Impacts of tea tree or lemongrass essential oils supplementation on growth, immunity, carcass traits, and blood biochemical parameters of broilers reared under different stocking densities

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ABSTRACT The effects of tea tree essential oil (TTEO) and lemongrass essential oil (LGEO) with different stocking densities on the growth performance, biochemical markers, antioxidants, and immunity state of broiler chickens were studied. Birds were housed at stocking densities of 25, 30, 35, and 40 kg/m^2. The treatments were, basal diet without any supplementation, the second and third groups were supplemented with 300 mg TTEO/kg feed, and 300 mg LGEO/kg feed, respectively. Results revealed that increasing stocking density from 25 to 40 kg/m^2 significantly reduced body weight and daily weight gain at different ages. The phagocytic index and activity were significantly higher under the lower stocking density (25 kg/m^2). Serum amyloid A (SAA), serum or liver transferrin (TRF), or C-reactive protein (CRP) were significant decreased when decreasing stocking density. Increasing stocking density from 25 to 40 kg/m^2 resulted in a significant increase in the serum urea, creatinine, uric acid, lactate dehydrogenase (LDH), alanine aminotransferase (ALT), aspartate aminotransferase (AST), malondialdehyde (MDA), and catalase (CAT) levels. However, there was a significant reduction in antioxidant enzyme activity, including glutathione peroxidase (GPx) and superoxide dismutase (SOD), as stocking density increased. The supplementation of TTEO produced significantly higher body weight and daily weight gain followed by LGEO. Additionally, the mortality rates were reduced in TTEO (27.4%) and LGEO (25%) groups. TTEO or LGEO supplementation significantly improved meat constituents and cellular immunity and reduced serum total lipids, serum and meat cholesterol, and triglycerides, SAA, TRF, and CRP. For all these measured parameters, superior results were obtained when TTEO was used compared to LGEO. TTEO or LGEO supplementation also significantly reduced serum urea, creatinine, uric acid, and the enzymatic activities of LDH, ALT, AST, MDA, and CAT (but not GPx and SOD) in comparison to the control treatment. Overall, our results showed the superiority of TTEO over LGEO as a feed supplement in broiler diets. In conclusion, TTEO treatment offers a better solution for raising broiler chickens in high stocking density.

Key words: broilers, essential oils, growth, immunity, stocking density

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INTRODUCTION

The stocking density is expressed as a mass for each floor space unit instead of the number of birds raised in a specific area (Thaxton et al., 2006). Increasing the stocking density may be more economic for producing a higher meat yield per space area. However, it may be a primary cause for other adverse effects such as increased respiratory problems and the incidence of cannibalism (Thaxton et al., 2006). It is also well known that high stocking density increases the requirement for improved ventilation inside the poultry houses (Thaxton et al., 2006). Many studies evaluated the impact of different stocking densities (20−40 kg/m^2) on the broiler's productive performance.

It has been reported that reducing stocking densities may be beneficial for broiler and birds performance (Chmelnicna and Solcianska, 2007; Mtileni et al., 2007; Abo Ghanima et al., 2020a). However, some investigators revealed no effect of reducing stocking density on broiler performance (Thomas et al., 2004), while others reported adverse effects (Mtileni et al., 2007).

In the recent decade, based on their various functional characteristics (such as antimicrobial, antioxidant, and anticancer activities), research has focused on the application of essential oils (**EOs**) derived from different herbs and spices as poultry feed supplements (Swamy et al., 2016; Abo Ghanima et al., 2020b). EOs have been commonly used in fumigants, cosmetics, and aromatherapy agents. Research is now oriented towards using plant-derived materials in poultry feed as natural alternatives to synthetic drugs (Popovíc et al., 2016).

The tea tree essential oil (**TTEO**), owing to its wide range of activities, is one of the most important subjects studied (Holliday, 2004). *Melaleuca alternifolia*, frequently called tea tree, is the tree species or tall myrtle tree species of the Myrtaceae family (Holliday, 2004). TTEO has antimicrobial, antioxidant, and acaricidal properties. The poultry production showed a substantial increase in daily weight (around 7%), while there were decreased mortality and morbidity rates when TTEO was used as a dietary supplement (50−150 mg/kg) in broiler chicken feed (Puvača et al., 2019). The outlook for TTEO as a supplement to poultry diets is strongly optimistic regarding efficiency and output (Puvača et al., 2019). Khattak et al. (2014) demonstrated that sufficient concentrations of the TTEO in broiler chicken diets increased the average daily feed intake by 7% compared to the control group.

Lemongrass (*Cymbopogon citratus*) is used in Asian cuisine as a flavoring agent (Singh et al., 2011). Lemongrass essential oil (**LGEO**) arises from lemongrass. It contains citrus, as the active ingredient, in the combination of isomeric forms like geranial (α-citral) and neral (β-citrus), as active ingredients (Tajidin et al., 2012; Verma et al., 2015). The LGEO includes limonene, citronella, ß-myrcene, and geraniol at low concentrations (Schaneberg and Khan, 2002).

Also, a high amount of linalin and limonene is found in citrus EOs (Zantar et al., 2015). Other scientists have acknowledged the substantial improvement in broiler's live weight, feeding efficacy, and carcass characteristics by adding herbal additives like essential citrus oils into their diets (Alcicek et al., 2004). *Citrus sinensis* peel mixtures were reported to increase the live body weight and the feed conversion ratio in broilers (Cabuk et al., 2014; Erhan and Bölükbasi, 2017).

