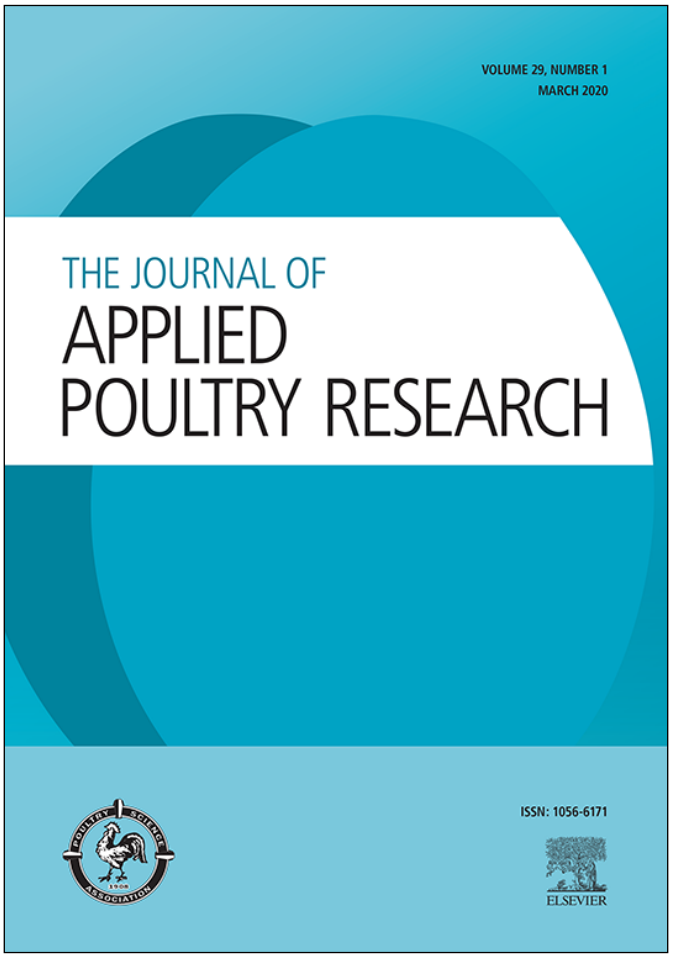

Effects of an Essential Oil Mixture Added to Drinking Water for Temperature-Stressed Broilers: Performance, Meat Quality, and Thiobarbituric Acid-Reactive Substances

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Primary Audience: Researchers, Broiler Breeder Companies, Nutritionists, Veterinarians and Food Engineers

SUMMARY

This study investigated the effects of an essential oil mixture (EOM; *Eucalyptus globulus* labill, *Tymus vulgaris*, *Cymbopogon nardus,* and *Syzgium aromaticum*) added to drinking water on temperature-stressed broilers. The performance parameters (body weight, average daily weight, feed intake, and feed conversion ratio), meat quality, and thiobarbituric acid-reactive substances (TBARS) were evaluated. In a 42-d study, 400 Ross-308 male chickens (1-d-old) were randomly assigned to 8 different groups ($n = 50$), each containing 4 subgroups ($n = 8$) (22°C Control (C), C + 250 mL/1,000 L, C + 500 mL/1,000 L, C + 750 mL/1,000 L), 36°C (stress control (SC), SC + 250 mL/1,000 L, SC + 500 mL/1,000 L, SC + 750 mL/1,000 L). Adding 750 mL/1,000 L at 22°C and 250 mL/1,000 L at 36°C was more beneficial to the fattening performance parameters than those in the control group. EOM reduced liver weight but increased abdominal fat in the SEOM-250 groups but did not affect other organ weights. EOM had no effect on the TBARS or the b^* color parameter while it augmented the a^* and L^* coordinates of meat color.

Key words: broiler, essential oil mixture, internal organ weight, TBARS

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DESCRIPTION OF THE PROBLEM

Stress causes physical and chemical changes in an organism by affecting the homeostatic balance of the organism [1]. One of the most important stress factors in poultry breeding is temperature. An increase in the ambient temperature of broilers above 35°C increases mortality and morbidity rates [2]. To this end, various feed additives have been used to prevent stress in poultry and to increase the quantity and quality of the products obtained. Antibiotics have been used for this purpose for over 50 yr. However, antibiotics are now banned from

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animal feeds by the European Union because their misuse has led to resistant bacteria, which has consequences on animal welfare, environment, food chain, and human health [3]. Consequently, research has begun to investigate new alternative agents that may be helpful to regulate the intestinal microflora in birds, increase fattening performance, and protect animal health. Numerous products have been used, but the increase in consumer preference toward organic products has increased interest in organic feed additives [4, 5].

