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Animal Health and Well Being

Role of oral phytogenic supplementation to protect cardiac, hepatic, nephrotic, and splenic oxidative stress in broiler chickens

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Abstract

This study investigated the effects of adding essential oils of garlic, ginger, turmeric, and cinnamon to drinking water on cardiac, hepatic, nephrotic, and splenic oxidative status of broiler chickens. A batch of 200 1-d old Arbo acre broiler chicks was administered with Control (Water: no additive), 30 ml/L of cinnamon, ginger, turmeric, or garlic essential oils in drinking water for 42 d. On day 43, three broiler chickens/replicates were sampled randomly, sacrificed, and eviscerated. The hearts, spleens, kidneys, and livers were excised and assayed for glutathione peroxidase, total antioxidant activity, catalase, superoxide dismutase, and lipid peroxidation using standard protocols. In spleen broiler chickens, all additive essential oils increased ($P < 0.05$) total antioxidant activity. Catalase, superoxide dismutase, and glutathione peroxidase significantly increased ($P < 0.05$) in garlic, ginger, and turmeric essential oils except cinnamon. In kidney broiler chickens, lipid peroxidation was significantly reduced ($P < 0.05$) in all the additive essential oils. Garlic, cinnamon, and ginger essential oils increased ($P < 0.05$) catalase, superoxide dismutase, and glutathione peroxidase in kidney broiler chickens. In liver broiler chickens, lipid peroxidation, and glutathione peroxidase were higher ($P < 0.05$) in cinnamon essential oil than other additive essential oils. Superoxide dismutase and catalase were higher ($P < 0.05$) in turmeric essential oils. In heart broiler chickens, all the additive essential oils significantly decreased ($P < 0.05$) lipid peroxidation and increased ($P < 0.05$) total antioxidant activity. In conclusion, oral garlic, turmeric, and ginger essential oils supplementation did not reduce lipid peroxidation in spleen, whereas cinnamon essential oil caused lipid peroxidation in liver of broiler chickens.

Key words: antioxidant enzymes, essential oils, liver, peroxidation, spleen

Background Information

In recent years, poultry production has provided essential animal protein for human consumption. There has been restriction on antibiotics usage in chicken production, which has pushed poultry farmers to source for an alternative plant feed additives to tackle challenges facing poultry farmers (Pan et al., 2022). The ban on the use of antibiotics in poultry production was due to the residual effects on animal meat and tissue resulting in resistance to antibiotics. This restriction has led to utilization of herbal supplements competent to enhance utilization of nutrients and reduce antibiotic use (Alagawany et al., 2021). The alternative plant feed additives which are herbs, spices, and essential oils contain bioactive compounds, medicinal, and pharmacological properties required for poultry production (Mnisi et al., 2023). Plant feed additives added to chicken feeds help in improving the immunity and performance of birds with their anti-microbial, anti-inflammatory, antioxidant, and cholesterol-lowering properties (Rafeeq et al., 2023).

When birds are exposed to chronic illness/disease or thermal stress, their oxidative processes increase (Surai et al., 2019). Production of free radicals in the body are reactive oxygen species (ROS) produced during mitochondrial oxidative metabolism. Oxidative stress occurs when the amount of ROS exceeds the antioxidant capacity (enzymatic and non-enzymatic), oxidative stress surfaces (Jimoh, 2022). Malondialdehyde (MDA), the result of lipid peroxidation, is a good indicator of the level of oxidation in the blood, and its concentration in the liver is an indicator for the assessment of antioxidant activity. Resultant disorders in body's normal function, metabolism, and physiology are caused by damage to nucleic acids, lipids membrane, and proteins, which is defined as oxidative stress (Mostafa Abd El-Aal, 2012). Dietary oxidation stability can affect the feed shelf life during and after the processing and storage of feedstuffs through oxidative reactions, which will negatively influence animal health and performance (Zhao et al., 2011). Therefore, antioxidant supplements like plant feed additives are recommended for poultry production as management practices to ameliorate oxidative stress (Bhagwat et al., 2021).

Garlic (*Allium sativum*) contains many bioactive compounds (Poojary et al., 2017), and organic sulfide

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compounds (Ajanaku et al., 2022). Garlic is rich in saponin, tannin, alkaloid, and flavonoids as phytochemicals. Garlic contains enzymes, minerals, and vitamins (Shang et al., 2019). Bioactive compounds in garlic have shown antioxidant, anticancer, antidiabetic, neuroprotective, and hepatoprotective effects on the cardiovascular, renal, and digestive systems (Gudalwar et al., 2021; El et al., 2021).

