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REGULAR ARTICLES

Influence of dietary supplementation of clove and rosemary essential oils or their combination on growth performance, immunity status, and blood antioxidant of growing rabbits

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Abstract

This study aimed to assess the dietary effects of rosemary and clove essential oils separately and in combination on the growth performance; immunological, hematological, and physiological responses; and antioxidant status of growing rabbits. One hundred forty-four of 42-day-old growing V-line rabbits (both sexes with initial live body weights of 765 ± 6 g) were randomly allocated into four treatment groups of 36 rabbits each. Each group was further sub-divided into 12 replicates of 3 rabbits in a completely randomized design. The 1st group was fed a basal diet free of additives and served as the control group, the 2nd and 3rd groups were fed basal diets supplemented with rosemary and clove essential oils, respectively, at doses of 400 mg/kg diet. The 4th group received a basal diet supplemented with a combination of clove and rosemary essential oils at doses of 200 mg/kg diet each. The results showed that the different supplementations did not influence rabbit performance or immunological traits. Opposite to performance or immunological traits, differences in red blood cells and hemoglobin value among all dietary treatments were improved ($P < 0.05$). Dietary essential oil supplementation with clove, rosemary oil, or a mixed of both increased ($P < 0.05$) blood concentrations of catalase, superoxide dismutase, and glutathione peroxidase by 47, 42, and 7%; 56, 35, and 36%; and 40, 39, and 37%, respectively, in supplemented rabbits versus control rabbits. In conclusion, clove and/or rosemary essential oils can potentially be used in rabbit diets to improve antioxidant status without change in rabbit's growth performance or immunological parameters.

Keywords Antioxidant · Essential oil · Immunity · Performance · Rabbits

Introduction

Rabbit exposure to many stress-inducing factors with high cellular metabolism causes oxidative stress. Enhancing the antioxidant status of rabbits could be a way to improving the immunity and thus the productivity. Supplementation of animal feed with essential oils, or also called volatile oil, is an alternative solution to antibiotic use to increase immunity and animal health, reduce metabolic problems, and maximize feed efficiency (de Oliveira Monteschio et al. 2017). Recently, essential oils and medicinal plants in the animal sector have increased due to their antioxidant and beneficial immunomodulatory effects (Nehme et al. 2021; El-Gindy et al. 2020). There is evidence of positive impact of essential oils on digestibility and gut function that was found that could be used as natural growth promoters in animal diets (Giannenas et al. 2013). Essential oils, mixtures of compounds whose chemical compositions and concentrations are variable, are hydrophobic volatile aromatic compounds. The therapeutic properties of aromatic plants could be mainly due to the antioxidant substances such as phenolic and polyphenolic compounds (Giannenas et al. 2013). Many studies have focused on natural antioxidants in medicinal herbs that are healthy, popular, and accepted by customers (Chaves et al. 2011; de Souza et al., 2019, Souza et al. 2019), such as clove and rosemary oil, as replacements for pharmaceuticals.

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Clove (*Syzygium aromaticum*) oil has attracted much attention due to its high and varied content of approximately 36 bioactive constituents and phenolic compounds (Table 1), including eugenol, the main compound; eugenol acetate; β-caryophyllene; caryophyllene oxide; caryophyllene; and α-humulene (Issac et al. 2015). Clove oil may act as an antifungal (Campaniello et al. 2010), antiviral, and anti-inflammatory agent (Han et al.2017); in addition, it exhibits antibacterial activity against several types of pathogenic bacteria as a replacement for carcinogenic preservatives (Radünz et al. 2019) and exhibits antioxidant activity, due to the presence of eugenol, gallic acid, flavonoids (quercetin) and kaempferol, and phenolic acids like ferulic, caeic, ellagic, and salicylic acids (Batiha et al. 2020).

Rosemary (*Rosmarinus officinalis*) oil has high antioxidant activity and contains several phenolic compounds, such as carnosol, rosmanol, rosmaridiphenol, and rosmarquinone (Assis et al. 2009). The main chemical compounds of rosemary oil are borneol, α-pinenen, α-caryophyllene, ledol, eucalyptol, camphor, γ-terpinene, and D-verbenone as shown in Table 1 (Abozid and Asker 2013; Nie et al. 2020). Rosemary is widely used for its antimicrobial bioactivity, but its significance is largely unknown as a phytomedicinal with regard to its antimicrobial properties and antioxidant properties (Dorman et al. 2003). To the best of our knowledge, this is the first available report in the literature evaluating dietary clove oil with or without rosemary oil on growth performance, immunity, and antioxidant status of growing rabbits. The aim of this study was to investigate the effects of dietary supplementation with clove and rosemary essential oils separately and in combination as antioxidant and anti-inflammatory alternatives in rabbit diets. It was hypothesized that the antioxidants in these essential oils would improve growth performance and prevent oxidative stress.