One of these alternative feed sources is essential fatty acid derived from plants with medical and aromatic characteristics. These are volatile fatty acids. It was reported that there was no accumulation in the tissues of the animals, no drug resistance, or no risk for human health after use [6]. Essential fatty acids have antioxidant effects in monogastric animals [7, 8], as well as anti-microbial [9–11], anti-inflammatory [12, 13], anti-viral [14, 15], anti-tumoural [15], anti-fungal [16, 17] and anti-parasitic [18, 19] effects. In addition, it can be used as growth promoters in poultry by stimulating endogenous digestive secretions (enzymes, bile, and mucus) or by hold on to the intestine to affect epithelial structure in a beneficial way [20–22].

This study investigated the nutritional benefits of an essential oil acidic mixture (**EOM**) (*Eucalyptus globulus* labill, *Thymus vulgaris*, *Cymbopogon nardus,* and *Syzygium aromaticum*) added at different levels to the drinking water of temperature-stressed broilers (22 and 36°C, respectively). The animals' performance (live weight, daily live weight gain, feed consumption, and feed utilization rate), internal organ weight, and breast meat quality (meat color parameters and thiobarbituric acid-reactive substances [**TBARS**] values) were investigated.

MATERIALS AND METHODS

Birds, Diets, and Management

Four hundred 1-d-old Ross-308 male chickens were utilized in this study. The chickens were held in a 110 × 110 × 100 cm pen at the poultry unit of the Bayburt University Food, Agriculture and Animal Husbandry Application and Research Centre for 7 d of exercise and 35 d of fattening. The control, EOM-250, EOM-500, and EOM-750 groups at 22°C and the SC, SEOM-250, SEOM-500, and SEOM-750 groups at 36°C were used during the study period [23]. The basal diet feeds given in Table 1 were fed at the same time every day, and the new feeds were offered after the animals were weighed. Drinking water was removed at the same time every day and replaced with fresh water containing the EOM, which was provided from a commercial company in Ankara, Turkey. The feed used in this study was analyzed according to standard AOAC methods [24].

Table 1. Basal Diet Ration Nutrient Content and Analysis (g/kg).

Raw Material	Starter (0–14 d)	Grower (14–28 d)	Finisher (28–42 d)
Maize	52.70	54.60	58.12
Maize gluten feed	15.21	21.20	26.14
Soybean residue	26.35	18.90	10.65
Di-calcium phosphate	1.95	1.70	1.60
Calcium carbonate	1.18	1.10	1.04
Sodium chloride	0.31	0.31	0.31
Sodium bicarbonate	0.20	0.20	0.20
Salt	0.2	0.2	0.2
Methionine	0.50	0.50	0.44
Lysine	1.20	1.10	1.10
Vitamin–mineral premix[1]	0.20	0.20	0.20
ME (kcal/kg)	3100	3150	3225
Crude protein (%)	24	22	20
Crude oil (%)	2.61	2.30	2.50
Ash (%)	5.19	4.63	3.85
Moisture (%)	13.20	13.20	13.20

[1]The vitamin–mineral premix provided the following (per kg of diet): vitamin A, 12,000 IU; vitamin D3, 1,500 IU; vitamin E, 35 mg; vitamin K3, 5 mg; vitamin B1, 3 mg; vitamin B2, 4 mg; vitamin B6, 4 mg; vitamin B12, 0.03 mg; calcium-D-pantothenate, 15 mg; folic acid, 1 mg; niacin, 25 mg; D-biotin, 0.115 mg; Mg 80 mg; I, 0.15 mg; Co, 0.2 mg; Cu, 5 mg; Fe, 60 mg; Se, 1 mg; Zn, 60 mg.

EOM Composition

The EOM contained 26.70% durenol, 23.89% eugenol, 16.49% gamma terpinene, 8.35% hieptaethylene glycol, 6.42% hexaethylene glycol, 3.31% cymene, 3.08% pentaethylene glycol, 2.87% caryophyllene, 2.30% D-limonene, 2.18% betapinene, and 0.95% eucalyptol.

Internal Organ Weights

At the end of the experiment, 20 broilers were randomly selected from each group

Table 2. Effect of Essential Oil Mixture (EOM) Added to Drinking Water on Fattening Performance of Groups Fed in Stress Conditions.

