, while V-line rabbits showed greater benefits at 0.5 ml/kg BW. These results suggest peppermint oil as a natural alternative to antibiotics for enhancing rabbit health and performance.

Keywords | Peppermint, Rabbit, Antioxidant activity, Growth performance, Gut health

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INTRODUCTION

Oxidative status of animals is a multifaceted physiological parameter which is influenced by diet, age, production levels, management practices, and environmental conditions (Bernabucci *et al*., 2002). Oxidative stress results when there is an imbalance between the generation of reactive oxygen species and the antioxidant defense mechanisms disrupt cellular integrity and physiological functions (Pizzino *et al*., 2017; Hendawy *et al*., 2020). Adverse effects of this phenomenon include reduced animal health, depressed performance, diminished production efficiency, and thus negative economic consequences on livestock systems (Lee *et al*., 2017; Hendawy *et al*., 2022). Therefore, it is important to identify the determinants of oxidative stress and to implement effective mitigation strategies in order to improve animal welfare and production outcomes.

In recent years, natural agents with strong antioxidant properties have gained much attention as alternatives to antibiotics in animal nutrition fields (Hendawy *et al*., 2019; Hassan *et al*., 2023a, b). Herbal plants and their extracts are one of the available sources of potential antioxidants. Herbs possess diverse bioactive properties that can beneficially influence contemporary animal nutrition (Jachimowicz *et al*., 2022). Among these, flavonoids and other plant-derived phenolic compounds function as potent free radical scavengers and inhibitors of lipid peroxidation (Formica and Regelson, 1995). Peppermint (*Mentha piperita* L.), a perennial aromatic medicinal herb belonging to the Lamiaceae family, contains approximately 1–3% essential oil. The primary constituents of this oil are menthol (37.69%) and menthone (21.79%) (Khodambashi *et al*., 2012).

Peppermint essential oil has shown antioxidant, antimicrobial, antiviral, anticarcinogenic, antiparasitic, anti-inflammatory, and immunomodulatory actions on animal physiology with particular positive effects on the digestive system (Attia *et al*., 2017; Hesabi Nameghi *et al*., 2019). Elspeiy *et al*. (2020) observed that rabbits receiving peppermint oil exhibited higher average daily gain (ADG) and improved feed conversion ratio (FCR) compared with those on a basal diet. Their findings also indicated that peppermint oil supplementation lowered serum levels of cholesterol, low-density lipoprotein (LDL), triglycerides, and malondialdehyde (MDA), while elevating high-density lipoprotein (HDL), total antioxidant capacity (TAC), and immunoglobulin G concentrations. Ciliberti *et al*. (2024) reported that peripheral blood mononuclear cells (PBMCs) treated with 10% peppermint oil and stimulated with concanavalin A and lipopolysaccharide exhibited higher proliferation rates than the positive control (PBMCs stimulated with concanavalin A alone). Additionally, peppermint oil treatment increased the concentration of anti-inflammatory cytokines and reduced pro-inflammatory cytokine levels compared with the positive control. Based on these findings, the present study was conducted to evaluate the impact of peppermint oil supplementation on antioxidant status, growth performance, and carcass characteristics in two strains of growing rabbits.

MATERIALS AND METHODS

The experiment was conducted at El-Bostan Poultry Farm, Faculty of Agriculture, Damanhour University. All experimental procedures were performed in accordance with the protocols approved by the Institutional Animal Care and Use Committee of Damanhour University (Approval No. DUFA-2024-10). Peppermint oil (*Mentha piperita*) was purchased from a local commercial supplier in Egypt. The oil was food-grade, with no additives, and was used as received for preparing the experimental doses.

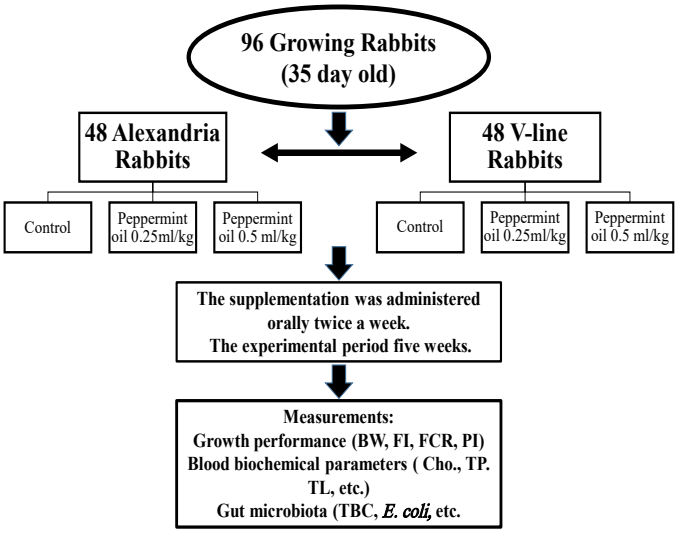

Figure 1: Flowchart of the experimental design.

Animals and experimental design

As shown in Figure 1, a total of 96 growing rabbits (48 Alexandria and 48 V-line) were included in the study. At 35 days of age, their average initial body weights were 1112 g and 1023 ± 29.77 g, respectively. Galvanized wire cages with a single tier (55 × 45 × 35 cm) were used for housing the experimental rabbits within a naturally ventilated building. Each breed was divided into three groups: a control group receiving a placebo (2 ml of water), a second group receiving 0.25 ml peppermint oil/kg of BW, and a third group receiving 0.5 ml peppermint oil/kg of BW. Each group was composed of 16 rabbits per breed divided into four replicates (4 rabbits per cage). The supplementation was administered orally twice a week (Sunday and Wednesday). The experimental period extended for five weeks. Ventilation and temperature were

naturally regulated. The temperature, relative humidity, and photoperiod varied from 23 to 27˚C, 60 to 65%, and 14 hours, respectively, during the experimental period.

