Chapter

Investigation of the Effects of Some Herbal Extracts Used in Different Ratios on Meat Fatty Acid Profile Level in Experimental Heat Stress Created in Broilers

Emre Tekce, Bülent Bayraktar and Vecihi Aksakal

Abstract

Stress is the biological or external alteration of the organism against the factors that make it possible to achieve hemostasis or normal physiological balance. In our world, temperature increase due to climate change has become one of the most important stress factors in poultry sector. This research investigated the effects of essential oil mixture (EOM; *Eucalyptus globulus* Labill, *Thymus vulgaris*, *Cymbopogon nardus*, and *Syzygium aromaticum*) broilers adding to the drinking water under heat stress conditions. The fatty acid profile was evaluated. In a 42-day study, 400 Ross-308 male chickens (1-day-old) were randomly assigned to eight different groups (n = 50), each containing five subgroups (n = 10). As a result of the research, in stress-free groups 22°C rations of myristic acid ($C_{14:0}$), palmitic acid ($C_{16:0}$), stearic acid ($C_{18:0}$), oleic acid ($C_{18:1}$), linoleic acid ($C_{18:2n\text{-}6t}$), and Cis 11 eicosapentaenoic acid (C 20: 1n9) increased, whereas MUFA, UFA, and behenic acid ($C_{22:0}$) reduced. However, in stressed groups, 36°C rations of myristic acid ($C_{14:0}$), palmitic acid ($C_{16:0}$), stearic acid ($C_{18:0}$), and arachidonic acid ($C_{20:0}$) decreased, increased the UFA ratio, and had no effect on the MUFA and PUFA.

Keywords: essential mix oil, broilers, heat stress, water drinking, fatty acid profile

1. Introduction

For the world population to have a balanced and nutritious diet, it is essential that animal food, and products obtained from them, be consumed. Improving food quality, quantity of the products, and nutritional value is an important tool used to not only feed an ever increasing world population but also enhance health and longevity [1]. according to who do not exceed 30% of people's daily energy needs however, not more than % 10 of these oils should be saturated fatty acid (SFA) and % 3–7 polyunsaturated fatty acid (PUFA) [2]. Because of the metabolic diseases and disturbances associated with nutrition (cardiovascular diseases, low-density lipoprotein (LDL), arteriosclerosis, and diabetes), the human population brought about an increased interest for the functional foods [1, 3].

Stress is associated with endogenous (nutrition, rapid growth, sexual maturation period, and infection) and exogenous (climate, high-density insufficient ventilation) factors in living beings [4–6]. Heat stress is one of the most critical environmental factors in poultry all over the world. Stress disrupts the balance between oxidation and antioxidant defense systems, causing lipid peroxidation, protein structure, and consequently DNA oxidative damage [7, 8]. Stress responses in poultry occur mainly by activation of the hypothalamic pituitary adrenal axis and the orthotic nervous system. Furthermore, heat stress causes a series of physiological and metabolic changes in broiler chickens, such as high body temperature, rapid breathing, and respiratory alkalosis [8]. İn addition high ambient temperature, especially such as feed consumption, growth, immune system disorder, physiological effects show. Therefore, it has become necessary to develop alternative strategies and modulations in animal nutrition for animal feeding, disease prevention, and heat tolerance. Within this concept, antibiotics have been used in stockbreeding for over 50 years to protect animal health and increase the quality and quantity of the products. However, the European Union has banned the use of antibiotics as productivity enhancers because microorganisms have developed resistance against antibiotics and there is a risk of residue in the final products. Various alternative feed additives have been used to compensate for the production impacts of this ruling [9, 10]. In addition, a growing sensitivity toward human health, food safety, and environmental pollution has emerged among consumers despite the progresses in food production techniques and slaughterhouse hygiene in European Union countries [11, 12]. Therefore, many studies have been conducted recently with the aim of achieving organic products using natural feed additives as an alternative to synthetic additives.