To obtain a reasonable economic return, the ultimate goal of global poultry production is to improve the weight of chicken produced per square metre while minimizing production losses due to overcrowding. Accordingly the current study was conducted to investigate the effects of dietary TTEO and LGEO supplementation in broiler diets and different stocking densities on growth performance, carcass characteristics, serum, and tissue biochemistry. The current study hypothesized that TTEO or LGEO supplementation to broiler diets positively impacts birds' health and physiological status.

MATERIALS AND METHODS

This study was approved by the Native Experimental Animal Care Committee and the Ethics Committee of the Animal Husbandry and Animal Wealth Development Department, Faculty of Veterinary Medicine, University of Damanhour, Egypt (DMU/VetMed-2019-/0145).

Birds and Experimental Design

A total of 4,200 one-day-old Cobb 500 chicks (El-Wataniya Hatcheries, Alexandria, Egypt) were subdivided into 12 groups in a 4 × 3 factorial arrangement (4 stocking desities × 3 groups of additives). Each group had 10 replicates each of 35 birds (4 × 3 × 10 × 35 = 4,200 chicks). Birds were housed at stocking densities of 25, 30, 35, and 40 kg/m^2. The treatments were, basal diet without any additive which served as control, the second and third groups were fed on basal diet supplemented with 300 mg TTEO/kg feed, as 100% pure steam distillation extracted leaf oil (Manufactured by Nature Naturals India, Delhi, India) and 300 mg LGEO/kg feed as 100% pure steam distillation extracted leaf oil (Manufactured by Expo Essential Oils, Delhi, India), respectively. The chemical composition and ingredients of the basal diet are shown in Table 1.

Productive Performance and Carcass Traits

The overall mortality for each replicate was reported as the total mortality for all weeks. The body weight of 7, 21, and 42-day-old chicks and the total feed intake was recorded for each replicate. The overall increase in daily weight gain and total weight gain was determined at 42 d. Feed conversion ratio (**FCR**) was calculated as a total feed intake/total gain. Broilers were starved for 12 h before they were slaughtered, then weighed. Five birds from each replicate were then scalded, plucked, and eviscerated, then separately measured for weight and dressing inputs.

Abdominal fats were entirely obtained from the carcass in the pelvic and abdominal cavities and then weighed. The carcass was cut into breast, thigh (2 thighs

Table 1. Chemical composition and ingredients of the basal diet provided.

Items	Starter (1 d–21 d)	Finisher (22–42 d)
Ingredients		
Yellow corn %	54.10	58.7
Soya bean meal (44% protein) %	34.50	29.5
Corn germ (60% protein) %	5.50	5.50
Dicalcium phosphate %	2.00	1.75
Limestone %	1.08	0.95
Soya oil %	1.80	2.30
Nacl %	0.30	0.30
Lysine (98%) %	0.29	0.24
Methionine (88%) %	0.20	0.18
Premix %	0.30	0.30
Calculated analysis		
Crude protein %	23.12	20.99
Metabolizable energy (kca/kg)	3001	3180
Calcium %	0.99	0.89
Potassium %	0.54	0.52
Available phosphorus %	0.51	0.46
Digestible methionine + cysteine %	0.93	0.89
Digestible lysine %	1.43	1.24
Digestible methionine %	0.59	0.52
Digestible arginine %	1.25	1.07
Digestible tryptophan %	0.19	0.17

Minerals and vitamins premix manufactured by Multi Vita Animal Nutrition (Tenth of Ramadan City, Sharkia Governorate, Egypt) provides vitamin A 12,000 IU, vitamin D3 2,500 IU, vitamin E 20 mg, vitamin K3 2 mg, vitamin B1 2 mg, vitamin B2 5 mg, vitamin B6 2 mg, vitamin B12 0.05 ug, niacin 30 mg, biotin 0.05 ug, folic acid 1 mg, pantothenic acid 10 mg, manganese 60 mg, zinc 50 mg, iron 40 mg, copper 10 mg, iodine 0.6 mg, selenium 0.3 mg per 1 kg diet. DL-methionine (manufactured by Evonik Industries, Essen, Germany) and contains 99 % methionine. Lysine = lysine hydrochloride (Evonik Industries) and contains 70 % Lysine.

average weight), shoulder (2 shoulders average), and left fillet.

Serum and Tissue Biochemistry

At 42 d, two blood samples were independently collected in separate centrifuge tubes from the wing vein from 5 birds of each replicate. Sodium citrate solution (3.2%) was added to the first sample to determine phagocytic index and activity as described by Kawahara et al. (1991). The other blood sample was left to clot and then centrifuged at 4,500 × *g* for 15 min. Commercial kits were used to determine total lipids (**TL**), cholesterol (**CHO**), and triglycerides (**TG**) in the serum. Kits were purchased from BIO-Diagnostic Ltd, Giza, Egypt. The analyses were carried out as recommended by the manufacturer (Spectronic Corporation, Ivyland, PA). Serum samples were collected and preserved in a deep freezer at −20°C until the analysis.

To determine the activity of oxidative stress markers, including malondialdehyde (**MDA**), glutathione peroxidase (**GPx**), superoxide dismutase (**SOD**), catalase (**CAT**), and lactate dehydrogenase (**LDH**), ELISA Kits (Quanti Chrom, BioAssay Systems, Hayward, CA) were used. The acute protein levels in the serum (C-reactive protein; **CRP**), serum amyloid A (**SAA**), and transferrin (**TRF**) were measured using ELISA kits (Wuhan Fine Biotech Co., Ltd., Wuhan, Hubei Province, China). Levels of alanine aminotransferase (**ALT**), aspartate aminotransferase (**AST**), creatinine, and urea were measured using the BIO-Diagnostic kits. All procedures were carried out according to the manufacturer's instructions for each kit.