Table 1 The main chemical components in clove and rosemary essential oils

Clove oil	%	Rosemary oil	%
Eugenol	76.23	Borneol	24.13
β-Caryophyllene	11.54	α-Pinene	9.72
Caryophyllene oxide	4.29	α-Caryophyllene	8.35
Eugenyl acetate	1.76	Ledol	6.42
α-Caryophyllene	0.64	Eucalyptol	5.11
β-Selinene	0.25	Camphor	5.01
Eucalyptol	0.14	γ-Terpinene	5.005
α-Selinene	0.16	D-Verbenone	4.17
4-Allylanisole	0.13	Limonene	2.29
(−)-b-Cadinene	0.12	Methyl jasmonate	2.24

Clove oil compound identified by Xu et al. (2016) using GC–MS; rosemary oil compound identified by Abozid and Asker (2013) using GC/MS analysis

Materials and methods

Experimental animals and management

This study was carried out at the Rabbit Production Laboratory, Animal and Fish Department, Faculty of Agriculture Saba Basha, Alexandria University, Egypt, throughout the period from January to March (temperature ranging from 9 to 20 °C and humidity from 60 to 70%). One hundred forty-four growing six-week-old V-line rabbits of both sexes with an initial live body weight of 764.79 ± 6.00 g were randomly allocated into four treatment groups of 36 rabbits each. Each treatment was further sub-divided into 12 replicates of 3 rabbits. The rabbits were housed in 3-level wire batteries (45 *L*, 36 W, 36 *H*, in cm) equipped with automatic drinkers and feeding hoppers and were offered diets for the duration of the feeding trial (42 days) until they reached 84 days of age. All rabbits were kept under similar hygienic conditions in a well-ventilated block building (with natural ventilation through windows). The rabbits were kept under a cycle of 16-h light and 8-h dark (after natural sunlight lighting hours, an artificial lighting was used to ensure a 16 lighting hour). Dangerous diseases were largely avoided, and the rabbits were never subjected to any kind of systematic vaccination or medication. The rabbit house had an average air temperature and relative humidity of 18.2 °C and 50.6%, respectively, that were measured daily by room dry-bulb thermometer and hygrometers.

Animal diets

Four pelleted diets were prepared, the 1st group was fed a basal diet free of feed additives, antibiotics, and antioxidants and served as the control group, while the 2nd and 3rd groups were fed basal diets supplemented with clove and rosemary oils, respectively, at a dose of 400 mg/kg diet. The 4th group received a basal diet supplemented with a combination of clove and rosemary oils at doses of 200 mg/kg diet each. These doses were chosen according to the results of Rivaroli et al. (2016) for clove oil and Gogary et al. (2018) for rosemary oil. The organic clove and rosemary essential oils were purchased from ElCAPITAN Company for Extracting Natural Oils, Plants and Cosmetics, Al Obour City, Cairo, Egypt. The oil extracting was by cold pressing to obtain extra virgin oil. The experimental diets were offered to the rabbits ad libitum. The pellets were 4 mm in diameter and 5 mm in length, and to ensure homogeneity, the essential oils were mixed with sunflower oil free of added antioxidants. Pelleting of the experimental diets was initiated by addition of molasses

as a binding material; then, all the diet ingredients were pressed at 60 °C. The basal experimental diet was formulated to support optimal rabbit growth according to the recommendations of nutrient requirements of domestic animals (NRC 1977) (Table 2).

Growth performance

The initial and final live body weights and daily feed intake were measured. The daily gain and feed conversion ratio were calculated. The mortality rate was measured as the number of dead rabbits in each treatment group during the entire experimental period. Blood samples were taken at the end of the experiment, and 12 overnight fasted rabbits (one rabbit from each replicate) were selected from each treatment group.

Respiration rate and rectum temperature

Respiration rate per minute rectal temperature of 12 rabbits/treatment was measured twice (70 and 84 days of age) at mid-day (between 12 am and 3 pm). Respiration rate was evaluated by calculating the flank movement per minute by using a hand counter, while rectal temperature was recorded by using a digital thermometer scale inserted into the rectum for 2 min at depth of 2 cm, in contact to the mucous membrane.