In this context, people's interest in natural products has been increasing in recent years. At the beginning of these products are volatile fatty acids derived from medicinal aromatic plants. Primary metabolites (such as carbohydrate, fat, and protein) in medicinal plants have small molecule secondary metabolites (alkolites, essential oils, glycosides, flavonoids, and resins) that are not very important in plants, which are not vital in plants [13]. It has been stated that animals do not pose risks to human health after accumulation, drug resistance, and use in their tissues [14]. These essential oil fatty mix acids have been reported to have properties that include antimicrobial [15, 16], anti-inflammatory [17, 18], antiviral [19, 20], antitumoral [15], antifungal [21, 22], and antiparasitic [23, 24] effects. In addition, UYA obtained from medical aromatics plants; metabolic reactions and metabolism reactions in the organism and metabolism analysis is done in the body tissue quickly does not accumulate [25].

This study investigated the fatty acid profile 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).

2. Materials and methods

2.1 Animals, experimental design, and feeds

Four hundred 1-day-old Ross-308 male chickens were placed in a 110 × 110 × 100 cm pen in the poultry unit at the Bayburt University Food, Agriculture and Animal Husbandry Application and Research Center, during 7 days of exercise and 35 days of fattening. On day 7 of the experiment, the animals were randomly assigned to eight groups (n = 50) (C (Control), EOM-250 (22°C 250 ml/1000 L), EOM-500 (22°C 500 ml/1000 L), EOM-750 (22°C 750 ml/1000 L), SK (36°C Control), SEOM-250 (36°C 250 ml/1000 L), SEOM-500 (36°C 500 ml/1000 L),

SEOM-750 (36°C 750 ml/1000 L)), each containing five subgroups (n = 10). For each of the experimental periods, four treatments were prepared by supplementing the drinking water with 0 (control), 250 mL/1000 L, 500 mL/1000 L, and 750 mL/1000 L of EOM. During the study period was applied to the C, EOM-250, EOM-500 and EOM-750 groups at 22°C and SC, SEOM-250, SEOM-500 and SEOM-750 groups at 36°C. The nutritional content and ratios of broiler rations were shown in **Table 1**. Each experimental drinking water was offered to birds housed at either 22°C (normal temperature) or 36°C (heat-stressed conditions), and the birds with drinking water with 0 EOM were considered the control groups (22°C, positive control; 36°C, negative control). The feed used in this study was analyzed according to the standard AOAC methods [26].

2.2 EOM composition

The volatile oil of EOM (contained 26.70% durenol, 23.89% eugenol, 16.49% gamma-terpinene, 8.35% heptaethylene glycol, 6.42% hexaethylene glycol, 3.31% cymene, 3.08% pentaethylene glycol, 2.87% caryophyllene, 2.30% D-limonene, 2.18% beta-pinene, 0.95% eucalyptol) was provided from a commercial company in Ankara, Turkey.

2.2.1 Poultry house temperature, humidity, and illumination

The general temperature of the poultry house was maintained at 32–33°C during the first 2 days and at 27–28°C during the next 5 days. However, thereafter, a temperature of 36°C and humidity of 75–85% were applied to the groups subjected to

Raw material	Starter (0–14 days)	Grower (14–28 days)	Finisher (28–42 days)
Maize	52.70	54.60	58.12
Maize gluten feed	15.21	21.20	26.14
Soybean residue	26.35	18.90	10.65
Dicalcium 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

The vitamin-mineral premix provided the following (per kg of diet): vitamin A, 12000 IU; vitamin D3, 1500 IU; vitamin E, 50 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; Co, 0.2 mg; Cu, 6 mg; Fe, 60 mg; K, 0.75 mg; Mn, 80 mg; Se, 0.15 mg; Zn, 60 mg.

Table 1.
Composition and analyses of the basal diet (g/kg).

heat stress, and a temperature of 22°C and humidity of 55–60% to the other birds. Throughout the experimental period, all groups were housed under lighted (60 W) conditions constantly.