Meat sampling, storage, and extract preparation were carried out, as described by Folch et al. (1957). Meat TG and CHO levels were estimated using different Bio-Diagnostic kits. Immediately after slaughter, livers were weighed, and samples were mechanically homogenized in a phosphate-buffered saline solution (**PBS**) containing 0.05% sodium azide and 0.5% triton X-100 (Sigma—-Aldrich Chemie GmbH, Taufkirchen, Germany) (pH 7.4).

Homogenized samples were then sonicated for 10 min. After sonication, the samples were centrifuged for 10 min at 12,000 × *g* to remove all solid particles. Dichloromethane (0.4 mL) was then applied to 1 mL of the supernatant, followed by centrifugation for 10 min at 12,000 × *g*. The supernatant was then promptly removed and checked. According to the manufacturer's instructions, ELISA kits (Wuhan Fine Biotech Co., Ltd.) were used to determine the activity of CRP, SAA, and TRF.

Statistical Analysis

Data were performed using the SPSS program (Version 20.0, SPSS Inc., Chicago, IL). Two-way analysis of variance (**ANOVA**) was used to evaluate the effect of different treatments and their interactions on different measured parameters. The data were analyzed using general linear model (GLM) according to the following model:

$$Y_{ijk} = \mu + S_i + T_j + S_i * T_j + e_{ijk}$$

where; Y_{ijk} = observed value; Si = fixed effect of stocking densities (25, 30, 35, and 40 kg/m^2), Tj = treatment effect (control, 300 mg TTEO/kg feed and 300 mg LGEO/kg), S_i*T_j = the interaction between stocking densities and treatment; e_{ijk}= random error. The differences between means were compared by LSD at 5% level of probability.

RESULTS

Productive Performance and Carcass Traits

Data in Table 2 illustrated that increasing stocking density from 25 to 40 kg/m^2 significantly ($P < 0.0001$) reduced body weight at d 21 and 42 days of age and daily weight gain at different intervals. However, increasing the stocking density from 25 to 40 kg/m^2 did not significantly affect body weight on d 7 (Table 2).

On the other hand, the total FCR and mortality percentage were substantially ($P < 0.0001$) increased with increasing the stocking density from 25 to 40 kg/m^2 (Table 2). Nonetheless, total feed intake was significantly higher under the highest (40 kg/m^2) and lowest

Table 2. Effect of stocking density and supplementation with lemongrass essential oil (LGEO) or tea tree essential oil (TTEO), and their interactions on broilers' growth performance.

Effect		Body weight (g) D7	D21	D42	DWG (g)	TFI (g)	TFCR(g feed/ g gain)	Mortality %
Stocking density (SD)								
SD25 (25 kg/m^2)		42.40	940.24^{a}	$2,190.81^{a}$	51.18^{a}	$3,694.05^{a}$	1.72^{c}	1.80^{d}
SD30 (30 kg/m^2)		42.35	924.48^{b}	$2,178.29^{b}$	50.86^{b}	$3,678.67^{b}$	1.72^{c}	2.34^{c}
SD35 (35 kg/m^2)		42.41	897.48^{c}	$2,151.86^{c}$	50.23^{c}	$3,681.95^{ab}$	1.75^{b}	2.57^{b}
SD40 (40 kg/m^2)		42.48	845.95^{d}	$2,139.48^{d}$	49.92^{d}	$3,694.00^{a}$	1.76^{a}	2.99^{a}
SEM		0.12	2.34	2.84	0.07	4.36	0.003	0.01
Treatment (TR)								
Control		42.42	890.71^{c}	$2,157.18^{c}$	50.35^{c}	$3,692.36^{a}$	1.75^{a}	3.03^{a}
LGEO		42.38	902.89^{b}	$2,164.89^{b}$	50.54^{b}	$3,690.39^{a}$	1.74^{b}	2.27^{b}
TTEO		42.44	912.50^{a}	$2,173.25^{a}$	50.74^{a}	$3,678.75^{b}$	1.73^{c}	2.20^{c}
SEM		0.10	2.03	2.46	0.06	3.78	0.002	0.01
Interaction effect								
SD	TR							
SD25	Control	42.40	926.86	2,183.57	50.99	3,696.42	1.73	2.27^{ef}
	LGEO	42.34	937.43	2,188.14	51.09	3,707.14	1.73	1.67^{h}
	TTEO	42.44	956.43	2,200.71	51.40	3,678.57	1.71	1.60^{h}
SD30	Control	42.34	914.29	2,169.29	50.63	3,694.86	1.74	2.81^{c}
	LGEO	42.29	929.00	2,178.00	50.87	3,676.57	1.72	2.30^{def}
	TTEO	42.43	930.14	2,187.57	51.08	3,664.57	1.71	1.91^{gh}
SD35	Control	42.34	884.00	2,150.14	50.19	3,692.43	1.75	3.16^{b}
	LGEO	42.44	900.57	2,151.85	50.23	3,677.57	1.74	2.47^{de}
	TTEO	42.45	907.86	2,153.57	50.27	3,675.85	1.74	2.09^{fg}
SD40	Control	42.58	837.71	2,125.71	49.60	3,685.71	1.77	3.86^{a}
	LGEO	42.43	844.57	2,141.57	49.97	3,700.29	1.76	2.64^{cd}
	TTEO	42.44	855.57	2,151.14	50.20	3,696.00	1.75	2.46^{de}
SEM		0.20	4.06	4.93	0.12	7.55	0.005	0.11
P-value								
SD		0.8826	<0.0001	<0.0001	<0.0001	0.0225	<0.0001	<0.0001
TR		0.9021	<0.0001	<0.0001	<0.0001	0.0272	<0.0001	<0.0001
SD × TR		0.9948	0.3450	0.4399	0.4337	0.0607	0.3308	0.0144

Abbreviations: D7, body weight on day 7; D21, Body weight on day 21; D42, body weight on day 42; DWG, daily weight gain; TFI, total feed intake; TFCR, total feed conversion ratio.