Ginger (*Zingiber officinale*) is rich in niacin, vitamin C, thiamin, and riboflavin (Zhukovets and Özcan, 2020) It has saponins, tannins, and oxalates (Nutakor et al., 2020). The main bioactive substances in ginger are gingerdione, shogaol, gingerdiol, and gingerol and their derivatives with pharmacological activity (Mao et al., 2019). Medicinal properties of ginger include antioxidant, analgesic, radioprotective, antitumorigenic, antiapoptotic, antihyperglycemic, antilipidemic, and antiemetic properties (Abd El-Hack et al., 2020b; Abd El-Hack et al., 2022).

Cinnamon (*Cinnamomum zeylanicum*) contains cinnamaldehyde, cinnamic acid, cinnamate, and many other essential oils. Cinnamon leaf oil has a high eugenol content (80%–88% in Ceylon, 87%–96% in Seychelles) while cinnamon bark oil contains cinnamaldehyde, cinnamyl acetate, eugenol acetate, and benzaldehyde (Abd El-Hack et al., 2020a). Cinnamon has been shown to have anti-inflammatory properties due to polyphenols that help the body repair damaged tissue (Ali et al., 2021). In addition, Cinnamon has antioxidant properties that help reduce problems that cause oxidative stress in humans, as well as beneficial properties that are useful in preventing ROS damage to cellular membranes (Saeed et al., 2018).

Turmeric (*Curcuma longa*) is rich in nutrients and contains carbohydrates, protein, vitamin C, polyphenols, beta-carotene, fatty acid volatile oils, and essential oils (Patil et al., 2019; Umar et al., 2020). The bioactive substances in *Curcuma longa* are curcumin, tetrahydro curcuminoids, bisdemethoxycurcumin, and demethoxycurcumin (Jyotirmayee and Mahalik, 2022). It has been reported that turmeric essential oils have antioxidant, anticancer, and anti-inflammatory properties (Liju et al., 2011).

The above plant feed additives are used as an alternative to antibiotics in poultry production, which has shown greater animal health, potential stress alleviators, feed safety, and economic benefits with minimal toxicity and no adverse effects. The reports on antioxidative effects of these herbs on tissue physiology, suggest their potential to influence oxidative stability of animals exposed to thermal discomfort-induced oxidative stress. On this note, we are conducting an experiment to evaluate the roles of garlic, ginger, turmeric, and cinnamon essential oils on cardiac, hepatic, nephrotic, and splenic oxidative stress in broiler chickens.

Materials and Methods

Ethical Approval

The institutional research and ethics committee of the Federal Polytechnic Ado-Ekiti approved the experimental protocols of this study, and was performed in conformity with the ARRIVE 2.0 and NRC guidelines (National Research Council. 2011; Du Sert et al., 2020). The experiment was performed in line with guidelines of University Animal Scientific procedures [Animal (Scientific Procedures) Act 1986]. Adequate management practices were taken to reduce discomfort of the laboratory animals.

Phytogenics Processing and Evaluation

Fresh and matured garlic (*Allium sativum*), turmeric (*Curcuma longa*), ginger (*Zingiber officinale*), and cinnamon (*Cinnamomum zeylanicum*) were procured from spices vendors in Ado-Ekiti; a tropical rainforest agro-ecological zone in the south–western part of Nigeria. They were diced, air-dried, blended, and thereafter soaked with distilled water (40% w/v) and essential oils of garlic, turmeric, ginger, and cinnamon were extracted using steam distillation (Dehariya et al., 2020). The same distillation procedures were used across all the herbs.

Experimental Animals and Management

Two hundred (200) day-old Arbo acre broilers were purchased from a reputable hatchery/breeder. They were weighed and assigned to 5 treatments with 10 replicates and 4 birds per replicate in a completely randomized design. Birds were fed on a standard diet to meet the nutrient requirement of broiler chickens at the starter and finisher phases (National Research Council, 2012). Garlic, turmeric, ginger, and cinnamon essential oils were added to chicken drinking water (30 mL/L) for 5 treatments noted as treatment 1: control (no additives), treatment 2: garlic essential oil, treatment 3: ginger essential oil, treatment 4: turmeric essential oil, treatment 5: cinnamon essential oil throughout the 42-d feeding trial study. Feed and freshwater were offered ad libitum. The hatchery recommended vaccination programs were followed.