2.3 Lipid profile in meat and blood

Fatty acid composition of the 40 breast meat filets, 5 from each group, was measured using a gas chromatograph with flame ionization detector (GC-FID) provided from a commercial company in İzmir, Turkey. For the fatty acid and conjugated linolenic acid analysis of the collected samples, fat transmission to organic solvents within the samples was performed by homogenizing in chloroform:methanol mixture (2:1) using a homogenizer at 24,000 rpm. Samples were kept frozen until methylation, following Folch et al. [27]. Subsequently, 0.5 ml of the fat was taken from the frozen samples and placed in centrifuge tubes with 1 ml 2 N methanolic KOH solution and 7 ml n-heptane and then centrifuged at 5000 rpm for 10 min with a closed lid. The gas formed at the top of the centrifuge tube was collected and transferred to viola after being filtered with anhydrous Na_2SO_4. A capillary column (100 m, HP88) was used to separate the fatty acids in viola. Gas chromatography was measured using an automated injection GC-FID FID (HP-6890 N, Agilent).

2.4 Statistical analysis

The measures were all normally distributed and data are expressed as means and standard errors of the mean. Univariate general linear model was used to identify if differences existed in the fatty acid profile groups. Duncan multiple range tests were applied to identify differences among groups. All statistical tests were performed at 5% level of statistical significance by IBM SPSS statistics 20.0.

3. Results

Table 2 shows the levels of monounsaturated fatty acids (MUFA), polyunsaturated fatty acid (PUFA), saturated fatty acids (SFA), and total unsaturated fatty acids (UFA) within the fatty acid profiles of samples collected from breast meat filets of broilers fed at 22 and 36°C. EOM; (*Eucalyptus globulus* Labill, *Thymus*

	Fatty acid profile							
	SFA		MUFA		PUFA		UFA	
	22°C	36°C	22°C	36°C	22°C	36°C	22°C	36°C
Control	34.23	38.53	50.71	47.70	15.05	13.77	65.77	61.47
EOM 250 mL/L	38.48	35.60	43.17	51.15	18.14	13.26	61.31	64.41
EOM 500 mL/L	40.23	33.96	45.32	51.71	14.92	14.51	60.24	66.22
EOM 750 mL/L	33.10	34.52	52.12	50.00	14.76	15.48	66.89	65.48
Source of variation (*P*-values)								
Diet	0.00**		0.16		0.61		0.71	
Temperature	0.10		0.07		0.07		0.13	
Temperature × Diet	0.00**		0.01**		0.09		0.00**	
Main effect means diet								
Control	36.38[b]		49.21[ab]		14.42		63.62[a]	

	Fatty acid profile							
	SFA		**MUFA**		**PUFA**		**UFA**	
	22°C	**36°C**	**22°C**	**36°C**	**22°C**	**36°C**	**22°C**	**36°C**
EOM 250 mL/L	37.04[b]		47.16[a]		15.71		62.86[a]	
EOM 500 mL/L	37.10[b]		48.52[ab]		14.71		63.23[a]	
EOM 750 mL/L	33.82[a]		51.06[b]		15.12		66.18[b]	
Temperature								
22 °C	36.51		47.83		15.72		63.55	
36 °C	35.65		50.14		14.25		64.39	
SEM	0.32		0.77		0.49		0.36	

*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.*

Table 2.
Fatty acid profile of the experimental groups (7–42 days).