All rabbits across the experimental treatments were provided with identical standard diets formulated according to NRC (NRC, 1977). The diets were provided in pelleted form and offered ad libitum, and fresh drinking water was also available ad libitum throughout the trial. The feed composition and proximate chemical analysis were consistent with those reported in the previous study by Shahba and Mansour (2022). The commercial pelleted diet had 2464 kcal/kg of metabolizable energy, 17.24% crude protein, 13.46% crude fiber, and 2.80% fat.

Feed intake and body weight

Feed intake was recorded per cage weekly throughout the research period. Rabbits were weighed individually at the beginning of the experiment as an initial weight and weekly. Initial and final weight of individual rabbits were used to determine ADG as follows: ADG = (Final weight – Initial weight)/Experimental period (d). The FCR was calculated as: FCR = average dry matter (DM) intake per rabbit/ADG. Performance index = (final live BW (kg)/ feed conversion ratio) x 100.

Blood biochemical constituents

Six rabbits were chosen at random from each treatment at the conclusion of the trial, and blood samples were taken through the inner ear vein in sterile tubes devoid of anticoagulants. Samples were stored in an ice tank right away. Serum was separated from blood samples by centrifuging them for 15 minutes at 4000 rpm. The serum was then kept at -20˚C until analysis. As directed by the manufacturer, assay kits were used to measure the levels of total cholesterol, LDL, HDL, VLDL, triglycerides, total protein, albumin, TAC, superoxide dismutase (SOD), and MDA in serum (Biodiagnostic Company, Dokki, Giza, Egypt). The variation between total protein and albumin was used to determine the serum globulin level (g/dl).

Carcass characteristics

To assess carcass characteristics, including the weights of the carcass, head, full and empty stomachs, abdominal fat, intestine, cecum, pancreas, liver, kidney, heart, spleen, and lung, six rabbits were chosen at random from each treatment at the conclusion of the experiment. The weight of the liver, kidney, lungs, spleen, heart, pancreas, and abdominal fat was reported as a percentage of live body weight.

Gut microbial count

The total cecal bacterial count was determined following the method described by the American Public Health Association (Richardson and Association, 1985) Specific bacterial groups, including *Clostridium* spp., *Escherichia coli,* and *Proteus* spp., were identified according to the procedures outlined by Mackie and McCartney (1953). The colony-forming unit (CFU) technique was employed, with incubation conducted at 30 ˚C for 2 to 7 days.

Statistical analysis

The following formula was used to statistically analyze the data using the General Linear Model (GLM) process of the SAS Institute's statistical analysis system (SAS, 2002) employing factorial analysis of variance: Y_{ijk} is equal to $\mu + B_i + T_j + E_{ijk}$. Where Y_{ijk} is the statistical measure's observation, μ is the overall mean, B_i is the breed effect, T_j is the treatment effect, and E_{ijk} is the experimental random error. Arcsine transformation was used to standardize the data distribution prior to analysis. Duncan (1955) was used to determine the least square mean (LSM) + standard errors and test for significance at ($p \leq 0.05$).

RESULTS

Body weight and body weight gain

Peppermint oil supplementation did not significantly affect BW from week 5 to week 10 or overall BWG (Table 1). However, during weeks 8–9 and 9–10, the 0.25 m/kg group exhibited higher BWG compared with both the control and 0.5 ml/kg groups. Breed differences were also observed: Alexandria rabbits had higher BW at weeks 5, 6, 8, and 9, and greater BWG between weeks 9–10, whereas V-line rabbits gained more during weeks 6–7. A tendency for treatment × strain interaction was detected for BWG during weeks 9–10.

Feed intake, feed conversion ratio, and performance index

Peppermint oil significantly influenced FI at weeks 6, 9, and 10, as well as total FI (Table 2). The 0.25 ml/kg group consumed more feed at week 9 and overall compared with the control and 0.5 ml/kg groups, while the 0.5 ml/kg group had the lowest intake at week 10. The FCR was affected at week 10, where the control group showed the highest value and the 0.5 ml/kg group the lowest. Strain effect was significant for FCR at week 8 and week 10, with V-line rabbits showing higher FCR at week 8 and lower FCR at week 10. The PI and live body weight were not significantly affected by peppermint oil or strain. A significant treatment × strain interaction was observed for total FI and FCR at week 10.

Blood parameters

Peppermint oil supplementation significantly reduced cholesterol, LDL, and MDA concentrations (Table 3), with the 0.25 and 0.5 mL/kg groups showing lower values

Table 1: Effect of peppermint oil (PO) level on body weight and body weight gain in Alexandria and V-line rabbits during the growing period.

