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Antioxidant properties of the fennel essential oil nanoemulsion: Effect on European production efficiency factor, blood metabolites, immune system and cecal microbial population of heat stressed broiler chickens

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Abstract In this study, the antioxidant properties of fennel essential oil nanoemulsion (FEON) and its effect on European production efficiency factor, blood metabolites, immune system, and cecal microbial population of broiler chickens under heat stress conditions were investigated. Two hundred one-d-old Ross broiler chickens were arranged in a completely randomized design with 5 treatments, 4 replicates, and 10 birds each. The birds were fed on a basal diet (control), basal diets supplemented with 50, 100, and 200 mg kg^{-1} fennel essential oil nanoemulsion (FEON50, FEON100, and FEON200) and 200 mg kg^{-1} fennel essential oil (FEO). The results showed that compared with FEO, all concentrations of FEON had greater antioxidant activity (P≤0.001). During the starter, grower, and the whole phase of the rearing period, the performance indicators such as the European production efficiency factor and European broiler index, were not affected by dietary treatment. Further, blood total cholesterol and low-density lipoprotein contents were significantly decreased by supplementation of broiler diets with FEON (P≤0.01). Primary antibody titer against sheep red blood cells (SRBC) was affected by the addition of FEO and FEON50 into broiler diets (P≤0.05). Moreover, the *Lactobacillus* population of cecal content was significantly increased and coliform populations were decreased when FEON was added to the diet. The *Escherichia coli* population of cecal content decreased when FEO and FEON were supplemented into the diets (P≤0.05). The jejunal, ileal, and cecal digesta pH decreased significantly in the FEON50 treatment. The results of this experiment showed that under heat stress conditions, supplementation of broiler chickens' diets with FEON can enhance primary humoral immunity and improve the broiler gut health and FEON50 was the optimal level.

Keywords: broiler, gut microbiota, heat stress, plant secondary metabolites

Introduction

Heat stress is one of the major problems of the poultry industry in countries in tropical regions. Such conditions can adversely affect the growth rate, feed efficiency, mortality, and welfare of poultry, and consequently economic profitability (Wang et al., 2008). Generally, heat stress can indu-

ce oxidative stress and negatively affect poultry performance. Oxidative stress occurs when the balances between free radical production and antioxidant defenses become impaired. Such a situation is also associated with damage to a wide range of molecular species, including proteins, lipids, and nucleic acids.

Antioxidants are stable molecules donating an electron to free radicals to reduce their damaging potential. Many free radicals are scavenged by several body enzymatic systems, with a non-enzymatic component such as micronutrient that have vital roles in this respect. Food plays an important role in protecting the oxidative system via the non-enzymatic system as well as phytochemicals, such as phenolic compounds, carotenoids, and vitamins E, and C (Brenes and Roura, 2010; Gharaghani et al., 2015). Previous studies have also shown that diet fortification with phytochemicals could reduce the harmful effects of oxidative stress and improve birds' feed intake (Wang et al., 2008). The use of medicinal plant component in poultry diets not only can improve their performance parameters but also affects the poultry products' quality and consumers' health (Gharaghani et al., 2015).

Fennel (*Foeniculum vulgar)* is an important herbal plant widely used as digestive, carminative, lactogogue, and diuretic medicine as well as a treatment for gastrointestinal and respiratory disorders (Rather et al., 2016). Fennel has different biological effects on poultry, including improved growth performance and egg quality, higher immune cell proliferation, and reduced oxidative stress (Khan et al., 2022). It has been reported that major constituents of fennel essential oil (FEO), including trans-anethole, fenchone, and estragole, can stimulate appetite and improve digestion and absorption of nutrients (Tollba and Hassan, 2003). In addition, according to the literature, supplementing the laying hens' diets with fennel extract could alleviate the side effects of carbon tetrachloride (a liver toxin, that increases the formation of reactive free radicals) by scavenging oxidants in the injured liver to improve liver health and egg production (Hadavi et al., 2017).