	Fatty acid profile													
	C14:0		**C16:0**		**C16:1**		**C17:0**		**C17:1**		**C18:0**		**C18:1**	
	22°C	**36°C**	**22°C**	**36°C**	**22°C**	**36°C**	**22°C**	**36°C**	**22°C**	**36°C**	**22°C**	**36°C**	**22°C**	**36°C**
Control	0.42	0.55	24.60	29.18	6.29	7.56	0.06	0.09	0.05	0.10	7.98	7.92	44.03	39.69
EOM 250 mL/L	0.67	0.51	28.26	28.03	6.56	7.84	0.14	0.09	0.07	0.09	7.95	6.51	36.15	42.92
EOM 500 mL/L	1.07	0.59	28.77	25.41	6.45	7.42	0.09	0.09	0.09	0.06	9.22	6.88	38.41	43.86
EOM 750 mL/L	0.38	0.30	25.29	25.58	7.34	7.02	0.07	0.09	0.06	0.06	6.72	7.02	44.39	42.56
Source of variation (*P*-values)														
Diet	0.00**		0.00**		0.91		0.18		0.61		0.08		0.11	
Temperature	0.00**		0.37		0.06		0.73		0.29		0.02		0.16	
Temperature × Diet	0.00**		0.00**		0.42		0.08		0.07		0.06		0.01**	
Main effect means diet														
Control	0.49[b]		26.89[b]		6.92		0.08		0.07		7.95[ab]		41.87[ab]	
EOM 250 mL/L	0.59[b]		28.15[c]		7.20		0.11		0.08		7.23[ab]		39.54[a]	
EOM 500 mL/L	0.83[c]		27.09[bc]		6.93		0.09		0.07		8.05[b]		41.13[ab]	
EOM 750 mL/L	0.34[a]		25.44[a]		7.18		0.08		0.06		6.87[a]		43.48[b]	
Temperature														
22 °C	0.63		26.73		6.66		0.09		0.06		7.97		40.74	

	Fatty acid profile													
	C14:0		C16:0		C16:1		C17:0		C17:1		C18:0		C18:1	
	22°C	36°C	22°C	36°C	22°C	36°C	22°C	36°C	22°C	36°C	22°C	36°C	22°C	36°C
36 °C	0.48		27.05		7.46		0.08		0.07		7.08		42.26	
SEM	0.02		0.24		0.26		0.01		0.01		0.23		0.69	

**Miristic: C14: 0, Palmitic: C16: 0, Palmitoleic: C16: 1, Heptadeconoic Acid (Margaric): C17: 0, Heptadesenoic: C17: 1, Stearic: C18: 0, Oleic: C18: 1, Linoleic: C18: 2n-6c, Linoleic: C18: 2n-6t, Arachidic: C20: 0, cis 11 Eicosapentaoic Acid: C 20: 1n9, Behenic: C22: 0, lignoceric: C24: 0. 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.*

Table 3.
Fatty acid profile of experimental groups.

	Fatty acid profile											
	C18:2n-6c		C18:2n-6 t		C20:0		C20:1n9		C22:0		C24:0	
	22°C	36°C	22°C	36°C	22°C	36°C	22°C	36°C	22°C	36°C	22°C	36°C
Control	14.49	13.39	0.56	0.38	0.31	0.32	0.34	0.35	0.28	0.17	0.56	0.30
EOM 250 mL/L	16.08	12.92	2.06	0.34	0.55	0.21	0.40	0.30	0.27	0.13	0.63	0.13
EOM 500 mL/L	14.47	14.15	0.44	0.35	0.35	0.26	0.38	0.37	0.16	0.25	0.57	0.50
EOM 750 mL/L	14.32	15.03	0.44	0.44	0.30	0.30	0.34	0.35	0.06	0.19	0.29	1.05
Source of variation (*P*-values)												
Diet	0.80		0.29		0.45		0.60		0.12		0.31	
Temperature	0.11		0.15		0.02*		0.25		0.75		0.85	
Temperature × Diet	0.16		0.24		0.05		0.22		0.01**		0.01**	
Main effect means diet												
Control	13.94		0.47		0.31		0.34		0.22^{b}		0.43	
EOM 250 mL/L	14.50		1.20		0.38		0.35		0.20^{ab}		0.38	
EOM 500 mL/L	14.31		0.40		0.31		0.38		0.21^{ab}		0.53	
EOM 750 mL/L	14.68		0.44		0.30		0.35		0.12^{a}		0.67	
Temperature												
22 °C	14.84		0.87		0.38		0.36		0.19		0.51	
36 °C	13.87		0.38		0.27		0.34		0.18		0.49	
SEM	0.38		0.22		0.03		0.01		0.02		0.08	

**Miristic: C14: 0, Palmitic: C16: 0, Palmitoleic: C16: 1, Heptadeconoic Acid (Margaric): C17: 0, Heptadesenoic: C17: 1, Stearic: C18: 0, Oleic: C18: 1, Linoleic: C18: 2n-6c, Linoleic: C18: 2n-6t, Arachidic: C20: 0, cis 11 Eicosapentaoic Acid: C 20: 1n9, Behenic: C22: 0, lignoceric: C24: 0. 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.*

Table 4.
Fatty acid profile of experimental groups.

vulgaris, *Cymbopogon nardus*, and *Syzygium aromaticum*) essential oil added at the drinking water in various dosages (250, 500, and 750 mL/1000 L). The broilers exposed to heat stress showed increased SFA and UFA compared to the control groups, while there is no statistically significant effect on PUFA and MUFA.