Hematological parameters and humoral immunity

A total of 6 ml (2 ml in a heparinized tube and 4 ml in a tube without an anticoagulant) of blood was taken from the ear vein with a sterile syringe. Hematological assays were carried out to determine several parameters. Red blood cell (RBC) counts were determined using an AO Bright-Line hemocytometer with a light microscope at 400 × magnification after diluting the blood samples 200-fold with physiological saline (0.9% NaCl solution). Hemoglobin (Hb) concentrations (g/dl) were estimated by the cyanomethemoglobin method as described previously (Eilers 1967). Packed cell volume (PCV) percentages were measured according to a previously described method (Provan et al. 2010). White blood cells (WBCs) were counted using an AO Bright-Line hemocytometer with a light microscope at

Table 2 Ingredients and chemical composition of basal diet fed to growing rabbits

Ingredients %	Control	Clove 400 mg/kg diet	Rosemary 400 mg/kg diet	Mix 200 g of each oil/kg diet
Ground yellow corn	17.90	17.90	17.90	17.90
Wheat bran	11.00	11.00	11.00	11.00
Barley grain	17.30	17.30	17.30	17.30
Soybean meal (44%)	20.00	20.00	20.00	20.00
Berseem hay	28.00	28.00	28.00	28.00
Molasses	3.00	3.00	3.00	3.00
Sunflower oil	1.0	0.96	0.96	0.96
Clove oil	0.00	0.04	0.00	0.02
Rosemary oil	0.00	0.00	0.04	0.02
Dicalcium phosphate	1.00	1.00	1.00	1.00
Sodium chloride (salt)	0.30	0.30	0.30	0.30
Premix[1]	0.30	0.30	0.30	0.30
DL-Methionine	0.10	0.10	0.10	0.10
L-Lysine	0.10	0.10	0.10	0.10
Calculated composition (% of DM basis)				
Crude protein	18.87			
Ether extract	3.33			
Crude fiber	13.57			
[2]**NDF**	37.84			
Nitrogen-free extract	54.89			
Organic matter	90.66			

[1]Premix contained the following vitamins and minerals mixture per kg (g/kg): vit A., 2000.000 IU; vit E, 10 mg; vit B1, 400 mg; vit B2, 1200 mg; vit B6, 400 mg; vit B12, 10 mg; vit D3, 180,000 IU; choline chloride, 240 mg; pantothenic acid, 400 mg; niacin, 1000 mg; folic acid, 1000 mg; biotin, 40 mg; manganese, 1700 mg; zinc, 1400 mg; iron, 15 mg; copper, 600 mg; selenium, 20 mg; iodine, 40 mg; and magnesium, 8000 mg

[2]NDF (neutral detergent fiber) = 28.924 + 0.657 × CF%

100 × magnification after diluting the blood samples 20-fold with a diluting fluid (1% glacial acetic acid solution with a small amount of Leishman's stain) (Hepler 1966).

Differential leucocytic counts were determined according to a method described previously (Lucky 1977). A drop of anticoagulated blood was quickly spread on a slide, air-dried, fixed in methyl alcohol for 3–5 min, and then stained with Giemsa stain for 20 min after being rinsed under a gentle stream of water and dried gently between two filter papers. Each stained blood sample was examined using an oil immersion lens. The percentage of each type of cell was calculated according to methods described previously (Weiss and Wardrop 2010). Blood indices such as the mean cell volume (MCV), mean cell hemoglobin (MCH), and mean cell hemoglobin concentration (MCHC) were calculated (Bauer et al. 1976). Serum IgG and IgM levels were determined using ELISA according to a method described previously (Siwicki 1993).

Twelve rabbits from each group (one rabbit per replicate) were immunized with 0.1 ml of 2.5% Sheep Red Blood Cells (SRBCs) 15 days after starting dietary supplementation to measure the antibody titers against SRBCs. The dosage of RBC for inoculation was pre-determined in a separate trial. Antiserum to SRBC was collected on the 7th, 14th, and 21st days post challenge. Blood (1 ml) was refrigerated to allow the RBC to settle. If complete sedimentation did not occur, the samples were centrifuged for 1 to 2 min at 3000 rpm to separate plasma and erythrocytes, and the supernatant was collected. Briefly, 50 µl of physiological saline solution was first added to the wells of 96-well plates. Then, 50 µl of antiserum was pipetted into the first well in duplicate, after which 50 µl from the first well was pipetted into the second well using an automatic pipette; this process was repeated for all wells. Finally, 0.75% of SRBC solution was added to each well. The plates were incubated at 37 °C for 3 h and then examined visually for agglutination (Wegmann and Smithies, 1966). The agglutination titer is expressed as the $\log^2$ of the reciprocal of the highest serum dilution giving complete agglutination (Nelson et al. 1995).