		BW5	BW6	BW7	BW8	BW9	BW10	BWG 5-6	BWG 6-7	BWG 7-8	BWG 8-9	BWG 9-10	Overall BWG
PO level													
0		1071	1279	1411	1697	1835	1943	208	133	309	138^{b}	108^{b}	893
0.25		1071	1259	1396	1665	1860	1994	188	137	269	196^{a}	134^{a}	923
0.5		1060	1268	1417	1704	1857	1955	208	149	287	153^{b}	98^{b}	895
SEM		36.47	33.30	39.42	31.78	29.85	31.06	9.51	20.41	24.22	12.18	7.57	20.40
Breed													
Alx		1112^{a}	1309^{a}	1428	1729^{a}	1894^{a}	1992	197	119	315	165	99^{b}	892
V-line		1023^{b}	1228^{b}	1388	1650^{b}	1811^{b}	1938	205	160	262	161	128^{a}	915
SEM		29.77	27.18	32.18	25.94	24.37	25.36	7.77	16.66	19.77	9.94	6.18	16.66
PO level and Breed Interaction													
0	Alx	1125	1333	1414	1743	1892	2001	208	81	377	150	108	913
	V-line	1016	1224	1409	1658	1785	1892	208	185	250	127	107	876
0.25	Alx	1111	1288	1412	1713	1910	2018	177	124	302	196	108	907
	V-line	1032	1230	1380	1616	1811	1971	198	150	237	195	160	940
0.5	Alx	1100	1307	1457	1732	1879	1958	207	151	275	146	80	859
	V-line	1021	1230	1377	1677	1836	1951	209	147	300	160	115	931
SEM		51.55	47.07	55.72	44.92	42.19	43.90	13.45	28.85	34.23	17.21	10.70	28.85
p- value													
Treatment		0.9731	0.9154	0.9226	0.6280	0.8650	0.5223	0.2287	0.8458	0.4545	0.0060	0.0046	0.5264
Strain		0.0403	0.0403	0.3925	0.0389	0.0222	0.1416	0.4798	0.0823	0.0542	0.7957	0.0025	0.3486
Interaction		0.9466	0.8571	0.7958	0.8952	0.7139	0.5227	0.6775	0.1722	0.1028	0.5889	0.0511	0.1855
Linear		0.9933	0.6761	0.7772	0.4401	0.6201	0.2926	0.1398	0.8922	0.2135	0.0022	0.0208	0.3287
Quadratic		0.8105	0.9921	0.7782	0.5776	0.8319	0.6867	0.3784	0.5759	0.8983	0.3519	0.0162	0.5843

Different superscripts in each column indicate significant differences ($p < 0.05$). 0: 0 ml peppermint oil /kg of body weight; 0.25: 0.25 ml peppermint oil /kg of body weight; 0.5: 0.5 ml peppermint oil /kg of body weight; SEM: standard error of the mean; Alx: Alexandria rabbits; V-line: V-line rabbits. BW: body weight (measured in g); BWG: body weight gain (measured in g).

Table 2: Effect of peppermint oil (PO) level on feed intake, feed conversion ratio and the production index in Alexandria and V-line rabbits during the growing period.

Item		FI6	FI7	FI8	FI9	FI10	Total FI	FCR6	FCR7	FCR8	FCR9	FCR 10	Total FCR	LBW	PI
PO level															
0		354.0^{a}	310.2	718.7	453.2^{b}	404.7^{b}	2241.0^{b}	1.72	2.61	2.51	3.27	3.76^{a}	2.51	1.95	77.80
0.25		307.0^{b}	328.8	713.0	593.7^{a}	466.5^{a}	2409.0^{a}	1.64	2.39	2.72	3.08	3.56^{b}	2.61	1.99	76.42
0.5		343.5^{ab}	354.5	750.7	478.0^{b}	333.0^{c}	2259.7^{b}	1.65	2.38	2.67	3.15	3.40^{c}	2.53	1.95	77.65
SEM		15.50	59.37	54.10	22.78	25.10	40.66	0.048	0.111	0.194	0.099	0.061	0.046	0.018	1.90
Breed															
Alx		328.83	291.0	758.3	523.8	378.8	2280.8	1.67	2.50	2.41^{b}	3.23	3.79^{a}	2.55	1.99^{a}	78.08
V-line		340.83	371.3	696.7	492.8	424.0	2325.7	1.66	2.42	2.85^{a}	3.09	3.36^{b}	2.54	1.94^{b}	76.50
SEM		12.66	48.47	44.18	18.60	20.49	33.19	0.039	0.090	0.158	0.080	0.050	0.037	0.015	1.55
PO level and breed interaction															
0	Alx	350.0	225.0	824.5	492.0	422.5	2314.0^{ab}	1.71	2.77	2.16	3.28	3.90^{a}	2.54	2.00	79.10
	V-line	358.0	395.5	613.0	414.5	387.0	2168.0^{b}	1.73	2.45	2.86	3.25	3.61^{b}	2.47	1.89	76.50
0.25	Alx	294.5	297.5	722.5	606.0	433.0	2353.5^{a}	1.66	2.38	2.40	3.14	4.01^{a}	2.59	2.02	77.75
	V-line	319.5	360.0	703.5	581.5	500.0	2464.5^{a}	1.62	2.39	3.03	3.01	3.11^{d}	2.63	1.97	75.10

Table continues on next page.....

Item		FI6	FI7	FI8	FI9	FI10	Total FI	FCR6	FCR7	FCR8	FCR9	FCR 10	Total FCR	LBW	PI
0.5	Alx	342.0	350.5	728.0	473.5	281.0	2175.0^{b}	1.66	2.34	2.67	3.28	3.46^{bc}	2.54	1.96	77.40
	V-line	345.0	358.5	773.5	482.5	385.0	2344.5^{a}	1.65	2.42	2.68	3.02	3.34^{cd}	2.52	1.95	77.90
SEM		21.92	83.96	76.51	32.22	35.50	57.50	0.068	0.156	0.274	0.139	0.086	0.065	0.026	2.69
p- value															
Treatment		0.0420	0.8124	0.8110	<.0001	0.0009	0.0039	0.3139	0.1472	0.6245	0.2650	0.0004	0.1340	0.0801	0.7905
Strain		0.4223	0.1684	0.2423	0.1661	0.0724	0.2574	0.8412	0.4853	0.0261	0.1467	<.0001	0.7289	0.0036	0.3867
Interaction		0.8144	0.4967	0.1300	0.2783	0.0692	0.0080	0.8616	0.2725	0.2616	0.6305	<.0001	0.6722	0.0577	0.7157
Linear		0.0171	0.7903	0.9277	<.0001	0.0472	0.0021	0.1561	0.0909	0.3654	0.1109	0.0122	0.0594	0.0392	0.5372
Quadratic		0.4126	0.5628	0.5273	0.0612	0.0007	0.1259	0.6022	0.3129	0.7504	0.8240	0.0006	0.5083	0.3603	0.7797

Different superscripts in each column indicate significant differences (p <0.05). 0: 0 ml peppermint oil /kg of body weight; 0.25: 0.25 ml peppermint oil /kg of body weight; 0.5: 0.5 ml peppermint oil /kg of body weight; SEM: standard error of the mean; Alx: Alexandria rabbits; V-line: V-line rabbits. FI: feed intake (measured in g); FCR: feed conversion ratio; LBW: live body weight; PI: performance index.