On the other hand, essential oils (EOs), as natural compounds, are used in poultry diets for their strong antioxidant, antifungal, and antibacterial activities. Although EOs have been shown to be promising alternatives to chemical compounds (antioxidants and antibiotics) in animal nutrition, they have presented special limitations, such as high volatility, low water solubility, strong odor, and chemical as well as physical instability. These features have made it difficult to include EOs in the diet (Amaral and Bhargava, 2015). Nanotechnology is one of the options to overcome these limitations. AbdEl-Hack et al. (2022) showed in a comprehensive review that nanoemulsions are carrier systems that can overcome the volatile nature of EOs and improve their bioavailability. In this regard, it was reported that the solubility and bioavailability of EOs in nanoemulsions is high (Calo et al., 2015) and they can exert more positive effects than bulk EOs. Therefore, a smaller amount of EOs is used which is more cost-effective. To the best of our knowledge, the application of EOs nanoemulsions has not been reported yet. Hence, in this research, we intended to evaluate the effect of different concentrations of FEON on heat-stressed broilers and compare them with bulk EO.

Materials and methods

Preparation of Nanoemulsion

Fennel EO was purchased from Barij Essence, Iran. For the preparation of nanoemulsion, all emulsions were prepared using two-stage homogenization. The primary coarse emulsion was first prepared using a high shear homogenizer so that EO, distilled water, and Tween 80 (1:8:1) were thoroughly mixed at 13,000 rpm using a high shear homogenizer for 3 min. Next, nanoemulsions were made by sonicating coarse emulsions (*Hielscher*, UP 200, Germany) for 1 min (Jafari et al., 2007). Then, the droplet size of microemulsions was measured using a dynamic light scattering method. To avoid multiple scattering, all samples were diluted using double distilled water. Furthermore, all measurements were made after overnight storage of samples at room temperature (Barzegar et al., 2018). After that, the antioxidant properties of FEONs and pure essential oil were measured using Keceli and Gordon's method (Keceli and Gordon, 2001). Briefly, 0.1 mL nanoemulsion or pure essential oil was added to 2.9 mL methanolic solution of 0.1mM 1, 1-diphenyl-2-picrylhydrazyl. After vortexing for 30 seconds, the mixture was kept in a dark place for 30 min and afterward the absorbance was measured at 517 nm. The scavenging properties of solutions were measured as:

$$\text{Scavenging properties} = (A_{blank} - A_{sample}/A_{blank}) \times 100$$

Where A_{blank} and A_{sample} are the absorbances of blank and samples at 517 nm.

Birds and housing

The present study was approved by the Animal Science Committee in the Agricultural Sciences and Natural Resources University of Khuzestan, Ahvaz, Iran. Two hundred one-d-old Ross 308 strain broiler chickens (mixed sexes) were used in a completely randomized design with five treatments, and four replicates for 42 days. Birds were fed a corn-soybean meal-based diet supplemented with 0, 50, 100, and 200 mg kg^{-1} FEON (Control, FEON50, FEON100, and FEON200) and 200 mg kg^{-1} fennel essential oils (FEO) as on top. The composition of the basal diets is shown in Table 1. At the beginning of the experiment, the mean body weight of all the replicates was kept similar. At the start of the experiment, the broiler's house temperature was 30°C. Then, every week, the temperature was lowered to the -

extent of 3°C until it reached 24 °C on day 21. After 3 weeks of age, the birds of all treatments were exposed to heat stress as suggested by Niu et al. (2009).

Table 1. The ingredients and chemical composition of the basal diets for broiler chickens

Item (gkg^{-1})	Starter diet (days 1-21)	Grower diet (days 22-42)
Corn	552.4	609.4
Soybean meal	377.0	318.0
Sunflower oil	30.0	36.0
Oyster shell	11.5	14.5
Sodium bicarbonate	2.3	2.3
Methionine	2.3	1.0
Dicalcium phosphate	16.0	11.5
Sodium chloride	3.0	1.8
Vitamin premix[1]	2.5	2.5
Mineral premix[2]	2.5	2.5
Coccidiostat	0.5	0.5
Calculated analysis		
ME[3] ($kcal\ kg^{-1}$)	2976	3086
Crude protein	214	192
Dry matter	898	898
Calcium	9.0	9.0
Available phosphorus	4.5	3.5
Methionine + Cystine	9.1	7.2