At the end of the feeding trial, three birds/replicates were randomly sampled and sacrificed with a sharp knife cutting through the jugular vein, carotid artery, and windpipe. The heart, kidney, liver, and spleen were eviscerated, excised, collected, and rinsed in cold phosphate-buffered solution. Twenty percent homogenates of heart, spleen, kidney, and liver from each broiler chicken were prepared by homogenizing at 4 °C in 0.15 M KCl, centrifuged at 12,000 rpm for 45 min at 0 to 4 °C to obtain supernatant (Venkatanarayana et al., 2012). Supernatants were analyzed for catalase, total antioxidant activity, superoxide dismutase (SOD), glutathione peroxidase (GPx), and lipid peroxidation as previously described by (Jimoh et al., 2021). All values were normalized to total protein in each sample for comparison within samples. Total protein was determined using Fortress Diagnostics commercial test kit and method.

Statistical Analysis

The data were tested for normality and homogeneity of variances with Levene's test to ascertain the suitability of one-way ANOVA. Before further analysis using IBM SPSS 20. Data obtained from the study were subjected to general linear model procedures of analysis of variance ($P < 0.05$). Mean differences were separated by the Duncan's Multiple Range Test (DMRT) using IBM SPSS 20.

Result

Table 1 shows the oxidative status in spleen of broiler chickens administered four spice essential oils. Total protein of spleen broiler chickens on cinnamon and ginger essential oils were similar ($P > 0.05$) to those in the control and were significantly ($P < 0.05$) higher than those in garlic and

turmeric essential oils groups. The total antioxidant activity of spleen broiler chickens on all additive essential oils were higher ($P < 0.05$) than those in control, while garlic essential oil group had the lowest ($P < 0.05$) value. Lipid peroxidation in spleen broiler chickens of all additive essential oils were higher ($P < 0.05$) than those in control except cinnamon essential oil, which was similar ($P > 0.05$) to the control. The GPx of spleen broiler chickens on turmeric essential oil was significantly ($P < 0.05$) higher, with the least ($P < 0.05$) values obtained in broiler chickens on cinnamon essential oils and control. The catalase of spleen broiler chickens on garlic and turmeric essential oils were significantly higher ($P < 0.05$) than other essential oil groups. SOD of broiler chickens on ginger essential oil had the highest ($P < 0.05$) value and the least ($P < 0.05$) values were obtained from the spleen of broiler chickens on control, cinnamon, and turmeric essential oils with statistically ($P > 0.05$) similar values.

Oxidative status of kidney broiler chickens administered with four spice of essential oils is shown in Table 2. Total protein in kidney broiler chickens on control was higher ($P < 0.05$) than those on essential oils-based treatments. The total antioxidant activity in kidney broiler chickens on garlic, ginger, and cinnamon essential oils were significantly higher ($P < 0.05$) than those on control and turmeric essential oils. The lipid peroxidation in kidney broiler chickens in control was higher ($P < 0.05$) than those in essential oils-based groups. GPx, catalase, and SOD in kidney broiler chickens on garlic, cinnamon, and ginger, respectively were significantly higher ($P < 0.05$) than in turmeric treatment with significantly ($P < 0.05$) least values obtained from control.

Table 3 shows the oxidative status in liver broiler chickens administered four spice essential oils. The total protein in liver broiler chickens on garlic and turmeric essential oils were statistically ($P < 0.05$) higher than other essential oil-based groups. The total antioxidant activity in liver broiler chickens on control and ginger essential oils were higher ($P < 0.05$) than those in other essential oil-based groups, which had statistically ($P > 0.05$) similar values. The lipid peroxidation and GPx in liver broiler chickens on cinnamon essential oils were higher ($P < 0.05$) than those in other essential oil-based groups which were statistically ($P > 0.05$) similar values. Catalase in liver broiler chickens on control were similar ($P > 0.05$) to those on turmeric and were significantly ($P < 0.05$) higher than those in other essential oil-based groups. The liver SOD on turmeric essential oils was higher ($P < 0.05$) across the group with significantly ($P < 0.05$) lower values obtained on the garlic and ginger essential oils.