Table 3: Effect of peppermint oil (PO) level on blood parameter in Alexandria and V-line rabbits during the growing period.

Item		Cholesterol, mg/dl	Triglyceride, mg/dl	HDL, mg/dl	LDL, mg/dl	VLDL, mg/dl	Total protein, g/dl	Albumin, g/dl	Globulin, g/dl	TAC, mg/dl	SOD, g/dl	MDA, g/dl
PO level												
0		154.9^{a}	118.9	37.50^{b}	93.68^{a}	23.77	4.638^{b}	1.870^{b}	2.768^{b}	128.60^{b}	1.21^{b}	1.53^{a}
0.25		142.6^{b}	114.0	40.197^{ab}	79.63^{b}	22.80	5.420^{a}	2.298^{a}	3.122^{b}	148.42^{a}	1.69^{a}	1.32^{b}
0.5		143.8^{b}	114.2	43.43^{a}	77.55^{b}	22.83	5.672^{a}	2.015^{b}	3.657^{a}	148.86^{a}	1.84^{a}	1.32^{b}
SEM		1.88	1.77	1.25	1.19	0.355	0.163	0.064	0.127	3.81	0.086	0.0511
Breed												
Alx		147.4	113.5^{b}	41.25	83.50	22.71^{b}	5.10	2.11	2.99^{b}	142.7	1.62	1.45^{a}
V-line		146.8	117.8^{a}	39.49	83.74	23.56^{a}	5.39	2.01	3.38^{a}	141.2	1.53	1.33^{b}
SEM		1.53	1.45	1.02	0.974	0.290	0.133	0.052	0.104	3.11	0.070	0.042
PO level and breed interaction												
0	Alx	156.3^{a}	116.0	39.43	93.62^{a}	23.20	4.703	2.067^{bc}	2.637	129.1	1.471^{c}	1.662^{a}
	V-line	153.6^{a}	121.7	35.57	93.73^{a}	24.34	4.573	1.673^{d}	2.90	128.1	0.945^{d}	1.397^{bc}
0.25	Alx	132.0^{b}	111.3	40.76	68.96^{c}	22.26	5.233	2.197^{ab}	3.037	151.6	1.527^{bc}	1.439^{b}
	V-line	153.3^{a}	116.7	39.61	90.30^{ab}	23.35	5.607	2.400^{a}	3.207	145.2	1.844^{a}	1.207^{d}
0.5	Alx	154.1^{a}	113.3	43.57	87.91^{b}	22.66	5.353	2.063^{bc}	3.290	147.3	1.863^{a}	1.253^{cd}
	V-line	133.5^{b}	115.0	43.29	67.19^{c}	23.01	5.990	1.967^{c}	4.023	150.4	1.814^{ab}	1.385^{bc}
SEM		2.66	2.52	1.78	1.69	0.504	0.232	0.091	0.181	5.40	0.122	0.072
p- value												
Treatment		<.0001	0.1068	0.0085	<.0001	0.1087	0.0003	0.0002	0.0001	0.0007	<.0001	0.0090
Strain		0.7639	0.0458	0.2333	0.8610	0.0458	0.1307	0.2067	0.0129	0.7487	0.3936	0.0483
Interaction		<.0001	0.6777	0.5785	<.0001	0.6808	0.2577	0.0098	0.2609	0.6840	0.0063	0.0175
Linear		<.0001	0.0625	0.1400	<.0001	0.0638	0.0020	<.0001	0.0591	0.0009	0.0005	0.0078
Quadratic		0.0385	0.3066	0.0056	<.0001	0.3073	0.0032	0.3854	<.0001	0.0340	0.0008	0.0974

Different superscripts in each column indicate significant differences (p <0.05). 0: 0 ml peppermint oil /kg of body weight; 0.25: 0.25 ml peppermint oil /kg of body weight; 0.5: 0.5 ml peppermint oil /kg of body weight; SEM: standard error of the mean; Alx: Alexandria rabbits; V-line: V-line rabbits. HDL: high density lipoprotein; LDL: low density lipoprotein; VLDL: very low-density lipoprotein; TAC: total antioxidant capacity; SOD: superoxide dismutase; MDA: malondialdehyde.

than the control. Meanwhile, the peppermint oil groups had higher levels of HDL, total protein, albumin, globulin, TAC, and SOD compared to the control group. No effect was observed on triglycerides or VLDL. Strain differences were noted for triglycerides, VLDL, globulin, and MDA. Several significant treatments × strain interactions were found, particularly for cholesterol, LDL, albumin, SOD, and MDA.

Cecal microbial populations

Peppermint oil supplementation significantly decreased total bacterial count, *Escherichia coli,* and *Clostridium* spp. (Table 4). Strain effect was also significant; the V-line rabbits had higher microbial counts than Alexandria rabbits in all microbial groups. A significant treatment × strain interaction was detected for *Clostridium* spp.

Table 4: Effect of peppermint oil (PO) level on the cecum microbial populations (log^{10}cfu/g) in Alexandria and V-line rabbits during the growing period.