Blood biochemical and antioxidant parameters

Total protein, albumin, total lipid, cholesterol, triglycerides, creatine, and urea were determined according to Gornall et al. (1949), Doumas et al. (1997), Zollner and Kirsch (1962), Allain et al. (1974), Fassati and Prencipe (1982), Bartles et al. (1972), and Tappel and Zalkin (1959) methods, respectively. Glutamic-pyruvic transaminase (GPT) and glutamic oxaloacetic transaminase (GOT) were determined according to Reitman and Frankel (1957). Serum antioxidant profiles such as total antioxidant capacity (TAC) and malondialdehyde (MDA) were measured by Koracevic et al. (2001) and Ohkawa et al. (1979) methods, respectively. Similarly, catalase (CAT), glutathione peroxidase (GPx), and superoxide dismutase (SOD) were determined using the methods of Aebi (1986), Chiu et al. (1976), and Misra and Fridovich (1972), respectively, by using commercial kits (Biodiagnostic, Egypt) according to the procedure outlined.

Statistical analysis

All data were analyzed using one-way analysis of variance (ANOVA) using the general linear model (with the diet as the fixed factor) followed by Duncan's test using SPSS 11.0 statistical software (SPSS 2001). The cage (replication) was used as a random effect in the statistical model. In the feed intake and feed conversion, replicate (containing 3 rabbits) within each treatment was the experimental unit, whereas rabbit within each treatment was the experimental unit in the other parameters. Significant differences between means were detected using new Duncan multiple range tests (Duncan 1955). The variances among mortality rates were analyzed with chi-square tests. Results were presented as means and standard error of the mean.

Results

Growth performance

No significant differences among treatments regarding the growth performance parameters (body weight, body gain, feed intake, and feed conversion) were found. The rosemary group exhibited the lowest mortality rate, as shown in Table 3.

Respiration rate and rectum temperature

Respiration rate of growing rabbits at the 1st month decreased by clove and rosemary treatments in comparison with the control and mixed groups. In the 2nd month, the mixed group recorded the highest value ($P < 0.05$) of respiration rate and rectum temperature compared to other groups (Fig. 1).

Hematological parameters and humoral immunity

The clove oil, rosemary oil, and combined essential oil treatments improved RBC counts and Hb concentrations ($P < 0.01$) by approximately 9.62, 10.99, and 8.78% and 10.55, 11.64, and 11.64%, respectively, compared to control rabbits. Clove oil addition increased ($P < 0.05$) the PCV (by 46.90%) versus control rabbits. The eosinophil percentage decreased with all essential oil treatments, while the WBCs and blood indices were unaffected by dietary essential oils supplementation. Rabbits treated with clove, rosemary oils,

Table 3 Effect of the essential oils[1] of clove and/or rosemary on growth performance of growing rabbits

	Control	Clove	Rosemary	Mix	SEM	*P* value
Initial BW (42 days old), g	760.56	767.22	764.72	766.67	5.995	0.980
Final BW (84 days old, g	2032.26	2078.90	2019.38	2069.64	9.453	0.070
Total gain, g	1280.56	1295.69	1256.81	1295.56	11.645	0.616
TFI, g	5051.19	4956.85	4775.99	5112.10	46.604	0.054
FCR	2.55	2.62	2.63	2.54	0.027	0.523
Mortality rate, %	13.8 (5)	13.8 (5)	11.11 (4)	22.2 (8)		0.233

BW, body weight; *T. gain*, total gain; *TFI*, total feed intake; *FCR*, feed conversion ratio; SEM, standard error of the mean

[1]Control—no essential oil added; Clove—clove oil was added at 400 mg/kg diet; Rosemary—rosemary oil was added at 400 mg/kg diet; Mix—essential oil was added at 200 mg of each oil (clove and rosemary)/kg diet