Oxidative status in heart broiler chickens administered four spice essential oils is shown in Table 4. The total protein in heart broiler chickens in control and cinnamon essential oil-based groups were similar ($P > 0.05$) and significantly ($P < 0.05$) higher than those on other essential oils. Cardiac

Table 1. Oxidative status in spleen of broiler chickens fed spices essential oils

	Control	Garlic EO	Ginger EO	Turmeric EO	Cinnamon EO	SEM
Total protein, g/l	10.61^{a}	4.59^{b}	8.71^{a}	3.85^{b}	8.67^{a}	13.832
Total antioxidant activity, ×10^{-2}mmol/L	19.57^{c}	40.22^{b}	96.99^{a}	65.59^{a}	70.32^{a}	9.586
Lipid peroxidation, ×10^{-7}MDA/mg protein	0.67^{c}	40.93^{a}	38.46^{a}	14.18^{b}	0.12^{c}	9.174
Glutathione peroxidase, µg GSH/min/mg protein	0.88^{c}	50.69^{b}	53.60^{b}	175.15^{a}	0.29^{c}	35.491
Catalase, ×10^{-2} nm H_2O_2/min/mg protein)	0.97^{b}	14.58^{a}	2.87^{b}	13.45^{a}	0.14^{b}	4.392
Superoxide dismutase, U/min/mg protein	4.53^{c}	54.09^{b}	104.71^{a}	10.08^{c}	0.24^{c}	20.853

^{a,b,c}: Mean in the same row with different superscripts are significantly ($P < 0.05$) different.
Treatments (n = 3/group); EO: Essential oil; SEM: Standard Error of Mean.
Treatment 1: Control (water: no additives) 0 mL/L,.
Treatment 2: Control + Garlic essential oil at 30 mL/L,.
Treatment 3: Control + Ginger essential oil at 30 mL/L,.
Treatment 4: Control + Turmeric essential oil at 30 mL/L and.
Treatment 5: Control + Cinnamon essential oil at 30 mL/L.

Table 2. Oxidative status in kidney of broiler chickens fed spice essential oils

	Control	Garlic EO	Ginger EO	Turmeric EO	Cinnamon EO	SEM
Total protein, g/l	42.69^{a}	19.56^{b}	13.23^{b}	9.82^{b}	7.92^{b}	6.748
Total antioxidant activity, ×10^{-2}mmol/L	0.81^{b}	1.44^{a}	1.28^{a}	0.74^{b}	1.02^{a,b}	0.193
Lipid peroxidation, ×10^{-7}MDA/mg protein	41.76^{a}	1.30^{b}	8.54^{b}	2.35^{b}	4.07^{b}	10.182
Glutathione peroxidase, µg GSH/min/mg protein	1.28^{c}	84.75^{a}	4.12^{c}	3.25^{c}	22.36^{b}	16.564
Catalase, ×10^{-2} nm H_2O_2/min/mg protein	5.54^{c}	27.90^{b}	6.29^{c}	11.96^{b}	62.72^{a}	11.709
Superoxide dismutase, U/min/mg protein	0.48^{b}	3.36^{b}	16.87^{a}	1.40^{b}	6.62^{b}	3.634

^{a,b,c}: Mean in the same row with different superscripts are significantly ($P < 0.05$) different.
Treatments (n = 3/group); EO: Essential oil; SEM: Standard Error of Mean.
Treatment 1: Control (water: no additives) 0 mL/L,.
Treatment 2: Control + Garlic essential oil at 30 ml/L,.
Treatment 3: Control + Ginger essential oil at 30 ml/L,.
Treatment 4: Control + Turmeric essential oil at 30 ml/L and.
Treatment 5: Control + Cinnamon essential oil at 30 ml/L.