Item		Total bacteria count	*Escherichia coli*	*Clostridium*
PO level				
0		12.90[a]	11.08[a]	3.12[a]
0.25		11.60[b]	8.84[b]	2.30[b]
0.5		9.05[c]	7.29[c]	2.03[b]
SEM		0.277	0.300	0.0933
Breed				
Alx		7.88[b]	5.87[b]	0.884[b]
V-line		14.48[a]	12.28[a]	4.085[a]
SEM		0.226	0.245	0.076
PO level and breed interaction				
0	Alx	9.49	8.09	0.888[d]
	V-line	16.31	14.07	5.355[a]
0.25	Alx	8.39	5.25	0.923[d]
	V-line	14.81	12.44	3.678[b]
0.5	Alx	5.78	4.27	0.841[d]
	V-line	12.32	10.32	3.22[c]
SEM		0.393	0.426	0.132
p- value				
Treatment		<.0001	<.0001	<.0001
Strain		<.0001	<.0001	<.0001
Interaction		0.8760	0.2874	<.0001
Linear		0.0024	<.0001	<.0001
Quadratic		<.0001	<.0001	<.0001

Different superscripts in each column indicate significant differences ($p < 0.05$). 0: 0 ml peppermint oil /kg of body weight; 0.25: 0.25 ml peppermint oil /kg of body weight; 0.5: 0.5 ml peppermint oil /kg of body weight; SEM: standard error of the mean; Alx: Alexandria rabbits; V-line: V-line rabbits.

Carcass traits

Peppermint oil significantly affected head weight, full and empty stomach weights, and pancreas weight, with the 0.25 ml/kg group showing higher head weight, while the 0.5 ml/kg group had greater full and empty stomach weights and lower pancreas weight (Table 5). Strain effect was significant for live body weight, with V-line rabbits being heavier than Alexandria rabbits. Significant treatment × strain interactions were observed for full and empty stomachs, pancreas, spleen, abdominal fat, intestine, and cecum.

Carcass percentage traits

Peppermint oil significantly affected full stomach percentage, pancreas percentage, spleen percentage, and abdominal fat percentage (Table 6). Strain effect was significant for head, pancreas, kidney, abdominal fat, intestine, and cecum percentages, with Alexandria rabbits generally showing higher relative weights. Significant treatment × strain interactions were detected for carcass, full stomach, pancreas, liver, spleen, kidney, abdominal fat, intestine, and cecum percentages.

DISCUSSION

In the present investigation, we examined the peppermint oil supplementation impact on rabbits' health and performance, studying its potential as a natural feed additive to improve feed efficiency, mitigate oxidative stress, and support the overall health and welfare of livestock. By examining several parameters, we aimed to clarify the mechanisms by which peppermint oil can contribute to the development of more sustainable and health-conscious animal husbandry practices. The findings of this study showed a complex interaction of how peppermint oil supplementation interacts with breed differences to influence several characteristics of rabbit physiology and performance. The enhancements in lipid profile, antioxidant status, and bacterial counts with peppermint oil supplementation suggest a multifaceted beneficial effect on rabbit health and metabolism. These effects likely contribute to the observed improvements in feed efficiency and body weight gain in late stages of growth.

With respect to growth performance, peppermint oil supplementation did not significantly affect overall BW, BWG, or FCR. However, rabbits receiving 0.25 ml/kg BW peppermint oil showed higher total FI compared with the other groups, and BWG was significantly increased during weeks 8–9 and 9–10. This pattern may reflect a biphasic (hormetic) response of peppermint oil, where a lower dose (0.25 ml/kg BW) stimulates appetite and digestive efficiency, while a higher dose (0.5 ml/kg BW) exerts stronger antimicrobial and antioxidant effects that might slightly influence palatability or fiber fermentation. The FCR at week 10 was improved in rabbits supplemented with 0.5 ml/kg BW compared with the control group. In accordance, Zeweil *et al.* (2017) reported that peppermint essential oil supplementation (400 mg of peppermint essential oil/ kg diet) to V-line rabbits had no significant effect on final BW, ADG, and FCR. In contrast, Elspeiy *et al.* (2020) showed that weaned male California rabbits

Table 5: Effect of peppermint oil (PO) level on carcass traits in Alexandria and V-line rabbits during the growing period.