[a-h]Means within a column with different superscripts are significantly different ($P < 0.05$).

(25 kg/m^2) stocking densities, followed by 35k g/m^2 density.

Concerning the impact of TTEO and LGEO on productive efficiency traits, the supplementation of TTEO in the broiler's diet produced significantly ($P < 0.0001$) the higher body weights and daily weight gain values followed by LGEO in comparison to the control treatment (Table 2). There were positive results for total feed intake, total feed conversion, and percentage mortality. The parameters described above were improved due to the dietary supplementation of TTEO or LGEO (Table 2). The mortality rate was significantly reduced by 27.4% and 25% when TTEO or LGEO were supplemented in the broilers feed, respectively (Table 2). These results in Table 2 demonstrated the benefit of TTEO over LGEO in improving the body weights at d 21 and 42 and daily weight gain. All performance traits were not significantly influenced by the interaction effect, except the mortality rate, which varied between the different supplements and had the same trend of the TTEO or LGEO effects ($P = 0.0144$).

Data in Table 3 demonstrated that dressing and left fillet percentages were significantly decreased with increasing stocking density from 25 to 40 kg/m^2 without significant difference between the 2 lowest stocking densities (25 and 30 kg/m^2) and the same for the 2 highest stocking densities (35 and 40 kg/m^2) (Table 3). However, all the other carcass characteristics were not significantly affected by increasing stocking density (Table 3).

The TTEO treatment showed a significantly higher dressing percentage than LGEO and the control treatments ($P < 0.0001$). Interestingly, LGEO did not significantly increase dressing percentage, liver or breast percentages for broilers (Table 3). Only breast and liver percentages for broilers under TTEO treatment were significantly higher than the control treatment. These results showed the superiority of TTEO over LGEO in improving some of the carcass characteristics (Table 3). On the other hand, the supplementation of TTEO or LGEO did not significantly affect the other carcass characteristics (gizzard, heart, spleen, abdominal fat, thigh, shoulder, and left fillet) compared to the control treatment without any supplementation (Table 3). The interaction effect on all carcass characteristics was not significant (Table 3).

Serum and Tissue Biochemistry

The highest stocking density (40 kg/m^2) significantly decreased the serum TL compared to 25 kg/m^2 and 30 kg/m^2 densities ($P = 0.0476$; Table 4). However, serum CHO and serum and meat TG were not significantly affected by stocking density levels (Table 4). Moreover,

Table 3. Effect of stocking density and supplementation with lemongrass essential oil (LGEO) or tea tree essential oil (TTEO), and their interactions on broilers' carcass characteristics at 42 days old.

Effect		DP	Liver	Gizzard	Heart	Spleen	Abdominal fat	Breast	Thigh	Shoulder	Left fillet
		Percentage of the slaughter weight									
Stocking density (SD)											
SD25 (25 kg/m^2)		69.90[a]	4.11	2.88	0.84	0.20	1.85	26.38	15.61	4.05	10.50[a]
SD30 (30 kg/m^2)		69.91[a]	3.98	2.90	0.86	0.21	1.89	25.73	15.19	4.31	10.74[a]
SD35 (35 kg/m^2)		68.60[b]	4.11	2.90	0.88	0.21	1.96	26.64	14.62	4.35	9.82[b]
SD40 (40 kg/m^2)		68.59[b]	4.14	2.93	0.87	0.21	1.82	26.71	14.87	4.31	9.95[b]
SEM		0.30	0.70	0.05	0.02	0.01	0.08	0.40	0.34	0.14	0.24
Treatment (TR)											
Control		68.61[b]	4.01[b]	2.85	0.86	0.22	1.92	25.68[b]	15.08	4.22	10.03
LGEO		68.82[b]	4.05[ab]	2.91	0.86	0.20	1.93	26.44[ab]	14.89	4.18	10.22
TTEO		70.32[a]	4.20[a]	2.95	0.87	0.20	1.78	26.98[a]	15.24	4.37	10.50
SEM		0.26	0.61	0.42	0.01	0.01	0.07	0.35	0.30	0.12	0.21
Interaction effect											
SD	TR										
SD25	Control	68.73	4.04	2.90	0.83	0.21	1.99	25.42	15.61	4.02	10.06
	LGEO	69.80	4.06	2.94	0.85	0.18	1.94	26.83	15.33	3.94	10.51
	TTEO	71.17	4.23	2.80	0.84	0.20	1.60	26.90	15.88	4.20	10.92
SD30	Control	70.26	4.02	2.93	0.89	0.22	1.94	26.23	14.96	4.57	11.12
	LGEO	68.47	3.94	2.80	0.82	0.21	1.91	24.68	15.07	3.89	9.99
	TTEO	70.99	3.97	2.97	0.87	0.19	1.83	26.28	15.55	4.46	11.12
SD35	Control	68.01	4.00	2.85	0.89	0.22	1.96	25.67	15.19	4.24	9.48
	LGEO	68.28	3.40	2.86	0.89	0.21	1.86	26.30	14.90	4.20	9.69
	TTEO	69.51	4.33	3.00	0.86	0.20	2.05	27.95	13.76	4.60	10.27
SD40	Control	67.44	3.97	2.74	0.83	0.21	1.80	25.39	14.57	4.03	9.44
	LGEO	68.72	4.20	3.02	0.88	0.21	2.02	27.93	14.26	4.68	10.71
	TTEO	69.60	4.26	3.03	0.92	0.21	1.63	26.80	15.76	4.21	9.69
SEM		0.51	0.21	0.08	0.03	0.02	0.13	0.70	0.58	0.24	0.41
P-value											
SD		0.0005	0.3591	0.9158	0.3198	0.7301	0.6116	0.3039	0.1899	0.4056	0.0222
TR		<0.0001	0.0738	0.3002	0.7719	0.4127	0.1965	0.0334	0.7006	0.4803	0.2761
SD × TR		0.0840	0.6352	0.1093	0.1775	0.9164	0.3292	0.0855	0.2965	0.1520	0.0855