Table 3. Oxidative status in liver of broiler chickens fed spice essential oils

	Control	Garlic EO	Ginger EO	Turmeric EO	Cinnamon EO	SEM
Total protein, g/l	7.13^{c}	21.23^{a}	10.85^{b}	17.19^{a}	11.48^{b}	2.683
Total antioxidant activity, ×10^{-2}mmol/L	9.91^{a}	4.71^{b}	11.13^{a}	7.37^{b}	5.18^{b}	1.556
Lipid peroxidation, ×10^{-7}MDA/mg protein	0.67^{b}	0.10^{b}	0.58^{b}	0.21^{b}	6.21^{a}	1.273
Glutathione peroxidase, µg GSH/min/mg protein	4.82^{b}	0.29^{b}	1.29^{b}	4.56^{b}	57.34^{a}	11.347
Catalase, ×10^{-2} nm H_2O_2/min/mg protein	1.05^{a}	0.26^{b}	0.02^{b}	2.16^{a}	0.43^{b}	0.526
Superoxide dismutase, U/min/mg protein	5.10^{b}	0.16^{c}	0.53^{c}	19.73^{a}	7.78^{b}	3.925

$^{a, b, c}$: Mean in the same row with different superscripts are significantly ($P < 0.05$) different.
Treatments (n = 3/group); EO: Essential oil; SEM: Standard Error of Mean.
Treatment 1: Control (water: no additives) 0 mL/L,.
Treatment 2: Control + Garlic essential oil at 30 mL/L,.
Treatment 3: Control + Ginger essential oil at 30 mL/L,.
Treatment 4: Control + Turmeric essential oil at 30 mL/L and.
Treatment 5: Control + Cinnamon essential oil at 30 mL/L.

Table 4. Oxidative status in heart of broiler chickens fed spice essential oils

	Control	Garlic EO	Ginger EO	Turmeric EO	Cinnamon EO	SEM
Total protein, g/l	19.75^{a}	14.26^{b}	6.82^{c}	12.83^{b}	29.84^{a}	2.684
Total antioxidant activity, ×10^{-2}mmol/L	0.82^{c}	2.21^{a}	1.66^{b}	2.03^{a}	1.65^{b}	0.329
Lipid peroxidation, ×10^{-7}MDA/mg protein	11.54^{a}	0.49^{d}	1.43^{c}	0.18^{d}	5.52^{b}	1.964
Glutathione peroxidase, µg GSH/min/mg protein	1.26^{b}	15.26^{a}	3.05^{b}	34.32^{a}	0.93^{b}	6.94
Catalase, ×10^{-2} nm H_2O_2/min/mg protein	0.05^{c}	2.08^{b}	0.00^{c}	5.04^{a}	0.15^{c}	1.272
Superoxide dismutase, U/min/mg protein	0.21^{d}	6.52^{b}	3.47^{c}	18.78^{a}	2.76^{c}	2.527

$^{a, b, c}$: Mean in the same row with different superscripts are significantly ($P < 0.05$) different.
Treatments (n = 3/group); EO: Essential oil; SEM: Standard Error of Mean.
Treatment 1: Control (water: no additives) 0 mL/L,.
Treatment 2: Control + Garlic essential oil at 30 mL/L,.
Treatment 3: Control + Ginger essential oil at 30 mL/L,.
Treatment 4: Control + Turmeric essential oil at 30 mL/L and.
Treatment 5: Control + Cinnamon essential oil at 30 mL/L.

total antioxidant activity in broiler chickens on garlic and turmeric essential oils were not significantly ($P > 0.05$) different but were higher ($P < 0.05$) than those on other essential oil-based groups with the significantly ($P < 0.05$) least values obtained in broiler chickens on control. The lipid peroxidation in heart broiler chickens in control was higher ($P < 0.05$) than those in essential oils-based groups. The GPx in heart broiler chickens on control, cinnamon, and ginger essential oils were statistically ($P > 0.05$) similar and significantly ($P < 0.05$) lower than those broiler chickens on garlic and turmeric essential oil-based groups. The catalase and SOD in heart broiler chickens on turmeric essential oil was higher ($P < 0.05$) than broiler chickens on other essential oil-based groups with the significantly ($P < 0.05$) least values obtained in broiler chickens on control.