		Body Weight (g)		Average Daily Weight (g)		Feed İntake (g)		Feed Conversion Ratio (g/g)	
	N	22°C	36°C	22°C	36°C	22°C	36°C	22°C	36°C
Control	100	1713.0	1804.2	48.94	51.55	95.22	93.02	1.95	1.81
EOM 250 mL/L	100	1715.2	1915.9	49.01	54.74	90.99	85.95	1.86	1.57
EOM 500 mL/L	100	1827.8	1450.6	52.22	41.45	84.79	77.54	1.63	1.88
EOM 750 mL/L	100	1904.6	1471.4	54.42	42.04	79.72	86.51	1.47	2.06
Source of variation (*P*-values)									
Diet		0.00**		0.00**		0.00**		0.19	
Temperature		0.00**		0.00**		0.46		0.07	
Temperature × Diet		0.00**		0.00**		0.26		0.00**	
Main effect means – Diet									
Control		1758.60^{a,b}		50.25^{a,b}		94.12^{a}		1.88^{a}	
EOM 250 mL/L		1815.56^{a}		51.87^{a}		88.47^{a,b}		1.72^{a}	
EOM 500 mL/L		1639.19^{c}		46.83^{c}		81.16^{b}		1.75^{a}	
EOM 750 mL/L		1688.00^{b,c}		48.23^{b,c}		83.12^{b}		1.76^{a}	
Main effect means – Temperature									
22°C		1790.15		51.15		87.68		1.73	
36°C		1660.52		47.44		85.76		1.83	
SEM		21.91		0.63		1.85		0.03	

$^{a–c}$Means within a column showing different superscripts are significantly different ($P < 0.05$): least significance difference test was applied to compare means.

* Significant at 0.05 level, ** Significant at 0.01 level, SEM = standard error of the mean.

(total = 160 broilers) and killed at the Laboratory of Bayburt University Food, Agriculture and Livestock Application and Research Centre. The liver, spleen, gizzard, and abdominal fat of the animals were excised and weighed (±0.001 g).

Meat Quality and Ethanol Antioxidant Properties

At the end of the trial, 160 animals (2 animals/group) were randomly selected, killed, and the breast was removed for analysis at the Bayburt University Department of Food Engineering. Color intensity (L*, a*, and b*) was measured in accordance with the specifications depicted by International Commission on Illumination CIELAB (Commision Internationale de L'e Clairage), which specializes in 3-dimensional measuring of color [25, 26]. According to these criteria, the following values signified different color intensities: L*; L* = 0 black, L* = 100 white (darkness/fairness); a*; a* = +60 red, a* = −60 green and b*; b* = +60 yellow, b* = −60 blue. The TBARS values were determined by a method developed by Tarladgis et al. [27] and modified by Lemon [28, 29].

Statistical Analysis

The parameters were all normally distributed, and the data are expressed as means and standard errors. A univariate general linear model was used to identify differences in feed intake [**FI**], feed conversion ratio [**FCR**], body weight [**BW**], average daily weight gain [**ADWG**], and meat color between the 2 temperature and 4 diet groups. Duncan's multiple comparison test was applied to compare differences between means. A P value <0.05 was considered significant [30].

RESULTS AND DISCUSSION

The performance data of the groups (BW, ADWG, FI, and FCR) and those of the EOM supplemented groups (250, 500, and 750 mL/1000 L) are given in Table 2. The groups exposed to temperature and diet stress effectively fattened in response to the EOM-750 mL/L and the (BW—ADWG and FCR) in the SEOM-250 mL/L group ($P < 0.05$).

In this study, the effect of the EOM mixture added at different doses (250, 500, and 750 mL/L) to drinking water of broilers under

Table 3. Effect of Essential Oil Mixture (EOM) on Meat Color Parameters and TBARS Added to Drinking Water of Groups Fed in Stress Conditions.

		L^*		a^*		b^*		TBARS	
	N	22°C	36°C	22°C	36°C	22°C	36°C	22°C	36°C
Control	40	44.07	38.55	6.05	5.49	17.29	12.81	1.33	1.23
EOM 250 mL/L	40	40.59	39.58	6.28	5.27	14.53	12.95	1.23	1.20
EOM 500 mL/L	40	40.26	40.94	6.68	6.37	14.65	13.96	0.95	1.02
EOM 750 mL/L	40	39.71	41.97	6.28	5.67	13.59	13.44	1.21	1.71
Source of variation (P-values)									
Diet		0.07		0.04*		0.00**		0.00**	
Temperature		0.01**		0.00**		0.00**		0.13	
Temperature × Diet		0.00**		0.71		0.00**		0.02*	
Main effect means – Diet									
Control		41.31[a]		5.77[b]		15.05[a]		1.28[a,b]	
EOM 250 mL/L		40.09[b]		5.78[b]		13.74[b]		1.21[b]	
EOM 500 mL/L		40.60[a,b]		6.52[a]		14.30[a,b]		0.98[c]	
EOM 750 mL/L		40.84[a,b]		5.98[a,b]		13.51[b]		1.46[a]	
Main effect means –Temperature									
22°C		41.16		6.32		15.01		1.18	
36°C		40.26		5.70		13.29		1.29	
SEM		0.23		0.15		0.21		0.05	