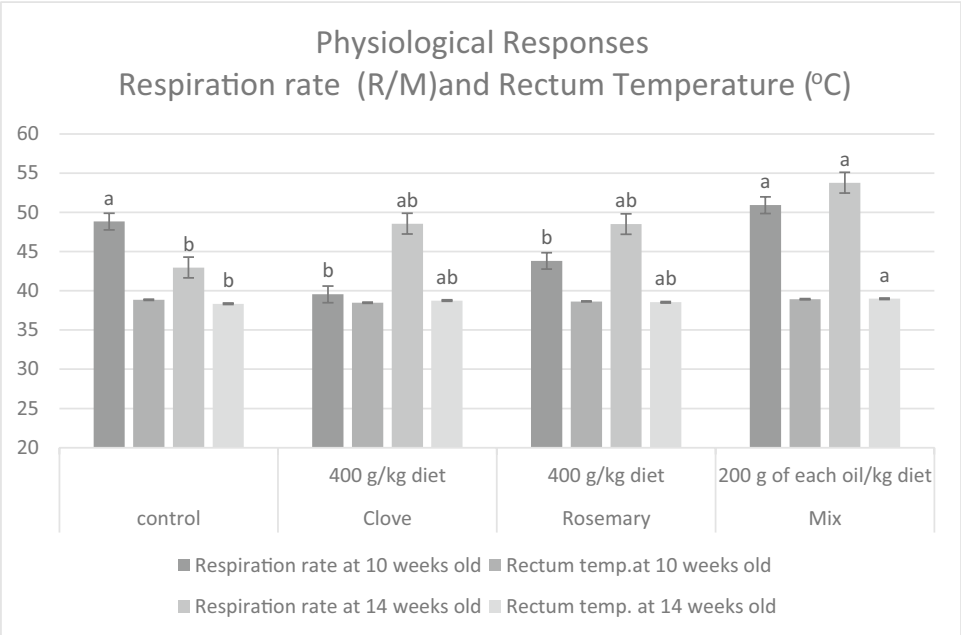

Fig. 1 The effects of clove and/or rosemary essential oils on physiological responses (respiration rate and rectum temperature) of growing rabbits (mean ± SEM). The bars of each parameter sharing a letter are significantly different at $P < 0.05$

and their mix did not affect the blood protein, lipid profile, and liver and kidney function level. All dietary treatments (clove, rosemary, and their mix) decreased ($P < 0.05$) the blood creatinine levels versus control rabbits (Table 4). The SRBC antibody titers were non-significant at all sample times (on the 7th, 14th, and 21st days) after challenge by clove, rosemary oils, or their combination (Fig. 2 and Table 5).

Blood biochemical and antioxidant parameters

Dietary essential oils supplementation (clove oil, rosemary oil, or both) increased ($P < 0.05$) blood concentrations of CAT, SOD, and glutathione (GSH) by 47.20, 42.35, and 7.16%; 56.36, 34.54, and 36.36%; and 39.80, 39.47, and 37.17%, respectively, compared to control rabbits. The blood concentrations of immunoglobulins (IgG and IgM) increased non-significantly in rabbits supplemented with essential oils.

Discussion

According to the obtained results, there were no significant differences among the treatments regarding growth performance parameters. This lack of a significant effect of essential oils may be related to the low oil doses. Similar results were found by Ghozlan et al. (2017) showing that average growth performance parameters were not significantly different in the clove- and rosemary-treated groups compared to the control rabbits, suggesting that clove and rosemary oils had no growth-promoting effects. Elnaggar et al. (2016) found that low levels (0.25%) of rosemary improved growth performance better than higher levels. The reasons for these discrepancies could be that the levels of active ingredients in the essential oils were different and that the oils were added to the feed at different levels. However, the rosemary group exhibited a lower

Table 4 Effect of the essential oils[1] of clove and/or rosemary on hematological parameters and blood index of growing rabbits

	Control	Clove	Rosemary	Mix	SEM	*P* value
RBCs, $\times 10^6$	5.82[b]	6.38[a]	6.46[a]	6.33[a]	0.069	0.001
Hb, mg/dl	12.03[b]	13.30[a]	13.43[a]	13.43[a]	0.159	0.001
PCV, %	34.70[b]	46.90[a]	39.95[ab]	38.18[b]	1.484	0.021
WBCs, $\times 10^3$	17.34	17.21	17.25	17.24	0.139	0.990
Eosinophil, %	0.75[a]	0.25[b]	0.00[b]	0.25[b]	0.083	0.007
Monocytes, %	8.18	7.63	7.00	7.25	0.189	0.138
Neutrophil, %	34.83	36.46	36.25	37.25	0.853	0.806
Lymphocytes, %	56.25	55.67	56.75	55.25	0.780	0.920
MCHC, g/dl	34.68	30.22	33.64	35.34	0.746	0.064
MCH, pg	3.47	3.02	3.36	3.53	0.069	0.065
MCV, fl	59.65	73.49	61.79	60.30	2.123	0.061