Discussion

This experiment investigated the effects of garlic, turmeric, ginger, and cinnamon essential oils on markers of oxidative stress in the heart, liver, kidney, and spleen of broiler chickens. Supplementation of garlic, ginger, and turmeric essential oils increased total antioxidant activity, lipid peroxidation, glutathione peroxidase, catalase, and superoxide dismutase in spleen broiler chickens except cinnamon compared to control. Ginger, garlic, turmeric, and cinnamon essential oil increased total antioxidant activity, glutathione peroxidase, catalase, and superoxide dismutase in broiler chicken kidneys. The addition of garlic, turmeric, and cinnamon essential oils reduced total antioxidant activity in liver of broiler chickens while ginger did not. Addition of garlic, ginger, and turmeric essential oils reduced lipid peroxidation and glutathione peroxidase in the liver of broiler chickens but improved in cinnamon. Garlic, ginger, and cinnamon essential oils decreased catalase and superoxide dismutase in broiler chicken livers but turmeric increased catalase and superoxide dismutase. In addition to the essential oils of garlic, turmeric, ginger, and cinnamon increased total antioxidant activity, glutathione peroxidase, catalase, and superoxide dismutase in heart of broilers. Poultry production is associated with nutritional and environmental stress, which often compromises the health status, reduced immunity, and reduced performance of broiler chickens. Oxidative stress caused by an imbalance of oxidants and antioxidants is a major stress in poultry production (Jimoh, 2022). The antioxidant system of broiler chickens consists of three levels of antioxidant defense. The first are antioxidant enzymes (as analyzed in our study)—SOD, GPx, and catalase, which initiate the process responsible for free radical detoxification. SOD is a "metalloprotein enzyme", GPx is a "seleno enzyme" that catalyzes the reaction of hydroperoxides with reduced glutathione to form glutathione disulfide. These are the primary enzymes that contribute to the antioxidant defense system. Antioxidant activity is an

important biomarker of physiological, pathological, and nutritional quality of animals. They can be used to demonstrate the effects of nutritional additives in poultry feeds.

The antioxidant activity of garlic supplementation added to broiler chicken has been attributed to the accumulation of antioxidant compounds such as alliums in garlic which are powerful antioxidants (Shang et al., 2019). Garlic has been adduced to low blood pressure by reducing oxidative stress, increasing nitric oxide and hydrogen sulfide production, and inhibiting angiotensin-converting enzymes (Ried and Fakler, 2014; Kravchuk et al., 2021). Interestingly, our study showed that garlic essential oil can reduce lipid peroxidation (MDA) in broiler chicken kidneys, liver, and heart, and increase total protein in broiler chicken liver. In our study, garlic essential oil increased total antioxidant capacity (TAC), glutathione peroxidase, catalase, and SOD in broiler chicken kidneys, as well as TAC, glutathione peroxidase, catalase, and superoxide dismutase in broiler chicken spleen and heart. According to our findings, the phytochemical nutrients, bioactive substances, and medicinal properties of garlic essential oil can reduce ROS, improve antioxidant enzymes, and reduce lipid peroxidation in broiler chicken. Our results are consistent with those findings of (Pourali et al., 2014) who stated that garlic supplementation reduced hepatic MDA levels in birds by 30% compared to birds fed a basic diet. In addition, another study showed that the intake of turmeric or garlic supplements (2, 4, and 6 g/kg) fed to growing rabbits linearly and quadratically resulted in an improvement in liver glutathione peroxidase, catalase, and superoxide dismutase. Likewise, glutathione and MDA levels decreased linearly compared to controls (Alagawany et al., 2016). Similarly, another study found that guanidinoacetic acid-fed chickens improved GPx activity in the liver and reduced MDA in stressed broilers (Nasiroleslami et al., 2018; Khalil et al., 2021; Majdeddin et al., 2023). These findings did support our results showing that the antioxidant activity of SOD and TAC were increased in broiler chickens supplemented with garlic powder (0.50 and 0.75 g/kg) compared to control diet and MDA was lowed in garlic powder groups (0.50 and 0.75 g/kg) (Ismail et al., 2021). However, it has been shown that garlic essential oils in the spleen induced higher lipid peroxidation, due to lymphatic activity. It is widely reported that garlic and ginger have anti-inflammatory activity which may be responsible for higher ROS generation in garlic and ginger essential oils-treated birds (Gardzielewska et al., 2003). The addition of garlic essential oils in chicken ration acted by immunomodulation via bioavailability of nutrients required to synthesize antibodies and promote development of the immune system of chickens (Elbaz et al., 2022). Claims by (Huang et al., 2023) demonstrated that garlic essential oil supports the immune system by improving the phagocytic activity of heterophages and explaining the rate of peroxidation induced by ROS production.