Abbreviation: DP, dressing percentage.
[a,b]Means within a column with different superscripts are significantly different ($P < 0.05$).

the highest content of meat CHO was reported in 35 kg/m^2 stocking density, and the lowest value was for 40 kg/m^2 group (Table 4).

Data in Table 4 also showed that both the phagocytic index and activity were significantly higher at the lower stocking density (25 kg/m^2). In contrast, serum and liver SAA, TRF, and CRP were significantly ($P < 0.0002$) decreased with decreasing stocking density level (Table 4).

It is noteworthy that serum urea, creatinine, uric acid, LDH, ALT, AST, MDA, and CAT were directionally proportional to stocking density (Table 5). These parameters were significantly increased as the stocking density level rose from 25 to 40 kg/m^2. In contrast, there was a significant reduction in the GPx and SOD activity when stocking density increased from 25 to 40 kg/m^2 (Table 5).

The incorporation of TTEO in broilers' diet as a feed supplement significantly reduced the serum TL, serum and meat CHO, serum and meat TG, serum and liver SAA, TRF, and CRP compared to the LGEO supplement or the control treatment (Table 4). On the other hand, TTEO significantly increased the phagocytic index and activity compared to LGEO supplement or the control treatment (Table 4). The interaction effects were not significant except for the phagocytic index, serum SAA, liver SAAM, and serum CRP (Table 4).

The supplementation of TTEO in broilers' diet as a feed supplement significantly reduced the serum urea, creatinine, and uric acid compared to the supplementation of LGEO or the control treatment (Table 5). The TTEO group had the highest levels of the antioxidant enzymes, GPx, and SOD followed by the LGEO and then the control group (Table 5). The opposite was found for the LDH, ALT, AST, MDA, and CAT activities ($P < 0.0001$; Table 5). Also, creatinine, LDH, MDA, and SOD were significantly influenced by the interaction effect (Table 5).

DISCUSSION

The demand for broiler meat as a cheap alternative for beef has increased recently (Thaxton et al., 2006). Researchers are investigating ways to achieve the most significant amount of broiler meat from the smallest possible floor area to decrease production costs (Thaxton et al., 2006; Chmelnicna and Solcianska, 2007). Additionally, organic feed additives are being investigated to improve birds' immunity and productive performance (Popović et al., 2016). Accordingly, our current study was established to achieve this aim by using different stocking densities, incorporating TTEO or LGEO in broiler diets as a feed supplement, and measuring their effects on performance, carcass characteristics, immune responses, antioxidant reactions, as well as serum and meat constituents.

Table 4. Effect of stocking density and supplementation with lemongrass essential oil (LGEO) or tea tree essential oil (TTEO), and their interaction on different serum and liver biochemical parameters of broilers at 42 days old.