Tables 3 and **4** show the fatty acids profile influence of the addition of EOM to the drinking water of the broilers on the stressed and non-stressed groups. EOM; (*Eucalyptus globulus* Labill *Thymus vulgaris*, *Cymbopogon nardus* and *Syzygium aromaticum*) essential oil added at the drinking water in various dosages stressed groups 36°C rations of myristic acid ($C_{14:0}$), palmitic acid ($C_{16:0}$), stearic acid ($C_{18:0}$), and arachidonic acid ($C_{20:0}$) decreased, increased the UFA ratio, had no effect on the MUFA and PUFA.

4. Discussion

Fatty acid composition in broiler meat generally reflects the fatty acid profile in the rations. Thus, increased linolenic acid (n-3) levels in PUFA of the rations reduces saturated fat ratio in broiler carcasses. Particularly in monogastric animals, n-3 fatty acid added to the rations is stored in tissues without transformation within the ileum. Thus, with the change of broiler carcass fat compounds, omega 3 fatty acid, which is very beneficial for humans and plays an important role in preventing cardiovascular diseases, is maybe ingested with the broiler meat [28]. Some studies have shown that the main EOM components reduce SFA rate in serum and thigh meat samples linearly, i.e., MUFA, PUFA, n-3, and n-6 increase with increased EOM dosage, but do not affect breast meat samples [29], whereas other studies report that EOM dosage reduces SFA and PUFA in breast and thigh meat but increases MUFA rate [30]. Studies have shown that the increase in unsaturated fatty acid in broiler rations produces increased oxidation in the meat, whereas this oxidation is hampered by EOM alkaloids [31]. Studies on rats have reported that adding thyme and thymol increases PUFA rate within the brain; DHA, which is found in brain tissues; microsomes; mitochondria; and synaptic vesicles and affects behavior, memory, and motor skills, in comparison to control group, and that free radicals are inclined to increase with age [32].

Although saturated and unsaturated fatty acid levels in abdominal and subcutaneous tissues of heat-stressed broilers decrease, fatty acids in intramuscular tissues are not affected [33]. On the other hand, palmitic acid ($C_{16:0}$), a saturated fatty acid, increases under heat stress and palmitoleic acid ($C_{16:1}$) and linoleic acid ($C_{18:2n\text{-}6c}$), unsaturated fatty acids, reduce [33]. Furthermore, oleic acid ($C_{18:1}$) decreased, whereas linoleic acid ($C_{18:2n\text{-}6c}$), linolelaidic acid ($C_{18:2n\text{-}6t}$), and PUFA increased in heat-stressed broilers [34]. Compared to cows and pigs, chicken saturated and unsaturated fatty acid rates in intramuscular tissues are higher [35]. The current study on breast meat samples collected from groups without heat stress showed decreased MUFA and UFA ($P < 0.05$), while SFA increased compared to the control groups and there is no statistically significant effect on PUFA ($P > 0.05$). Groups with heat stress showed increased SFA and UFA ($P < 0.05$) compared to the control groups, while there is no statistically significant effect on PUFA and MUFA ($P > 0.05$).

The current study is consistent in terms of the effects of EOM dosage on saturated (SFA) and unsaturated (MUFA, PUFA, and UFA) fatty acids with some previous studies [29, 32, 34] but inconsistent with some others [30, 33]. This is probably due to lipid peroxidation being prevented by EOM in the fatty acids, which are highly sensitive to lipid oxidation, and other variations, such as time, method, and dosages of EOM addition to the water drinking and other differences in experimental materials and methods.

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