[ab]Means with different superscripts within the same row differ significantly

RBCs, red blood cells; *Hb*, hemoglobin; *PCV*, packed cell volume; *WBCs*, white blood cells; *MCHC*, mean corpuscular hemoglobin concentration; *MCH*, mean corpuscular hemoglobin; *MCV*, mean corpuscular volume; *SEM*, Standard error of the mean

[1]Control—no essential oil added; Clove—clove oil was added at 400 mg/kg diet; Rosemary—rosemary oil was added at 400 mg/kg diet; Mix—essential oil was added at 200 mg of each oil (clove and rosemary)/kg diet

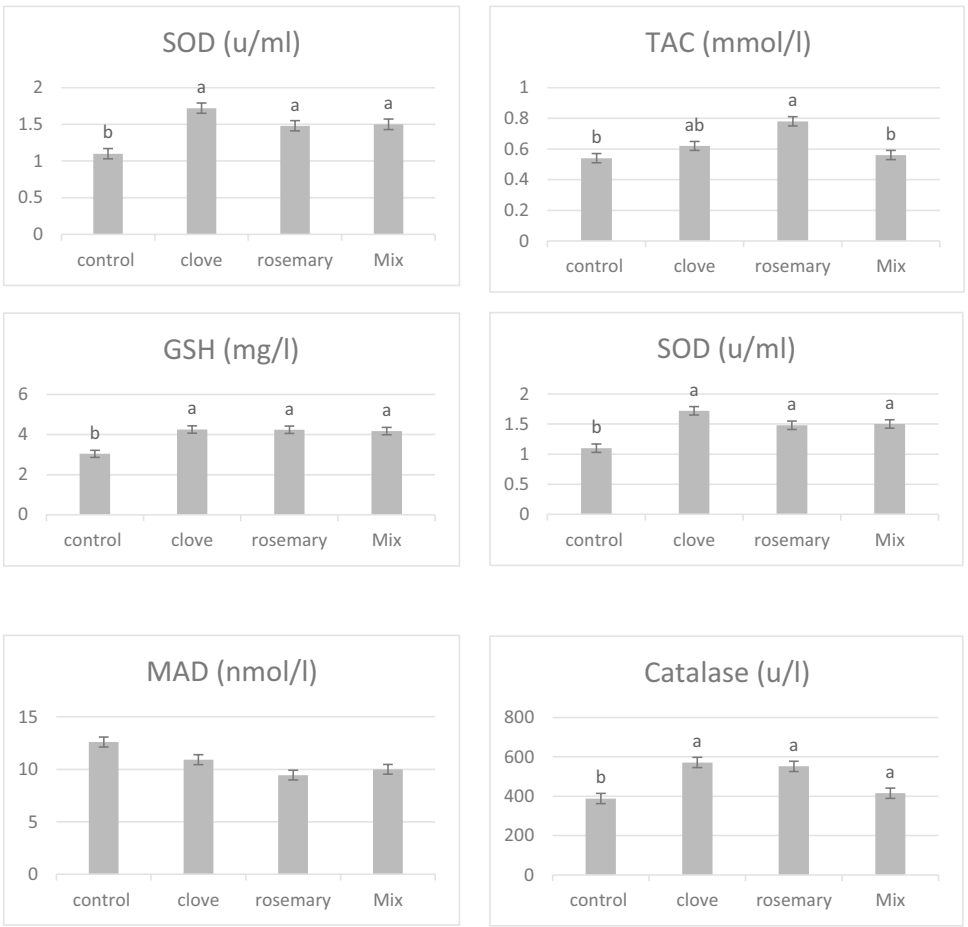

Fig. 2 The effects of clove and/or rosemary essential oils on antioxidant of growing rabbits (mean ± SEM). The bars of each parameter sharing a letter are significantly different at $P < 0.05$

Table 5 Effect of the essential oils[1] of clove and/or rosemary on protein, lipid profile, liver, kidney function levels, SRBCs, and immunoglobulin of growing rabbits