Effect		Serum TL (mg/dL)	Serum CHO (mg/dL)	Serum TG (mg/dL)	Meat CHO (mg/dL)	Meat TG (mg/dL)	Phagocytic Index (%)	Phagocytic activity (%)	Serum SAA (mg/l)	Liver SAA (mg/l)	SerumTRF (mg/l)	Liver TRF (mg/l)	Serum CRP (mg/l)	Liver CRP (mg/l)
Stocking density (SD)														
SD25 (25 kg/m^2)		586.02^{a}	152.08	147.71	106.40^{ab}	102.08	1.53^{a}	16.12^{a}	186.76^{d}	148.76^{d}	1.39^{d}	3.14^{b}	1.71^{c}	2.09^{d}
SD30 (30 kg/m^2)		579.59^{a}	153.51	143.33	106.85^{ab}	103.20	1.56^{a}	15.86^{a}	202.33^{c}	157.33^{c}	1.47^{c}	3.29^{b}	1.74^{c}	2.36^{c}
SD35 (35 kg/m^2)		568.36^{ab}	157.82	140.77	111.43^{a}	103.22	1.42^{b}	15.86^{a}	218.47^{b}	166.47^{b}	1.64^{b}	3.44^{a}	1.90^{b}	2.53^{b}
SD40 (40 kg/m^2)		541.03^{b}	154.21	144.75	100.05^{b}	102.94	1.38^{b}	15.47^{b}	257.09^{a}	185.33^{a}	1.79^{a}	3.47^{a}	2.37^{a}	2.74^{a}
SEM		11.93	2.65	3.24	3.22	2.89	0.02	0.13	2.12	1.04	0.02	0.04	0.03	0.04
Treatment (TR)														
Control		664.94^{a}	186.42^{a}	176.40^{a}	125.76^{a}	125.31^{a}	1.32^{c}	15.02^{c}	242.85^{a}	175.96^{a}	1.66^{a}	3.62^{a}	2.14^{a}	2.68^{a}
LGEO		570.16^{b}	150.24^{b}	139.18^{b}	105.88^{b}	102.09^{b}	1.49^{b}	15.87^{b}	210.46^{b}	165.89^{b}	1.56^{b}	3.29^{b}	1.89^{b}	2.36^{b}
TTEO		471.15^{c}	126.55^{c}	116.84^{c}	86.92^{c}	81.17^{c}	1.63^{a}	16.60^{a}	195.17^{c}	151.57^{c}	1.49^{c}	3.10^{c}	1.77^{c}	2.24^{b}
SEM		10.33	2.30	2.80	2.79	2.50	0.02	0.11	1.84	0.90	0.01	0.04	0.02	0.04
Interaction effect														
SD	TR													
SD25	Control	648.70	185.54	181.43	124.26	123.20	1.46^{cd}	15.43	210.57^{de}	158.71^{e}	1.46	3.29	1.89^{cd}	2.27
	LGEO	589.86	146.73	136.38	109.77	100.23	1.46^{cd}	16.20	178.86^{fg}	150.29^{f}	1.40	3.16	1.71^{e}	2.04
	TTEO	519.50	123.96	125.33	85.17	82.81	1.70^{a}	16.74	170.86^{g}	137.29^{g}	1.32	3.00	1.55^{f}	1.96
SD30	Control	668.46	186.62	177.03	124.89	126.33	1.40^{d}	15.17	221.14^{cd}	166.29^{d}	1.54	3.59	1.95^{c}	2.56
	LGEO	595.11	146.83	137.91	108.85	99.40	1.60^{ab}	15.57	202.28^{e}	159.86^{e}	1.47	3.21	1.65^{ef}	2.30
	TTEO	475.19	127.07	115.07	86.83	83.88	1.70^{a}	16.86	183.57^{f}	145.86^{f}	1.42	3.09	1.65^{ef}	2.21
SD35	Control	667.95	187.61	169.83	128.62	126.73	1.26^{e}	15.03	241.42^{b}	181.71^{b}	1.75	3.76	2.20^{b}	2.77
	LGEO	582.48	156.71	141.51	109.27	102.72	1.49^{bcd}	16.16	212.57^{de}	169.00^{d}	1.62	3.39	1.78^{de}	2.54
	TTEO	454.67	129.16	110.99	96.41	80.23	1.53^{bcd}	16.41	201.42^{e}	148.71^{f}	1.56	3.20	1.74^{e}	2.29
SD40	Control	674.66	185.94	177.31	125.29	125.00	1.17^{e}	14.46	298.28^{a}	197.14^{a}	1.92	3.87	2.53^{a}	3.16
	LGEO	513.19	150.68	140.94	95.64	106.03	1.42^{d}	15.57	248.14^{b}	184.43^{b}	1.78	3.43	2.44^{a}	2.56
	TTEO	435.24	126.02	115.99	79.24	77.78	1.57^{abc}	16.39	224.86^{c}	174.43^{c}	1.68	3.11	2.16^{b}	2.50
SEM		20.67	4.60	5.62	5.59	5.00	0.04	0.22	3.67	1.81	0.02	0.07	0.05	0.07
P-value														
SD		0.0476	0.4714	0.5005	0.1073	0.9912	<0.0001	0.0059	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
TR		<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
SD × TR		0.0754	0.9670	0.6916	0.7508	0.9014	0.0458	0.2475	<0.0001	0.0079	0.4537	0.0702	0.0159	0.0540

Abbreviations: CHO, cholesterol, CRP, C-reactive protein, SAA, serum amyloid A; TG, triglycerides; TL, total lipids; TRF, transferrin.
$^{a-d}$Means within a column with different superscripts are significantly different ($P < 0.05$).

Table 5. Effect of stocking density and supplementation with lemongrass essential oil (LGEO) or tea tree essential oil (TTEO) on antioxidant activity, liver, and kidney functions in 42 days old broilers.