Item		Live BW	After cavity	Head	Full stomach	Empty stomach	Pancreas	Lung	Liver	Heart	Spleen	Kidney	Abdominal fat	Intestine	Cecum
PO level															
0		1915.7	1030.8	110.5^{b}	78.63^{ab}	19.96^{b}	3.885^{a}	10.01	50.76	7.15	0.912^{b}	11.06	11.08	96.55^{b}	97.20
0.25		1953.0	1060.7	117.3^{a}	72.55^{b}	20.70^{ab}	3.418^{ab}	9.945	47.05	6.62	0.890^{b}	10.72	10.15	99.28^{ab}	103.7
0.5		1977.0	1095.0	111.8^{b}	83.04^{a}	22.02^{a}	2.980^{b}	10.15	47.67	6.61	1.122^{a}	10.95	8.410	101.54^{a}	97.97
SEM		47.17	43.37	1.74	2.81	0.503	0.172	0.491	1.75	0.431	0.056	0.318	0.792	1.33	3.73
Breed															
Alx		1888.9^{b}	1012.9	114.5	75.72	20.89	3.952^{a}	9.757	46.60	7.021	6.568	10.89	10.61	99.16	103.5
V-line		2008.2^{a}	1111.4	111.8	80.42	20.89	2.903^{b}	10.32	50.40	6.568	0.971	10.93	9.15	99.09	95.82
SEM		38.49	35.40	1.42	2.29	0.410	0.140	0.401	1.43	0.351	0.046	0.259	0.646	1.09	3.04
PO level and breed interaction															
0	Alx	1908.0	1023.3	113.8	71.38^{bc}	20.75^{ab}	4.627^{a}	10.27	45.86	7.823	0.797^{c}	10.97	12.45^{a}	99.29^{b}	100.8^{ab}
	V-line	1923.3	1038.3	107.2	85.88^{a}	19.16^{b}	3.143^{b}	9.760	55.67	6.493	1.027^{b}	11.13	9.710^{bcd}	93.82^{cd}	93.57^{bc}
0.25	Alx	1913.3	1050.0	118.4	69.80^{c}	19.50^{b}	4.243^{a}	8.847	47.27	6.773	0.833^{c}	10.64	12.32^{ab}	90.62^{d}	99.60^{ab}
	V-line	1992.7	1071.3	116.2	75.31^{bc}	21.90^{a}	2.593^{b}	11.04	46.84	6.460	0.947^{bc}	10.82	7.990^{cd}	107.9^{a}	107.9^{a}
0.5	Alx	1845.3	965.3	111.4	86.00^{a}	22.43^{a}	2.987^{b}	10.16	46.66	6.467	1.303^{a}	11.05	7.060^{d}	107.6^{a}	109.98^{a}
	V-line	2108.7	1224.7	112.2	80.08^{ab}	21.61^{a}	2.973^{b}	10.15	48.69	6.75	0.940^{bc}	10.86	9.760^{abc}	95.52^{bc}	85.97^{c}
SEM		66.62	61.26	2.46	3.97	0.710	0.242	0.694	2.48	0.608	0.079	0.449	1.12	1.88	5.26
p- value															
Treatment		0.6542	0.5830	0.0236	0.0425	0.0221	0.0033	0.9549	0.2909	0.5910	0.0107	0.7540	0.0688	0.0425	0.4063
Strain		0.0362	0.0581	0.1928	0.1573	0.9954	<.0001	0.3298	0.0700	0.3693	0.9180	0.9116	0.1219	0.9629	0.0853
Interaction		0.1721	0.0922	0.3254	0.0496	0.0201	0.0035	0.1330	0.1153	0.4186	0.0017	0.9045	0.0099	<.0001	0.0165
Linear		0.5794	0.6299	0.0102	0.1362	0.3042	0.0640	0.9220	0.1446	0.3810	0.7848	0.4646	0.4140	0.1589	0.2222
Quadratic		0.4654	0.3607	0.3269	0.0384	0.0099	0.0033	0.7756	0.5695	0.6006	0.0029	0.8854	0.0303	0.0339	0.5866

Different superscripts in each column indicate significant differences ($p < 0.05$). 0: 0 ml peppermint oil /kg of body weight; 0.25: 0.25 ml peppermint oil /kg of body weight; 0.5: 0.5 ml peppermint oil /kg of body weight; SEM: standard error of the mean; Alx: Alexandria rabbits; V-line: V-line rabbits.

Table 6: Effect of peppermint oil (PO) level on carcass percentage (%) traits in Alexandria and V-line rabbits during the growing period.

Item		Carcass	Head	Full stomach	Empty stomach	Pancreas	Lung	Liver	Heart	Spleen	Kidney	Abdominal fat	Intestine	Cecum
PO level														
0		63.75	5.773	4.097^{ab}	1.043	0.2017^{a}	0.523	2.643	0.375	0.047^{b}	0.578	0.577^{a}	5.04	5.06
0.25		64.08	6.012	3.732^{b}	1.063	0.1767^{b}	0.507	2.413	0.342	0.045^{b}	0.547	0.528^{ab}	5.10	5.33
0.5		64.34	5.720	4.248^{a}	1.137	0.1500^{c}	0.522	2.440	0.343	0.060^{a}	0.560	0.420^{b}	5.23	5.07
SEM		0.760	0.091	0.141	0.039	0.007	0.025	0.088	0.026	0.003	0.011	0.039	0.121	0.204
Breed														
Alx		63.59	6.076^{a}	4.013	1.112	0.2089^{a}	0.518	2.47	0.374	0.052	0.577^{a}	0.557^{a}	5.28^{a}	5.49^{a}
V-line		64.53	5.594^{b}	4.038	1.050	0.1433^{b}	0.517	2.52	0.332	0.049	0.547^{b}	0.460^{b}	4.97^{b}	4.82^{b}
SEM		0.620	0.075	0.115	0.031	0.006	0.020	0.072	0.021	0.0026	0.009	0.032	0.098	0.166
PO level and breed interaction														
0	Alx	63.61^{b}	5.977	3.73^{b}	1.09	0.243^{a}	0.537	2.40^{b}	0.413	0.040^{c}	0.577^{ab}	0.650^{a}	5.21^{bc}	5.27^{ab}
	V-line	63.90^{ab}	5.570	4.46^{a}	0.997	0.160^{b}	0.510	2.88^{a}	0.337	0.053^{b}	0.580^{ab}	0.503^{b}	4.87^{cd}	4.86^{bc}
0.25	Alx	64.85^{ab}	6.19	3.67^{b}	1.020	0.223^{a}	0.463	2.49^{b}	0.357	0.043^{bc}	0.553^{b}	0.653^{a}	4.76^{d}	5.24^{b}
	V-line	63.31^{b}	5.83	3.80^{b}	1.107	0.130^{c}	0.550	2.34^{b}	0.327	0.047^{bc}	0.540^{c}	0.403^{bc}	5.43^{b}	5.43^{ab}