	Control	Clove	Rosemary	Mix	SEM	P value
Total protein, g/dl	5.93	6.65	6.11	6.39	0.107	0.082
Albumin, g/dl	5.35	5.68	5.26	5.58	0.089	0.336
Globulin, g/dl	0.58	0.98	0.85	0.81	0.0596	0.111
Total lipid, mg/dl	264.44	270.46	276.35	262.98	3.480	0.529
Cholesterol, mg/dl	48.13	51.50	52.50	49.75	1.360	0.700
Triglyceride, mg/dl	92.90	91.25	94.88	87.75	1.269	0.242
GOT, mg/dl	34.38	30.25	30.75	31.75	0.917	0.406
GPT, mg/dl	39.25	38.50	32.50	32.25	1.229	0.062
Creatine, mg/dl	1.08[a]	0.94[b]	0.92[b]	0.93[b]	0.024	0.049
Urea, mg/dl	25.50	23.63	21.00	25.50	0.696	0.063
SRBCs—7th day	0.59	0.67	0.68	0.70	0.038	0.767
SRBCs—14th day	0.67	0.72	0.72	0.77	0.028	0.687
SRBCs—21st day	0.71	0.78	0.82	0.84	0.022	0.211
IgM, mg/dl	25.50	28.75	33.25	27.50	1.152	0.101
IgG, mg/dl	323.25	344.25	354.75	352.25	7.146	0.407

GOT, glutamic oxaloacetic transaminase; *GPT*, glutamic-pyruvic

transaminase; *SRBCs*, sheep red blood cells; *IgM*, immunoglobulin M; *IgG*, immunoglobulin G; *SEM*, standard error of the mean

Control—no essential oil added; Clove—clove oil was added at 400 mg/kg diet; Rosemary—rosemary oil was added at 400 mg/kg diet; Mix—essential oil was added at 200 mg of each oil (clove and rosemary)/kg diet

mortality rate than the other groups, which may have been due to improvements in blood antioxidant activity or due to borneol (a main active compound of rosemary oil) that improves the messenger agent and central nervous system (Zheng et al. 2018).

In the 1st month, clove and rosemary oils rabbits recorded the lowest rate of respiration that may be due to α-caryophyllene and β-caryophyllene (active compunants of clove and rosemary oils) which are useful in reducing oxidative stress and pain (Ullah et al. 2021 and Koyama et al. 2019). However, in the 2nd month, the mix of clove and rosemary oils rabbits recorded the highest value ($P < 0.05$) of respiration rate and rectum temperature compared to other treatments that could be due to increase in blood circulation.

Hematological parameters reflect the physiological responsiveness of an animal to its internal and external conditions. All complete blood count values fell within the normal ranges for healthy rabbits reported previously (Hewitt et al. 1989). The essential oil treatments improved RBC counts and Hb concentrations, suggesting that the clove and/or rosemary oils supported RBCs and hemoglobin synthesis. The present results get along with those of Ofem et al. (2018) who found that RBCs and Hb level were risen in treated animals with eugenol. Similar findings were seen in a study in rosemary extract by Shokrollahi et al. (2015). The active compounds of clove and rosemary oils such as eugenol, caryophyllene oxide, and α-pinene prevents the accumulation of lipid peroxidation (Salehi et al. 2019; Barboza et al. 2018; Salehi et al. 2019) substances in RBCs and maintains the activities of the antioxidant enzymes such as SOD, CAT, GPx, and glucose-6-phosphate dehydrogenase at regular levels (Kumaravelu et al. 1996) and by protecting the RBC membrane from ROS attack, removes the oxidative stress. Clove oil addition significantly increased the PCV compared to the control regimen by 46.90%. However, the eosinophil percentage was decreased in all essential oil treatments, probably due to the anti-inflammatory activity of clove and/or rosemary oil (Yaqoob and Calder 1995), while the WBCs and blood indices were not influenced by dietary essential oil treatments.

The blood concentrations of immunoglobulins non-significantly increased with essential oil treatment. These results disagree with a previous study (Gülçin et al. 2004) that found that rosemary significantly increased serum IgG and IgM levels in broiler chickens. Additionally, these are in accordance with those obtained in a study (ELnaggar et al. 2016) that found that addition of rosemary to basal diets at concentrations of 0.25, 0.5, 0.75, and 1.0% increased serum immunoglobulin (IgY, IgM, and IgA) levels. However, differences between IgG and IgM concentration can be due to the variation of species and duration of treatment.