[a–c]Means within a column showing different superscripts are significantly different ($P < 0.05$): least significance difference test was applied to compare means.

* Significant at 0.05 level, ** Significant at 0.01 level, SEM = standard error of the mean.

temperature stress on the performance parameters was investigated. Exercise was effective for fattening the groups supplemented with EOM at a dose of 750 mL/L in the non-stressed groups and in the 250 mL/L stressed groups. According to the data obtained, the highest feed consumption was found in the K (control) group (95.22) and the lowest feed consumption was in the EOM-750 group (79.72) ($P < 0.00$), while 36°C stress group the highest feed consumption in the SK (control) group (93.02) and the minimum feed consumption in the SEOM-500 groups (77.54) was determined to be ($P < 0.05$). The data obtained for the stress-free and stressed groups were similar to some previous studies [31–33] but contrasted with others [34–37]. These discrepancies were likely due to differences in the composition, administration routes, and doses of the essential fatty acid mixture under study.

The rate of utilization of feed decreased as heat stress increased, and these negative effects decreased the benefits of adding the EOM 250 mL/L (1.57) dose to drinking water ($P < 0.01$) and the 750 mL/L (1.47) dose increased the FCR rate compared to the control group ($P < 0.00$). These effects in the stress-free and stressed groups corroborate some studies [32, 37] but not others [34, 36], which may also be explained by the effects of the EOM on the intestinal microflora.

The negative effects of heat stress decreased in the SEOM-250 group (1915.9 g) when ADWG (daily live weight gain) (54.74 g) was added to the drinking water and in the stressed groups when EOM-750 (1904.6 g) and ADWG (54.42 g) were added. These results in the stress-free and stressed groups were similar to some studies [32], but contrasted with others [31, 33, 34, 36]. It is thought that these discrepancies were due to differences in the composition, administration routes, and doses of the essential fatty acid mixture under study.

Tables 3 and 4 show the effects of adding EOM to drinking water of the broilers in the stressed and non-stressed groups. The broilers exposed to heat stress had reduced liver weight but normal gizzard, splenic, and visceral weights. No effects on TBARS or the b^* color coordinate of breast meat were observed in the heat-stressed animals, whereas a^* and L^* color values increased.

The color of meat is affected by lipid oxidation, myoglobin concentration, and hemoglobin pigment within the muscles. The color change in the meat depends on the amount of these

Table 4. Effect of Essential Oil Mixture (EOM) on Internal Organ Weights Added to Drinking Water of Groups Fed in Stress Conditions.

		Abdominal Fat (g)		Gizzard Weight (g)		Liver Weight (g)		Spleen Weight (g)	
	N	22°C	36°C	22°C	36°C	22°C	36°C	22°C	36°C
Control	40	27.3	35.36	26.79	29.06	33.49	41.73	1.28	1.46
EOM 250 mL/L	40	30.22	40.08	27.41	28.70	42.38	33.11	1.55	1.21
EOM 500 mL/L	40	32.64	23.20	26.45	24.81	39.84	29.72	1.52	1.06
EOM 750 mL/L	40	27.51	25.06	25.12	26.77	44.18	28.67	1.45	1.18
Source of variation (*P*-values)									
Diet		0.24		0.42		0.55		0.85	
Temperature		0.64		0.50		0.00**		0.01**	
Temperature × Diet		0.13		0.72		0.00**		0.05*	
Main effect means – Diet									
Control		31.33		27.92		37.61		1.37	
EOM 250 mL/L		35.15		28.06		37.74		1.38	
EOM 500 mL/L		27.92		25.63		34.78		1.29	
EOM 750 mL/L		26.28		25.94		36.42		1.32	
Main effect means –Temperature									
22°C		29.41		26.44		39.97		1.45	
36°C		30.92		27.34		33.30		1.23	
SEM		2.24		0.91		1.15		0.06	