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Item		Carcass	Head	Full stomach	Empty stomach	Pancreas	Lung	Liver	Heart	Spleen	Kidney	Abdominal fat	Intestine	Cecum
0.5	Alx	62.31^{b}	6.06	4.64^{a}	1.227	0.160^{b}	0.553	2.53^{b}	0.353	0.073^{a}	0.600^{a}	0.367^{c}	5.85^{a}	5.95^{a}
	V-line	66.38^{a}	5.38	3.86^{b}	1.047	0.140^{bc}	0.490	2.35^{b}	0.333	0.047^{bc}	0.520^{c}	0.473^{bc}	4.60^{d}	4.19^{c}
SEM		1.07	0.130	0.200	0.055	0.010	0.035	0.124	0.037	0.004	0.016	0.055	0.173	0.288
p- value														
Treatment		0.8595	0.0733	0.0418	0.2175	0.0001	0.8743	0.1468	0.6000	0.0032	0.1740	0.0256	0.5405	0.5681
Strain		0.2920	<.0001	0.8819	0.1749	<.0001	0.9695	0.6337	0.1708	0.3597	0.0337	0.0419	0.0363	0.0086
Interaction		0.0421	0.4442	0.0027	0.0609	0.0023	0.1035	0.0192	0.7167	0.0002	0.0408	0.0097	<.0001	0.0063
Linear		0.7632	0.0767	0.0776	0.7179	0.0213	0.6406	0.0743	0.3729	0.7068	0.0647	0.3923	0.7424	0.3556
Quadratic		0.6486	0.1359	0.0629	0.0895	0.0001	0.8290	0.4186	0.6417	0.0008	0.8624	0.0101	0.2929	0.6058

Different superscripts in each column indicate significant differences ($p < 0.05$). 0: 0 ml peppermint oil /kg of body weight; 0.25: 0.25 ml peppermint oil /kg of body weight; 0.5: 0.5 ml peppermint oil /kg of body weight; SEM: standard error of the mean; Alx: Alexandria rabbits; V-line: V-line rabbits.

received peppermint oil had lower feed intake but exhibited enhanced final BW, ADG, and FCR. This discrepancy could be explained by the fact that *Mentha piperita L.* varies based on environmental parameters as sunshine exposure, soil and water quality, pH, humidity, pesticide use, and genetics (Waterman and Mole, 2019). Over all, the observed breed-specific responses may relate to physiological and genetic variations in metabolism and energy partitioning. V-line rabbits, being genetically selected for lean growth, might utilize nutrients more efficiently, whereas Alexandria rabbits with larger digestive organs may rely more on fiber fermentation and lipid deposition, leading to different responses to peppermint oil supplementation.

The increased total FI observed in the 0.25 g/kg peppermint oil group is noteworthy, particularly as this group also demonstrated enhanced FCR during specific growth stages. This indicates that peppermint oil could potentially boost appetite while also enhancing feed efficiency, likely due to its influence on gut health and metabolic processes. The increased weight gain noted in the groups supplemented with peppermint oil, especially the second group, supports the enhancements observed in feed efficiency. The impact was particularly evident during the later stages of growth, indicating that the advantages of peppermint oil may build up over time or become increasingly noticeable as the rabbits develop. The improvement in FCR at 0.5 ml/kg BW could also be attributed to reduced oxidative stress and metabolic energy loss, as evidenced by the lower MDA and improved antioxidant status.

Peppermint oil supplementation significantly reduced cholesterol, LDL, and MDA, while increasing HDL, total protein, globulin, TAC, and SOD compared with the control group. In agreement with our findings, Elspeiy *et al.* (2020) reported that peppermint oil supplementation at a level of 800 mg/kg diet decreased the concentrations of serum cholesterol, LDL, triglyceride, and MDA and increased the concentrations of serum HDL and TAC. In the same line, broiler chicks fed 0.1% peppermint oil exhibited a decrease in the concentrations of serum cholesterol, LDL, triglycerides, and MDA while serum total protein, glutathione activity, and HDL significantly increased (Hussein *et al.*, 2023). Conversely, Morshedy *et al.* (2019) revealed peppermint oil did not affect most of blood biochemical parameters including total lipids, triglycerides, cholesterol, LDL, VLDL, HDL, and MDA in the growing rabbits. Finally, the more pronounced improvement in HDL and reduction in LDL observed in Alexandria rabbits at 0.25 ml/kg BW may indicate that their hepatic metabolism and bile acid turnover are more responsive to the choleretic and hypolipidemic effects of peppermint oil compared with V-line rabbits.

It has been demonstrated that peppermint volatile oil, which contains menthol and thymol, reduces the activity of enzymes such hepatic reductase and hydroxymethyl glutaryl coenzyme A that are essential for the creation of cholesterol. It suggests that volatile phenolic components like menthol, menthone, mentyl acetate, menthofuran, limonene, polygen, cineole, and azulen may be the cause of a drop in total cholesterol levels in the case of a phenolic compound like peppermint extract (Arab *et al.*, 2016). Furthermore, the present compounds in peppermint stimulate liver cell activity, which increases bile acid concentration. Bile acids play a critical role in the digestion of lipids; therefore, elevated bile acid concentrations in the duodenum facilitate digestion of fats and fat-soluble vitamins (Crossland, 1980; Arab *et al.*, 2016). Mimica-Dukić *et al.* (2003) indicated that peppermint can stimulate bile secretion from the gall bladder because of its antioxidant and antibacterial activity and is subsequently able to decrease serum total cholesterol level. Thus, peppermint supplementation may help regulate cholesterol metabolism by reducing absorption in the intestine and

lowering serum lipid levels, as demonstrated in chickens (Zwain, 2022).