The diets with the different essential oils used in the present study failed to significantly affect SRBCs compared with the control diet. However, it was observed that the diets with the different essential oils used in the present study increased antibody titers against SRBCs non-significantly compared with the control diet. A previous study (Gandomani et al. 2014) indicated that the bioactive

components and highly polyunsaturated fatty acids of clove bud can improve the immunity of laying hens by fortifying mucosal and systemic immune functions such as health indices of the intestinal absorptive part. Eugenol (an active clove compound) damages the cell membrane, resulting in cell lysis in Gram-negative and Gram-positive bacteria and causing leakage of intracellular fluid along with lipid and protein contents (Hemaiswarya and Doble 2009).

Rabbits treated with clove, rosemary oils, and their mix did not affect the blood protein and lipid profile. The present findings agreed with Hosseinzadeh and Farhoomand (2014) and Petrovic et al. (2012) who, using eugenol, anethol, and menthol sources, showed no effect on protein levels.

Creatinine is the major waste product of the creatinine metabolism filtered by the glomerulus and actively excreted by the tubules (Miller et al. 2005). However, creatinine concentration as biomarkers of renal function is used to detect treatment-related toxic effects of compound on the kidney in experimental animals. All the essential oil treatments (clove, rosemary, and their mix) decreased the creatinine levels by 21.43, 17.39, and 13.89 as *versus* control. These results agreed with those presented by Rašković et al. (2014) and Sakr et al. (2019) who reveal that rosemary oil has the ability to repair damage of renal excretory function by reducing the level of creatinine in serum of rats.

Dietary essential oils treatments did not significantly affect GOT or GPT, suggesting the maintenance of proper liver function. Essential oils such as clove and rosemary oils have strong scavenging activity and can be used for the regulation of free radicals; this activity can be explained by synergistic effects between phenolic compounds, even at low levels (Radünz et al. 2019).

Dietary essential oils supplementation increased ($P < 0.05$) the blood concentrations of CAT, SOD, and GSH and decreased the blood levels of MDA non-significantly compared to the control regimen. However, blood MDA was often measured as an indicator of lipid peroxidation in the body. In biological systems, many oxidation reactions are critical for survival, and sometimes, inside normal cells, oxidation reactions proceed uncontrolled and produce unstable free radicals. These formed compounds react with many different important molecules in vital tissues, such as lipids, proteins, and DNA, producing new compounds that harm DNA (Ercegovac et al. 2010). Antioxidants are the first line of protection against free radical damage and are critical for sustaining optimum health. The present results disagree with Zeng et al. (2001) who mentioned rosemary inhibits lipid peroxidation via its active components, such as the phenolic compounds rosmanol, carnosol, and epirosmanol. In addition, rosemary exerts a protective effect against hyperglycemia and hypercholesterolemia induced by oxidative stress, thus maintaining blood homeostasis (Labban et al., 2014). Dietary supplementation with clove also significantly reduces blood sugar and lipid peroxidation in diabetic rats (Shukri et al. 2010). These effects may be due to the presence of phenolic compounds and flavonoids in clove extract and clove essential oil that scavenge free radicals (Gülçin et al. 2004).

In general, all active compounds of clove and rosemary oils such as eugenol, caryophyllene oxide, and α-pinene have a wide range of pharmacological activities, including, anti-inflammatory, anticarcinogenic, antimicrobial, analgesic, antioxidant, anticoagulant, antitumor, antimicrobial, antimalarial, and anti-*Leishmania* activities, by decreasing lipid peroxidation indices, protein oxidation, and inflammatory markers (reduction in the expression of COX-2, TNF-α, and IL-6) and by improving antioxidant status by maintaining antioxidants such as GPx, SOD, CAT, and GST (Barboza et al. 2018; Dougnon et al. 2021).

Conclusion

It can be concluded that supplementation of growing rabbit diets with essential oils (clove oil, rosemary oil, or both) produced a positive effect on some hematological parameters (RBCs and Hb) and blood antioxidant enzymes (SOD, GPx, and catalase), along with improved kidney function (blood creatine) with no beneficial effects on the studied performance or immunological parameters.

Author contribution YME, SMZ, and MAA conceived and designed the experiment. YME, SMZ, MA, and TRM conducted the experiment. YME, SMZ, and MAA supervised the experiment. YME, SMZ, MA, TRM, and AMZS prepared the manuscript. All authors approved of the manuscript.

Data Availability Not applicable.

Code availability Not applicable.

Declarations

Ethics approval All procedures were accomplished and estimated according to the Institutional Animal Care and Use Committee of Alexandria University under protocol no. 14/19/6/17/3/02 approved on 17/6/2019.

Consent to participate All authors agree to participate in the current work.

Consent for publication All authors agree to publish the findings of the current research.

Conflict of interest The authors declare no competing interests.

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