[1,2]Vitamin and mineral premix supplied the following per kilogram of diet: retinyl acetate, 1.5 mg; cholecalciferol, 0.025 mg; α-tocopheryl acetate, 20 mg; menadione, 2 mg; thiamine, 3 mg; riboflavin, 6 mg; cyanocobalamin, 0.016 mg; niacin, 15 mg; folic acid, 1.75 mg; pantothenic acid, 15mg; choline chloride, 250 mg; Mn, 120 mg; Zn, 100 mg; Cu, 16 mg; Se, 0.3 mg; and I, 1.25 mg.
[3]Metabolizable energy

Broilers in each pen were weighted weekly (at 7, 14, 21, 28, 35, and 42 days of age) and total body weight (TBW), body weight gain (BWG), and daily weight gain (DWG) were measured and calculated for starter (1-21 days), grower (22-42 days), and total period (1-42 days). Feed intake (FI) and feed conversion ratio (FCR) were measured weekly and calculated for starter, grower, and total periods. Broiler performance indicators such as European Broiler Index (EBI) and European Production Efficiency Factor (EPEF) were calculated according to the following equations (Kryeziu et al., 2018).

FCR (kg feed/kg gain) = Cumulative feed intake (kg) /Total weight gain (kg)
ADG (average daily gain) = TWG/days of the growth period
Viability (%) = 100- Mortality %
EBI= [Viability (%) x ADG (g per bird per day)]/ [FCR (kg feed/kg gain) x 10]
EPEF= [Viability (%) x BW (kg)] / [FCR (kg feed per kg gain) x age (day)] x100

To specify blood parameters at the end of the experiment, two chicks per pen were randomly selected and blood samples were taken from their brachial vein into sterilized tubes and serum was obtained by centrifuging the tubes (Hk 36, Hermle, Wehingen, Germany) at 3,000 rpm for 15 min. The sera were used for the colorimetric determination of blood sugar, triglyceride, total cholesterol, and high-density lipoprotein (HDL-C) using commercial kits (Pars Azmoon, Iran) according to the manufacturer's protocols (Autoanalyzer, Alison 300, Abbott, USA). Low-density lipoprotein (LDL-C) was calculated by the following equation (Friedewald et al., 1972).

LDL-C= TC-(HDL-C+TG/5)

On days 21 and 35 of the rearing period, two chicks per pen were randomly selected and 1 mL of 25% sheep red blood cell (SRBC) was injected into their breast muscle. Seven days after each injection, blood samples were taken from the brachial vein, and serum was obtained and stored at –20 °C until being assayed for antibody responses against SRBC by hemagglutination (HA) method as previously described by Yamuna and Thangavel (2011).

At the end of the experiment, one bird per cage that had not been injected with SRBC was randomly selected and killed and the digesta samples were collected from jejunum, ileum, and cecum, and mixed with distilled water (1:10), vigorously vortexed, and the samples' pH was measured using a standard pH meter (Izat et al., 1990).

At the end of the experiment, one bird per cage that had not been injected with SRBC was randomly selected and aseptically killed and one gram of cecal content was collected. The microbial population of the collected cecal content was also determined by serial dilution of cecal samples. *Lactobacillus* sp., *Escherichia coli,* and coliforms were cultured on Rogosa SL agar, eosin methylene blue agar, and MacConkey agar, respectively (Ghorbani et al., 2014).

Statistical analysis

The data were analyzed using the GLM procedure of SAS software. Differences between treatment means were tested using the Duncan's multiple range test, and statistical significance was declared at $P \leq 0.05$.

Results

The droplet size of FEON is shown in Fig. 1. The mean particle size in FEON50, FEON100, and FEON200 was 45, 152, and 186 nanometers, respectively. The scavenging capacity of different levels of FEON is shown in Table 2. In comparison with FEO (5 mg kg^{-1}), all concentrations of FEON (1.25, 2.5, and 5 mg kg^{-1}) had greater antioxidant activity (P≤0.001). Among FEON concentrations, the greatest antioxidant activity was associated with 5 mg kg^{-1} essential oil nanoemulsions.

The results of EBI and EPEF are shown in Table 3. European Broiler Index, EPEF, and FCR were not significantly affected by experimental treatments in any rearing periods. Although, in the starter period, the EBI and EPEF were increased numerically by supplementat-

ion broiler diets with FEON50 and FEON100 (P=0.09).