Breed differences were observed in the levels of triglycerides and VLDL, with V-line rabbits showing higher levels. This may suggest genetic variations in lipid metabolism between breeds, possibly related to differences in energy utilization or fat deposition patterns. Several pharmacological studies have demonstrated the potent antioxidant activity of peppermint, evidenced by its high antioxidant capacity, free radical hunting ability, and reducing power (Singh *et al*., 2015; Stringaro *et al*., 2018; Wu *et al*., 2019). Oils and extracts derived from *Mentha* species are capable of neutralizing free radicals and reactive oxygen species (Singh *et al*., 2015; Brown *et al*., 2019). This antioxidant potential is largely attributed to the presence of phytochemicals such as polyphenols, flavonoids, lignans, stilbenes, and monoterpenes within the plant (McKay and Blumberg, 2006; Pavlić *et al*., 2021). These phytochemical compounds mitigate oxidative stress through several mechanisms that include: The mechanisms by which antioxidants scavenge free radicals are classified primary into hydrogen atom transfer and single electron transfer, although most antioxidant reactions do not happen through a single mechanism but rather through a combination of the two (Prior *et al*., 2005; Brown *et al*., 2019). Furthermore, Mentha plants have antioxidant activity through direct radical scavenging, self-oxidation to less reactive species, singlet oxygen quenching, metal ion chelation, and neutralization of secondary oxidation products and the inhibition of pro-oxidative enzymes (Brown *et al*., 2019; Pavlić *et al*., 2021).

Concerning the antimicrobial effect of peppermint oil, the total bacterial count, *Escherichia coli,* and *Clostridium* spp., were significantly decreased in the cecum of growing rabbits fed peppermint compared to the control group. The significant reduction in pathogenic bacteria such as *E. coli* and *Clostridium* spp. likely created a more favorable gut environment, potentially reducing intestinal inflammation and competition for nutrients, which may have indirectly benefited the growth and activity of fiber-fermenting bacteria essential for rabbit health. This antibacterial effect was dose-dependent, with the highest dose showing the greatest impact. These findings have significant implications for gut health, nutrient absorption, and potentially for reducing the need for antibiotics in rabbits rearing. Similarly, total bacteria count and *Escherichia coli* were decreased in the jejunum of growing Japanese quail fed peppermint essential oil compared to growing Japanese quail fed basal diet only (Abbas *et al*., 2021). On the other hand, Ghazaghi *et al*. (2014) indicated that total count of microbial populations was increased in the ileal of growing Japanese quail fed dried spearmint (*Mentha spicata*) powder through reduction in *Escherichia coli* and increasing in *Lactobacillus* bacteria populations. The antibacterial effect of peppermint essential oil may be due to menthol and menthone which are the main active compounds of peppermint essential oil. Like antibiotics, essential oils particularly those with lipophilic character e.g., peppermint essential oil interact with the cell membranes of gram negative and gram-positive bacteria (Benchaar *et al*., 2006; Tajkarimi *et al*., 2010). Because the cyclic hydrocarbons found in several essential oils are hydrophobic, they can build up in the lipidic membrane of bacteria by taking up space between the fatty acid chains. The membrane structure undergoes conformational changes as a result of this contact, which causes it to expand and fluidify. Ions leak through the cell membrane as a result of this membrane instability, which lowers the transmembrane ionic gradient. Ionic pumps are typically used by bacteria to counterbalance these effects, preventing cell death. However, a significant amount of energy is diverted to this function with energy depletion, which slows down bacterial growth (Lv *et al*., 2011; Chouhan *et al*., 2017; Elfadadny *et al*., 2024).

Supplementation of peppermint oil did not affect live BW and carcass weight but altered organ weights. It decreased pancreas weight while increasing the weights of spleen, empty stomach, and intestine at a dose of 0.5 ml peppermint oil/kg of BW. The lower pancreas weight in treated groups may indicate improved digestive efficiency and reduced need for enzyme synthesis due to enhanced gut health. The heavier spleen at 0.5 ml/kg BW aligns with higher globulin levels and reduced pathogenic bacteria, suggesting enhanced humoral immunity. The increased intestinal weight could reflect improved mucosal integrity and gut-associated lymphoid tissue (GALT) stimulation resulting from a more balanced gut microbiota.

CONCLUSION

In conclusion, peppermint oil supplementation represents a viable natural approach to enhance the metabolic health and gut environment of growing rabbits. The present findings clearly demonstrate that its efficacy is breed-dependent. A supplementation level of 0.25 ml/kg BW is recommended for Alexandria rabbits to optimize feed intake and lipid metabolism, while 0.5 ml/kg BW is more suitable for V-line rabbits to improve feed efficiency and antioxidant capacity. This evidence-based, precision-nutrition strategy can contribute to more sustainable rabbit production and reduced dependence on in-feed antibiotics.

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NOVELTY STATEMENT

This study is the first to compare peppermint oil supplementation in Alexandria and V-line rabbits, revealing breed-specific responses. Peppermint oil improved lipid profile, antioxidant status, and gut microbial balance without adverse effects on growth, indicating its potential as a natural alternative to antibiotics in rabbit production.

AUTHOR'S CONTRIBUTION

All authors contributed to the conceptualization, methodology, resources, and formal analysis.
Amin O. Hendawy carried out the investigation, visualization, and writing of the original draft.
Abdalrahem A. Amrabit contributed to conceptualization, methodology, and resources.
Hossam A. Shahba handled data curation, investigation, and visualization.
Saber S. Hassan provided supervision and manuscript review.
Mohamed I. Hassan contributed to conceptualization, drafting, and editing of the manuscript.
Walid S. Habashy performed data curation, investigation, and visualization.

Generative AI and AI-assisted technology statement

All authors confirm that no generative AI tools, such as large language models (e.g., ChatGPT, Copilot) or text-to-image generators, were used in the conception, writing, or editing of this manuscript.

Conflict of interest

The authors have declared no conflict of interest.

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