Effect		Urea (mg/dL)	Creatinine (mg/dL)	Uric acid (mg/dL)	LDH (U/l)	ALT (U/l)	AST (U/l)	MDA (nmoles/mL)	GPx (U/gHb)	SOD (U/gHb)	CAT (nmoles/mL)
Stocking density (SD)											
SD25 (25 kg/m^2)		5.14^{c}	0.41^{d}	416.71^{d}	327.90^{d}	18.95^{d}	87.38^{d}	2.06^{d}	26.38^{a}	85.10^{a}	1.57^{d}
SD30 (30 kg/m^2)		5.18^{c}	0.47^{c}	424.38^{c}	337.05^{c}	20.52^{c}	92.57^{c}	2.19^{c}	24.33^{b}	82.29^{b}	1.86^{c}
SD35 (35 kg/m^2)		5.46^{b}	0.49^{b}	428.52^{b}	351.00^{b}	23.00^{b}	100.48^{b}	2.50^{b}	22.38^{c}	75.38^{c}	2.07^{b}
SD40 (40 kg/m^2)		5.62^{a}	0.55^{a}	434.23^{a}	372.28^{a}	24.71^{a}	104.62^{a}	2.83^{a}	20.29^{d}	68.81^{d}	2.23^{a}
SEM		0.34	0.01	1.26	2.69	0.30	0.81	0.04	0.35	0.60	0.30
Treatment (TR)											
Control		5.49^{a}	0.54^{a}	436.00^{a}	357.18^{a}	24.00^{a}	106.61^{a}	2.98^{a}	19.39^{c}	70.25^{c}	2.17^{a}
LGEO		5.31^{b}	0.47^{b}	425.57^{b}	348.64^{b}	21.82^{b}	94.29^{b}	2.23^{b}	24.00^{b}	77.86^{b}	1.91^{b}
TTEO		5.24^{b}	0.43^{c}	416.32^{c}	335.36^{c}	19.57^{c}	87.89^{c}	1.98^{c}	26.64^{a}	85.57^{a}	1.72^{c}
SEM		0.30	0.01	1.09	2.33	0.26	0.70	0.04	0.30	0.52	0.03
Interaction effect											
SD	TR										
SD25	Control	5.21	0.45^{e}	427.00	333.43^{def}	20.71	98.00	2.47^{de}	21.29	78.86^{de}	1.74
	LGEO	5.14	0.41^{fg}	416.00	330.57^{ef}	19.43	84.14	2.01^{fg}	27.86	84.71^{b}	1.54
	TTEO	5.05	0.38^{g}	407.14	319.71^{f}	16.71	80.00	1.69^{h}	30.00	91.71^{a}	1.41
SD30	Control	5.24	0.53^{cd}	433.29	346.86^{cd}	22.29	104.43	2.75^{c}	20.14	76.00^{ef}	2.07
	LGEO	5.21	0.46^{e}	426.57	332.71^{def}	20.86	91.00	2.00^{fg}	24.57	81.29^{cd}	1.91
	TTEO	5.09	0.42^{f}	413.29	331.57^{ef}	18.43	82.29	1.83^{gh}	28.29	89.57^{a}	1.59
SD35	Control	5.67	0.58^{ab}	439.57	361.71^{b}	25.71	110.29	3.10^{b}	18.86	67.43^{g}	2.31
	LGEO	5.34	0.46^{e}	427.00	350.57^{bc}	22.71	98.71	2.33^{e}	22.86	75.43^{f}	2.02
	TTEO	5.36	0.43^{ef}	419.00	340.71^{cde}	20.57	92.43	2.07^{f}	25.43	83.29^{bc}	1.88
SD40	Control	5.84	0.60^{a}	444.14	386.71^{a}	27.29	113.71	3.58^{a}	17.29	58.71^{h}	2.54
	LGEO	5.54	0.55^{bc}	432.71	380.71^{a}	24.29	103.29	2.57^{cd}	20.71	70.00^{g}	2.14
	TTEO	5.49	0.50^{d}	425.86	349.43^{bc}	22.57	96.86	2.34^{e}	22.86	77.71^{ef}	2.01
SEM		0.59	0.01	2.18	4.65	0.52	1.40	0.07	0.60	1.03	0.60
P-value											
SD		<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
TR		<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
SD × TR		0.1121	0.0143	0.7759	0.0399	0.4911	0.4429	0.0129	0.0681	0.0368	0.2609

Abbreviations: ALT, alanine transferase; AST, aspartate aminotransferase; CAT, catalase; GPx, glutathione peroxidase; LDH, lactate dehydrogenase; MDA, malondialdehyde; SOD, superoxide dismutase.

[a-h]Means within a column with different superscripts are significantly different ($P < 0.05$).

Increasing stocking density had adverse effects on broiler growth performance (Thaxton et al., 2006). Our results confirmed that the best body weight, weight gain, and FCR were obtained when broilers were housed at a stocking density of 25 kg/m^2 (Table 2). Our results were consistent with a previous study by Kryeziu et al. (2018), who examined the impact of stocking density on performance features at 3 levels (15, 18, and 22 chicks/m^2). They reported that 18 chicks/m^2 achieved the best results (Kryeziu et al., 2018). Additionally, Gholami et al. (2020) asserted that the highest body weight and weight gain were accompanied by the lowest stocking density (10 birds/m^2) and vice versa for the highest (20 birds/m^2) stocking density (Gholami et al., 2020). Our current study results can be attributed to a larger feeder space, allowing birds under low stocking density greater access to food and water with less anxiety. Broilers under the lowest stocking density of 25 kg/m^2 showed the lowest percentage of mortality. Our results were consistent with the former studies of Ferket and Gernat (2006), Heidari and Toghyani (2018), and Gholami et al. (2020).

The dressing and left fillet percentages were significantly decreased with increasing stocking density (Table 3). These results are in agreement with those of Li et al. (2019). The antioxidant enzyme activities (LDH, ALT, AST, MDA, and CAT) were also significantly reduced when the stocking density was decreased (Table 5). The SOD and GPx activities significantly (P < 0.05) declined side by side with increasing the stocking density level (Table 5). Our results are in agreement with those previously reported by Li et al. (2019).

The AST level is a key indicator of liver and muscle injury. Increasing levels of AST indicate cell damage (Nobakht and Fard, 2015; El Okle et al., 2018). Nevertheless, our results showed a rise in AST and ALT levels with increasing the stocking density (Table 5). Similar results were obtained by Gholami et al. (2020). This may be due to elevated densities creating difficulties for the birds to eat. Elevated density increases the risk of muscle injury and leads to increased liver enzyme concentration in the blood. Our data (Table 4) showed that the phagocytic index and activity were significantly reduced with increased stocking density. It is well known that high stocking densities are linked to inadequate immune response, as suggested by Heckert et al. (2002) who indicated that high stocking density causes immune suppression in broilers. Özbey et al. (2004) and Baghoyan (2006) also suggested that stress causes leukocyte destruction in poultry.