Table 4 shows that supplementing the broiler diet with FEON significantly decreased the serum total cholesterol content compared to control and FEO groups (P≤0.05). Low-density lipoprotein was decreased significantly with supplementation of the diet with FEO and the levels of 100 and 50 mg kg^{-1} of FEON (P≤0.05). Glucose, triglyceride, and HDL-C contents of serum were not significantly affected by treatments.

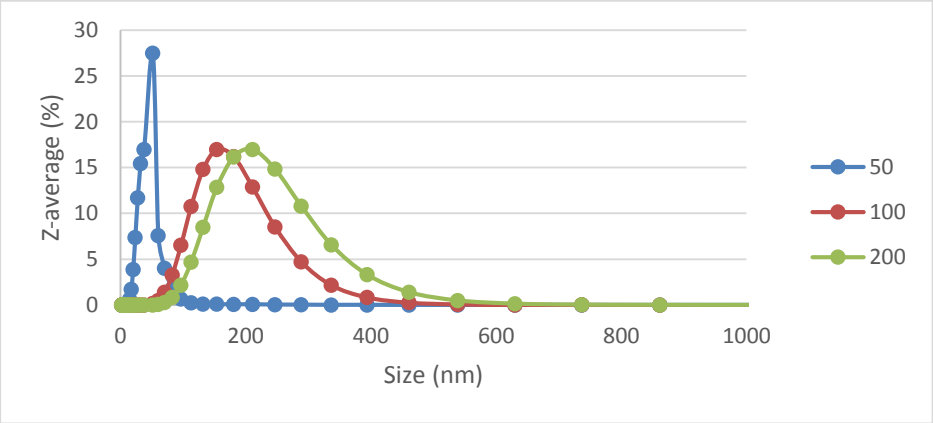

Figure 1. The droplet size of fennel essential oil nanoemulsion prepared at different concentrations (50, 100 and 200 mg kg^{-1})

Table 2. Antioxidant activity of the fennel essential oil and different levels of fennel essential oil nanoemulsion (%)

Treatment[1]	1	2	3	4	SEM[2]	P-value
Antioxidant evaluation	45.14	37.33	35.27	20.68	3.37	0.0005

[1] Refers to 5 ppm fennel essential oil nanoemulsion (FEON), 2 refers to 2.5 ppm FEON, 3 refers to 1.25 ppm FEON, and 4 refers to 5 ppm fennel essential oil
[2] Standard error of the mean.

Table 3. The effect of experimental treatments on EPEF, EBI, and FCR in broiler chickens

	European Production Efficiency Factor (EPEF)			European Broiler Index (EBI)			Feed conversion ratio (g of feed: g of gain)		
Treatment[1]	Starter (1-21 d)	Grower (22-42 d)	Total period (1-42 d)	Starter (1-21 d)	Grower (22-42 d)	Total period (1-42 d)	Starter (1-21 d)	Grower (22-42 d)	Total period (1-42 d)
Control	228.1	334.9	269.1	213.0	300.4	236.2	1.35	1.87	1.70
FEON50	254.0	389.9	312.3	239.5	349.9	272.7	1.32	1.80	1.65
FEON100	261.0	358.6	284.9	242.4	319.7	247.7	1.28	1.85	1.66
FEON200	230.9	348.5	268.5	214.6	309.8	228.8	1.33	1.88	1.70
FEO	221.9	425.9	304.8	204.0	378.5	263.9	1.41	1.73	1.62
SEM[2]	5.59	16.94	8.14	5.59	15.02	7.57	0.01	0.03	0.02
P-value	0.09	0.48	0.31	0.09	0.49	0.34	0.15	0.61	0.79

[1] Control (basal diet); FEON50= Basal diet plus 50 mg kg^{-1} fennel essential oil nanoemulsion; FEON100= Basal diet plus 100 mg kg^{-1} fennel essential oil nanoemulsion; FEON200= Basal diet plus 200 mg kg^{-1} fennel essential oil nanoemulsion; FEO = Basal diet plus 200 mg kg^{-1} fennel essential oil.
[2] Standard error of the mean.

By adding FEO and FEON50 to the diet, the primary antibody titer against SRBC was increased compared to control and FEON200 groups (P≤0.01); however, secondary antibody titer and relative weights of lymphoid organs were not affected by various concentrations of FEON as well as FEO (Table 5).