Many studies have shown that ginger has antioxidant, anti-inflammatory, and immunomodulatory properties and is therefore, beneficial for the function of chickens and prevents oxidative stress (Mao et al., 2019). In vitro studies have shown that ginger extracts can regulate lipid peroxidation levels. In addition, our study showed that the essential oil in ginger can reduce lipid peroxidation in broiler chicken kidneys, liver, and heart and increase total protein in the broiler chicken liver. Ginger essential oil increases total antioxidant activity, glutathione peroxidase, catalase, and superoxide dismutase in spleens and kidneys of broiler chickens. Ginger essential oil increased total antioxidant activity, glutathione peroxidase, and superoxide dismutase in broiler chicken heart. Our results are consistent with studies showing that ginger (1%) reduces lipid peroxides production in rats by promoting the activity of antioxidant enzymes (SOD, Catalase, and GSHPx) (Ahmed et al., 2000; Ahmed et al., 2008). Our findings are confirmed by research showing that the levels of antioxidative enzymes (SOD, catalase, and GPx) are increased in the liver of rats using ginger, leading to liver, and kidney lipid peroxidation. Protein oxidation products inhibited in the liver demonstrate the antioxidant benefits of ginger intake (Kota et al., 2008). Our findings confirmed the report that ginger supplementation increased SOD and GPx activity but decreased MDA in broiler chickens fed ginger root powder (Zhang et al., 2009). Habibi et al. (2014) and claimed, regarding our results, that ginger essential oil (150 mg/kg) in the broiler chicken diet improved liver SOD activity and decreased MDA while increasing TAC in the blood. A previous study showed that the use ginger extract increased TAC, GPx activity in the blood and breast muscle of 21 to 42-d-old broiler chicks under heat stress (Wen et al., 2020).

Cinnamon contains antioxidants that help prevent ROS damage to cell membranes (Kanani et al., 2017). However, according to our research, cinnamon essential oil reduces lipid peroxidation in broiler chicken spleen, kidney, and heart, while it increases total protein in broiler chicken heart and liver. The addition of cinnamon essential oil increases the total antioxidant activity, glutathione peroxidase, catalase, and superoxide dismutase in the kidneys of broiler chickens. Cinnamon essential oil supplementation increased lipid peroxidation despite increased glutathione peroxidase in broiler chicken liver. This may indicate more free radicals (singlet oxygen) in the liver, due to reduced SOD activity in broiler chickens fed cinnamon essential oil compared to control. GPx and catalase reduce H_2O_2 to H_2O, which may provide more GPx in broiler chickens treated with cinnamon essential oil. Cinnamon essential oil increases total antioxidant activity, glutathione peroxidase, and superoxide dismutase in broiler chicken heart. Our results showed that the decrease in lipid peroxidation and increase in TAC indicated a decrease in oxidative stress and an increase in total antioxidant activity in broiler chickens. Cinnamon bark contributes to lipid metabolism and antioxidant activity in rats fed a high-cholesterol diet (0.1g/100 g of diet) due to the presence of cinnamate. This suggests that phenolic compounds of cinnamate in cinnamon bark decrease lipid peroxidation by increasing hepatic antioxidant enzymes in the liver (Lee et al., 2003). Our findings are consistent with studies that showed a decrease in lipid peroxidation but an increase in TAC in the diabetic group treated with 200 mg/kg cinnamon compared to the control group (Salem et al., 2010, Salem et al., 2017). Our results confirm that cinnamon powder improves superoxide dismutase, TAC, and catalase while reducing MDA concentrations on study day 42. These findings are attributed to the antioxidant effects of cinnamaldehyde, which helps protect against liver ROS pressure (Sadeghi and Moghaddam, 2018).

Turmeric is used as a nutritional supplement in poultry, and the curcumin in turmeric powder is a good antioxidant. Recent studies have evaluated the effect of turmeric powder on blood biochemical parameters and antioxidant capacity of broiler chickens (Gowda et al., 2009, Hussein, 2013). However, our study showed that turmeric essential