* Significant at 0.05 level, ** Significant at 0.01 level, SEM = standard error of the mean.

pigments in the meat. It has been reported that poultry exposed to stress exhibit high post-cutting pH values [38, 39]. Broiler breast meat constitutes 5% of live broiler weight and is very susceptible to color deterioration [40]. In our study, there was an increase in the a^* color coordinate but no difference in the b^* color parameter of the breast meat in the stressed groups compared to the control group. Previous studies have reported that this difference is a consequence of reduced myoglobin oxidation by-products resulting from lipid oxidation [38, 39]. The brightness (L^*) of the breast meat decreased in the stress-free groups but increased in the stressed groups. This difference is due to the fact that the EOM-containing compounds added to drinking water have antioxidants with lipid oxidation inhibiting properties, which can change the luminal brightness value [41].

Because poultry meat is rich in highly unsaturated fatty acids, it has a higher rate of oxidative deterioration than other types of meat. The TBARS method is used to define the scale of rancidity (souring) that occurs as a result of autoxidation in fat and fatty parts of meat. TBARS values increase in parallel with the accumulation of short-chain fatty acids, which cause rancidity [42]. In our study, the phenolic compounds in the EOM mixture added to the drinking water have antioxidant properties [43, 44]. The phenolic hydroxyl (OH) groups of these phenolic compounds inhibit oxidation of unsaturated fatty acids leading to the formation of hydrogen peroxide, aldehydes, and ketones in the fatty tissues of the meat. Thus, it is possible to prevent changes in the pungency or taste of meat [45]. Studies have shown that some of the phenolic compounds in the EOM added to drinking water act like synthetic antioxidants [41] and reduce the amount of TBARS in tissues [42], thereby increasing antioxidant enzyme activities and inhibiting lipid oxidation [45]. We determined no difference between the TBARS values in the stressed groups and the control group after adding 750 mL/1000 L EOM to the broiler drinking water of the stressed groups. Although the meat color parameters and TBARS values were similar to some previous data [46], other authors have reported different findings [39, 43], which was thought to be due to the essential fatty acid contents administered to the animals.

Table 4 provides the weights of the internal organs of the chickens used in our study, including the intra-group and inter-group

statistical comparisons. Some studies have evaluated the effect of adding essential fatty acids at different feed concentrations to broilers under heat stress and found no effects [47–50]. In contrast, adding essential fatty acids to the feed of broilers under heat stress increases liver weight [51], highlighting the influence of the different EOM compounds. In our study, the EOM in the broiler drinking water decreased liver weight, but increased the abdominal fat in the 250 mL/1000 L group. The EOM had no effect on gizzard or splenic weights. The reason for this difference is thought to be caused by the effects of different compounds in the EOM mix, which is included in broiler rations.

CONCLUSION AND APPLICATIONS

1. The EOM had positive effects on the broiler performance parameters (BW, FI, and FCR).
2. Broiler liver weight decreased and abdominal fat increased in the SEOM-250 groups without affecting gizzard or spleen weight.
3. The TBARS and b^* color parameter of the breast meat were not affected by the added EOM, while it augmented the effect on a^* and L^* meat color coordinates.

İn order to achieve production at low costs, feed to increase performance and high quality furthers studies are required on anti-stress and antiooxidant features carrying EOM for the development of better nutritional strategies.

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23. On day 7 of the experiment, the animals were randomly assigned to 8 groups ($n = 50$) (22°C (K (control), EOM-250 (250 mL/1,000 L), EOM-500 (500 mL/1,000 L), EOM-750 (750 mL/1,000 L)), 36°C (SK (Stress Control), SEOM-250 (250 mL/1,000 L), SEOM-500 (500 mL/1,000 L), SEOM-750(750 mL/1,000 L)), each containing 5 subgroups ($n = 10$).

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29. Six ml of TCA solution (7.5% TCA, 0.1% EDTA and 50.1 1-Propil Gallat) was added to a 1-g ground sample of breast fillet. The mixture was homogenized with an Ultro-turrax for 20–30 s and was filtered through Whatman filter paper. A 1 mL aliquot of 0.02 M thiobarbituric acid was added to the filtered mixture. The mixture was held in a hot water bath for 40 min. Once cooled, the mixture was centrifuged for 5 min at 2,000 rpm, and absorbance values were measured at 532 nm (UV 160; Shimadzu, Tokyo, Japan). The TBARS values were reported in μmol malondialdehyde/kg.

30. IBM SPSS statistics 20.0 software (IBM Corp., Armonk, NY, USA) was used for the analyses.

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