Supplementation of the diet with FEON50 tended to increase the *Lactobacillus* sp. count (P=0.06). *Escherichia coli* and coliform populations were decreased significantly by the experimental treatments (P≤0.05). Supplementing the diet with FEON50 decreased the pH of the digesta in jejunum, ileum, and cecum content compared to FEO (P≤0.05, Table 6).

Discussion

During the production of nanoemulsion, the droplet size increased from 45 to 186 nm when the concentration of EO increased at the same preparation condition and surfactant concentration (Figure 1). The figure also shows that the distribution of all nanoemulsion droplets was monomodal and polydispersity increased by raising

the EO concentration. The increases in droplet size as well as polydispersity index may be due to a lower concentration of surfactant to cover newly formed droplets at higher oil concentrations. In the high energy method, for preparing nanoemulsions, after droplet formation, the surfactant must move toward the droplet and cover its surface. By increasing the EO concentration, more droplets will be formed, but there isn't enough surfactant to cover their surface, and consequently bigger droplets will be formed due to the coalescence of newly formed droplets (Jafari et al., 2007; McClements and Rao, 2011).

It was observed that scavenging capacity increased from 21 to 45% for the treatment with 5 mg kg^{-1} FEON when compared to 5 mg kg^{-1} FEO. The increased antioxidant properties of nanoemulsions may be due to the increase in droplet numbers, and consequently, the increase in surface-to-volume ratio. It was reported that nanomaterials can transport and protect EOs from degradation, and improve their stability (AbdEl-Hack et al., 2022).

Table 4. The effect of experimental treatments on serum parameters (mg/dL) of broiler chickens at day 42

Treatment[1]	Glucose	Triglyceride	Cholesterol	HDL[3]	LDL[4]
Control	237.00	42.175	153.66^{a}	86.73	35.33^{a}
FEON50	227.75	46.75	128.33^{b}	93.30	20.33^{c}
FEON100	230.75	47.00	128.66^{b}	94.23	26.00^{bc}
FEON200	251.50	56.25	126.33^{b}	95.30	29.00^{ab}
FEO	241.00	58.00	156.33^{a}	111.20	27.33^{bc}
SEM[2]	3.52	3.98	438	2.97	1.47
P-value	0.13	0.76	0.01	0.19	0.009

[1] Control (basal diet); FEON50= Basal diet plus 50 mg kg^{-1} fennel essential oil nanoemulsion; FEON100= Basal diet plus 100 mg kg^{-1} fennel essential oil nanoemulsion; FEON200= Basal diet plus 200 mg kg^{-1} fennel essential oil nanoemulsion; FEO = Basal diet plus 200 mg kg^{-1} fennel essential oil.
[2] Standard error of the mean. Within the column, means with a common letter (s) do not differ (P>0.05).
[3] HDL-C: High-density lipoprotein.
[4] LDL-C: Low-density lipoprotein.

Table 5. The effect of experimental treatments on immune responses in broiler chickens

	Response to SRBC[3] (log2)		Lymphoid organs (% of live weight)	
Treatment[1]	Day 28	Day 42	Spleen	Bursa of Fabricius
Control	3.12^{c}	7.16	0.10	0.21
FEON50	5.71^{ab}	7.57	0.13	0.19
FEON100	4.12^{bc}	7.57	0.11	0.18
FEON200	4.62^{abc}	7.57	0.11	0.17
FEO	6.71^{a}	8.20	0.11	0.20
SEM[2]	0.36	0.20	0.01	0.01
P-value	0.01	0.29	0.96	0.83

[1] Control (basal diet); FEON50= Basal diet plus 50 mg kg^{-1} fennel essential oil nanoemulsion; FEON100= Basal diet plus 100 mg kg^{-1} fennel essential oil nanoemulsion; FEON200= Basal diet plus 200 mg kg^{-1} fennel essential oil nanoemulsion; FEO = Basal diet plus 200 mg kg^{-1} fennel essential oil.
[2] Standard error of the mean. Within the column, means with a common letter (s) do not differ (P>0.05).
[3] SRBC: Sheep red blood cell.