oil supplementation decreased lipid peroxidation in broiler chicken spleen, kidney, liver, and heart and increased total protein in broiler chicken liver. In our study, turmeric essential oil supplementation increased total antioxidant activity, glutathione peroxidase, catalase, and superoxide dismutase in the spleen and heart of broiler chickens. Turmeric essential oil supplementation increases catalase and superoxide dismutase in the broiler chicken liver. Addition of turmeric essential oil improves glutathione peroxidase, catalase, and superoxide dismutase in broiler chicken kidney. Curcumin supplementation was found to inhibit lipid peroxidation in rat liver microsomes, erythrocyte membrane, and brain homogenate but more potently inhibited superoxide dismutase, catalase, and glutathione peroxidase in liver homogenates of rats fed a diet containing turmeric compared with controls (Reddy and Lokesh, 1994). Quiles et al. (2002) found that *curcuma longa* can reduce oxidative stress and control the development of fatty streaks in rabbits. Additionally, rabbits were fed a diet supplemented with turmeric-reduced lipid peroxides (Quiles et al., 2002). Our results support Lin et al. (2003) who found that 0.05 to 1.0 mg/mL aqueous and ethanol extracts of dried bark of *Cinnamomum cassia* exhibit anti-superoxide forming activity and are an excellent hepatic enzymatic and non-enzymatic antioxidant (Lin et al., 2003). In addition, our findings support the report that administration of turmeric extract (1.66 mg/kg of body weight) to rabbits fed on high-fat diet reduces oxidation of erythrocyte, hydroperoxide, and thiobarbiutric reactive substance (TBARS) in liver microsomes (Mesa et al., 2003). Our study agreed with some published reports that showed the addition of turmeric powder (0.5%) and curcuminoids (222 mg/kg) to 1 mg aflatoxin B1/kg contaminated diet increased total antioxidant and superoxide dismutase of broiler chickens (Gowda et al., 2008, Gowda et al., 2009). Furthermore, our findings confirm that turmeric powder (0.5% and 1%) has protection against pro-oxidants and increases glutathione in aflatoxin-contaminated duck feed (Ayoub et al., 2011). In another study, increased glutathione peroxidase and SOD and decreased MDA were reported in broiler chickens fed 4 and 8 g/kg turmeric powder before (28) and after (42) heat stress (Hosseini-Vashan et al., 2012). Our study supports another study showing high antioxidant activity in liver of curcumin in turmeric powder diet. Turmeric supplementation alone or in combination reduced erythrocyte malondialdehyde levels in broiler chickens (Sahoo et al., 2019). Our results differ from the study, which showed that the TAC of tissues (liver, heart, and kidney) and blood were not affected by turmeric powder (Qasem et al., 2016). Quails-fed nano-curcumin demonstrated showed higher SOD and GSH activities and lower MDA in blood (Reda et al., 2020).

Collectively, our study showed a high antioxidant enzyme activity, suggesting that broiler chickens have evolved to inhibit oxygen free radicals. In addition, MDA decreased and TAC increased, indicating a reduction in oxidative stress and an increase in total antioxidant defense. In addition to the essential oils of garlic, ginger, turmeric, and cinnamon, it increased the antioxidant capacity of the broiler chickens due to the antioxidant components of the herbal feed additives. The antioxidant enzymes—catalase, superoxide dismutase, and glutathione peroxidase are synthesized and regulated endogenously. SOD plays an important role in protecting cells from damage caused by ROS. It is also clear that the essential oils of garlic, ginger, turmeric, and cinnamon can act as exogenous antioxidants that prevent tissue damage. In our study, the addition of garlic, ginger, cinnamon, and turmeric essential oils to the drinking water of broiler chickens improved the cardiac, hepatic, nephrotic, splenic antioxidant enzymes of superoxide dismutase, glutathione peroxidase, and catalase, and reduced the lipid oxidation in comparison with the control group of broiler chickens. However, a combination of essential oils should be explored to overcome the deficiencies of cinnamon, ginger, and garlic essential oils in their ability to reduce lipid peroxidation in liver and spleen of broiler chickens.

Conclusion

The supplementation of garlic, ginger, turmeric, and cinnamon essential oils elevated glutathione peroxidase, catalase, and superoxide dismutase and reduced lipid peroxidation in the spleen, kidney, liver, and heart of broilers. Therefore, the study concluded that antioxidant activity on vital tissues of broiler chickens were positively influenced by the inclusion of garlic (*Allium sativum)*, ginger *(Zingiber officinale)*, turmeric *(Curcuma longa)*, and cinnamon *(Cinnamomum zeylanicum)* essential oils in their drinking water. Further studies are recommended to evaluate composite mixtures of the essential oils to combat deficiencies observed in lipid peroxidation ability of cinnamon, ginger, and garlic essential oils in the liver and spleen of broiler chicken.

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Conflict of Interest Statement

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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