The supplementation of TTEO or LGEO in our study recorded a massive influence on the dynamic performance characteristics, as it improved body weights and daily weight gains (Table 2). Still, overall feeding and overall feed conversion were significantly reduced, and better outcomes were recorded when TTEO was supplemented than LGEO (Table 2). Khattak et al. (2014) demonstrated that the application of sufficient concentrations of TTEO in broiler chicken diets increased the average daily weight by about 7% and feed efficiency usage by about 6%. In comparison, feed intake was lowered by about 2% compared to the control group, with an augmented concentration of TTEO. Furthermore, Hashemipour et al. (2013) reported similar results for thymol and carvacrol EOs. Puvača et al. (2019) showed similar results for a new herbal tea based on thyme, rosemary, and oregano EOs. However, these findings could be due to the digestion-stimulating properties of the EOs active components. These active components could increase broilers' growth by influencing the equilibrium of the gut microbial environment and stimulating endogenous digestive enzyme secretion (Puvača et al., 2019).

Our results showed that the mortality percentages were significantly decreased when TTEO (27.4%) or LGEO (25%) were added to the broilers' diet, compared to the control treatment (Table 2). This confirms the superiority of TTEO over LGEO (Table 2). Similar findings previously attributed the positive effects of TTEO to its critical antimicrobial activity (Cross et al., 2007; Mumu and Hossain, 2018; Puvača et al., 2019).

Dressing and breast percentages were significantly better only when TTEO was supplemented to broiler diets (Table 3). Similarly, Khattak et al. (2014) reported an improvement in the carcass characteristics with the high doses of a natural blend of EOs than the control and the low doses.

EOs supplementation was reported to improve cellular immunity (Puvača et al., 2019) regarding the phagocytic index activity (Toghyani et al., 2012). Sadeghi et al. (2016) reported a positive impact on birds' immune responses after administering plant-derived material (Sadeghi et al., 2016). Our results showed a similar trend, where LGEO and TTEO significantly improved the phagocytic index and activity (Table 4), with TTEO being superior to LGEO (Table 4). It has been shown that broilers (Kirkpinar et al., 2011) and animals (Abdel-Latif et al., 2020) nourished on oregano-based diets had significantly lower CHO and TG levels than the control treatment in animals. In contrast, the impact of thyme and oregano EOs on TG levels had not been confirmed. Still, this disparity can be caused by a range of doses used in thyme, rosemary, and oregano EOs.

The introduction of TTEO in the broilers' diet in our study significantly increased GPx and SOD compared to the LGEO application (Table 5); however, the reverse was true for MDA. It is well-known that antioxidant enzymes are often used to assess oxidative damage (Jensen et al., 1997). Our results were in agreement with Li et al. (2019), who reported that the intake of herbs in chicken feed resulted in an increase in the activity of antioxidant enzymes and a decrease in MDA level. Also, our results were in line with Hashemipour et al. (2013) who reported that thymol and carvacrol supplementations significantly ($P < 0.05$) increased the activity of SOD and GPx and significantly ($P < 0.05$) decreased MDA in muscle, liver, and serum of broilers (Hashemipour et al., 2013).

Investigating the antioxidant activity of TTEO (*in vitro*), Zhang et al. (2018) indicated that it might be a beneficial natural-base resource for decreasing the oxidative stress in growing poultry. They also assessed the antioxidant activity by inhibiting 1,1-diphenyl 2-picrylhyorazyl (**DPPH**), nitric oxide (**NO**), and reactive oxygen species (**ROS**) in 5 mixed limonene, lavender, peppermint, eucalyptus, and TTEO. The TTEO and the eucalyptus oil had higher DPPH and NO levels relative to the other EOs. The TTEO displayed the highest ROS level of all EOs products examined (Zhang et al. 2018). Barbarestani et al. (2020) found that the dietary supplementation of lavender essential oil (**LEO**) at both levels (LEO300 or LEO600 mg/kg LEO) improved antioxidant status in serum and liver.

Our current study successfully incorporated either 300 mg/kg of TTEO or LGEO in broiler diets as a feed supplement. The supplementation of these 2 EOs reduced mortality rates, significantly improved body weights, daily weight gain, cellular immunity, and reduced serum total lipids, serum and meat cholesterol and triglycerides, SAA, TRF, and CRP.

In general, superior results were obtained using TTEO as a feed supplement in broiler diets compared to LGEO. Future work is required to determine the optimum concentration of TTEO to be used as a feed supplement in broiler diets.

CONCLUSION

It could be concluded that TTEO treatment offers a positive solution for raising poultry in high stocking densities. The TTEO supplement improved the growth performance traits, meat constituents and cellular immunity of broilers with a concurrent reduction in the serum and meat CHO and TG, serum and liver SAA and TRF. It was shown to be superior to LGEO in broiler diets.

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Ethics statement: The animal study was reviewed and approved by the Native Experimental Animal Care Committee and the Ethics Committee of the Animal Husbandry and Animal Wealth Development Department, Faculty of Veterinary Medicine, University of Damanhour, Egypt (DMU/VetMed-2019-/0145).

DISCLOSURES

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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