In the present study, the performance indicators such as EBI, EPEF, and FCR were not significantly affected by experimental treatments in any rearing periods. In the starter period, the EBI and EPEF were increased numerically by supplementation the diet with FEON50 and FEON100 (P≤0.09). In this period the birds that received FEON50, and FEON100, had respectively 11 and 12.3 percent more EBI and 10 and 12.6 percent more EPEF than control birds (P≤0.09). As shown in the formula, these indicators increase when the body weight and viability percent are high or the FCR is low. The greater values of these indicators show that the birds were in good health (Bhamare et al., 2016). Therefore, it can be concluded that the small droplet size in FEON50 and FEIN100 positively affects the performance of broilers. It was reported that the solubility and bioavailability of essential oils in nanoemulsion form were high (Calo et al., 2015) and can exert more positive effect than bulk EOs. In agreement with our findings, it has been reported that plant secondary metabolites or their active components such as anethole in fennel can stimulate the birds' appetite, increase gut digestive secretions, and improve performance and carcass quality (Tollba and Hassan, 2003, Çabuk et al., 2006). Al-Sagan et al. (2020) reported that the broiler performance improved with the addition of 3.2 % fennel seed powder to the diet and this improvement may be due to enhanced nutrient digestibility and enriched body antioxidants status.

Several serum parameters such as cholesterol, glucose, and corticosterone have been used as stress indicators in broiler chickens (Buijs et al., 2009). In the present study, the total cholesterol content in serum decreased with the inclusion of different levels of FEON in the diet. Birds in the control group showed the greatest blood LDL-C which was decreased with FEO or FEON at

100 and 50 mg kg^{-1}. Birds fed 50 mg kg^{-1} of FEON had the lowest LDL-C (42% lower than the control). It might be due to the small droplet size in FEON that could reduce the blood concentration of cholesterol and LDL-C. Indeed, the small size of the droplets in FEON can change the activity of any encapsulated components for example, by increasing the fraction of the encapsulated component, which reaches the intended site of action (Chang et al., 2012). Similar to the present study results, Hadavi et al. (2017) reported that laying hens' blood concentrations of triglyceride, cholesterol, and LDL-C were increased in stress conditions, and dietary supplementation of fennel extract could scavenge the oxidants in the injured liver, improve liver health, and reduce lipid leakage to the serum. Consistent with some of the present findings, Gharaghani et al. (2015) showed that fennel consumption significantly lowered triglyceride and cholesterol concentration in the eggs. The effect of herbal plants and their derivative on blood lipid parameters has been ascribed to their active compounds. Anethole is the main component in FEO and like other plant sterols, changes the body metabolic pathways, leading to triglyceride and cholesterol reactions (Gharaghani et al., 2015).

In the present study, the lowest primary antibody responses against SRBC (the first week of heat stress) were in the control group; however, when the diet was supplemented with FEO and FEON50, these responses increased. Therefore, it can be concluded that the small droplet size in FEON50 (in fourfold lower density) positively affects the same raw FEO and this effect is due to the high solubility and bioavailability of nanoemulsions. Safaei-Cherehh et al. (2020) reported that supplementation of broilers' diet with fennel extract (100, 200, 300, and 400 mg kg^{-1}) did not affect total antibody, IgG, and IgM production, except for IgM at 42 d. In line with our results, Kazemi Fard et al. (2013) reported that the addition of 50 mg kg^{-1} fennel extract improved immune response in post-molted broiler breeder hens. In this regard, Rezaei et al. (2016) also asserted that supplementation of broiler diets with anise seed, which has the same active component, anethole, as fennel seed, stimulated the primary immune responses, but had no effect on the secondary antibody responses. As shown in Table 6, the beneficial bacterial population was increased when FEON was added to the diet. This change in bacterial population can, in turn, improve the immune system. The interaction between the gut microbiome and the host innate immune system can also lead to subsequent adaptive immune response (Pan and Yu, 2014).

Table 6. The effect of experimental treatments on cecal microbial population and gut digesta pH in broiler chickens

	Microbial population in the cecum (Log CFU[3] g^{-1})			Digesta pH		
Treatment[1]	Lactobacillus	Coliform	E. coli	Jejunum	Cecum	Ileum
Control	7.87	8.68^{a}	8.66^{a}	7.37^{bc}	7.45^{bc}	7.53^{a}
FEON50	8.77	8.33^{b}	8.39^{c}	7.25^{c}	7.30^{c}	7.15^{b}
FEON100	8.69	8.30^{b}	8.50^{bc}	7.52^{ab}	7.60^{ab}	7.55^{a}
FEON200	8.53	8.33^{b}	8.50^{bc}	7.45^{abc}	7.60^{ab}	7.74^{a}
FEO	8.41	8.50^{ab}	8.53^{b}	7.60^{a}	7.76^{ab}	7.55^{a}
SEM[2]	0.11	0.05	0.02	0.04	0.05	0.04
P-value	0.06	0.03	0.002	0.01	0.006	0.001

[1] Control (basal diet); FEON50= Basal diet plus 50 mg kg^{-1} fennel essential oil nanoemulsion; FEON100= Basal diet plus 100 mg kg^{-1} fennel essential oil nanoemulsion; FEON200= Basal diet plus 200 mg kg^{-1} fennel essential oil nanoemulsion; FEO = Basal diet plus 200 mg kg^{-1} fennel essential oil.
[2] Standard error of the mean. Within the column, means with a common letter (s) do not differ (P>0.05).
[3]CFU: colony forming units

The gastrointestinal microflora has been shown to have an important role in the health and growth of birds. Environmental stresses are also considered to be an indirect factor affecting this community (Burkholder et al., 2008). The intestinal microbial ecology can also be affected by diet and different feed additives, such as prebiotics, probiotics, and plant secondary metabolites (Pan and Yu, 2014). Our findings showed that the *Lactobacillus* sp. population was increased significantly by supplementation of the diet with FEON so that its maximum population was observed at FEON50 level. A possible explanation for the stimulatory effect of polyphenolic compounds on bacterial growth is that these bacteria are able to use these compounds as nutritional substrates (Ghorbani et al., 2014), and these compounds are more soluble and bioavailable in their nanoform. On the contrary, the *Escherichia coli* and coliform populations in the present study were decreased significantly by the experimental treatments, and the minimum *Escherichia coli* population was observed at 50 mg kg^{-1} FEON level. AbdEl-Hack et al. (2022) reported that emulsions stabilize and improve the antimicrobial effects of oils in aqueous solutions. In line with our results, Diao et al. (2014) demonstrated that the gram-negative and gram-positive bacteria had different sensitivities to fennel seeds EOs. Furthermore, it has been shown that trans-anethole, in fennel seeds EOs, is the most abundant volatile compound responsible for the antibacterial activity of fennel EOs against *Streptococcus mutans* (Park et al., 2004).

Nanoemulsions showed antibacterial activity, and *E* -

scherichia coli and coliform counts decreased by supplementation of broilers' diets with FEON. Moghimi et al. (2016) reported that the antibacterial activity of nanoemulsions of *Thymus daenensis* EOs against *Escherichia coli* was considerably enhanced in comparison with bulk EOs. They also attributed the ability of EOs in nanoemulsion form to easier access of these components to the bacterial cells and their power to disrupt cell membrane integrity (Moghimi et al., 2016). In this regard, Topuz et al. (2016) showed that the long-term physicochemical stability and antimicrobial activity of anise nanoemulsion EOs were better than bulk anise oil. In general, the antimicrobial efficacy of EOs nanoemulsions strongly depends on tested microbial strain, EO components, and emulsion size as well as its formulation (Donsi and Ferrari, 2016).

Additionally, the pH value of the digesta contents can affect the gastrointestinal tract health in such a way that at lower pH, the colonization of beneficial bacteria as well as the growth rate of the birds might be improved (Pan and Yu, 2014). Further, in terms of gastrointestinal content pH, our findings suggested that supplementing the diet with FEON50 decreased significantly the digesta pH in the jejunum, ileum and cecum. The decrease in gastrointestinal content pH by supplementation of the diet with FEON was expected and may be related to short-chain fatty acid production by *Lactobacillus* sp. bacteria. In agreement with our findings, Mikulski et al. (2008) reported that *Lactobacilli* and *Bifidobacteria* could produce short-chain fatty acids in the intestine lumen to reduce the intestinal pH.

Conclusions

The results of this experiment showed that supplementation of the broiler diet with FEON in heat stress conditions can decrease the blood total cholesterol and LDL-C and improve the balance of the microbial population in the cecal digesta content, and primary humoral immunity.

Acknowledgments

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