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Impact of Dietary Thyme (*Thymus Vulgaris*) on Broiler Chickens Concerning Immunity, Antioxidant Status, and Performance

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Broilers, Immunity, Antioxidant, Performance, Thyme

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ABSTRACT

The current study aimed to determine the immune and antioxidant status, and performance of broiler chickens fed diets supplemented with thyme (*Thymus vulgaris*) leaves powder, as an alternative growth promoter. Therefore, a total of 120 one-day-old Cobb chicks were fed for 35 days with four experimental diets containing thyme powder (0, 5, 10 and 15 g/kg: these were designated groups Control, Thyme I, Thyme II and Thyme III, respectively). The GC-MS analysis of *n*-hexane extract of thyme showed the presence of isocaryophyllene (33.73%) as a major active ingredient with other antioxidant ingredients. Serum total protein and globulin content was significantly increased in thyme-supplemented chicks at the 3rd week and significantly increased in Thyme I and III at the 5th week. Serum albumin and creatinine content and ALT activities were non-significantly increased in the thyme-supplemented chicks at the 3rd and 5th weeks. Moreover, serum total cholesterol and triacylglycerol levels were significantly decreased in the thyme groups at the 3rd and 5th weeks. Dietary thyme supplementation for broiler was significantly improved serum IgG, IgM, INF-γ, and IL-10 levels, and muscle glutathione content, superoxide dismutase, and glutathione S-transferase with significantly reduced malondialdehyde levels. Regarding to growth performance, thyme 0.5% was non-significantly improved the final body weight, body weight gain, feed conversion ratio, and protein efficiency ratio of broiler chicks. So, these data indicated that incorporation of thyme into the broiler's diet was improved the immune status and antioxidant activities in broilers. Also, production of broilers meat with low levels of lipid peroxidation products.

1. INTRODUCTION

Antibiotics have been extensively used as feed additives and growth promoters in animal feed industry. The use of antibiotics as feed additives is of many hazardous due to cross-resistance and multiple resistances of pathogens (Schwarz *et al.*, 2001). Therefore, European Union has banned the application of most of the antibiotics in poultry diets. Thus, during the past decade, many studies investigated the use of new and promising feed additives including probiotics, prebiotics, enzymes, and plant extracts in animal feeding (Sarica *et al.*, 2007). Medicinal plants and their extracts were introduced to the animal feeding that improving performance immune system of animals that could be used as antibiotic alternatives (Mikulski *et al.*, 2008). Essential oils derived from herbs have antimicrobial properties (Faleiro *et al.*, 2003). It has been reported that herb extracts have antibacterial characteristics, antioxidant activity, and enhance digestibility by stimulating endogenous enzyme activity and

facilitating nitrogen absorption (Azaz *et al.*, 2002; Botsoglou *et al.*, 2004).

Medicinal plants are resources of new drugs and many of the modern medicines that improve the health status of animals (El-Far *et al.*, 2016b; El-Far *et al.*, 2017). Thyme (*Thymus vulgaris*) is a flowering plant in the mint family *Lamiaceae*. It is growing up to 15-30 cm with about 40 cm of width that cultivated in most of the European countries (Reddy, 2014). Thymol (5-methyl-1-2-isopropyl phenol) and carvacrol (5-isopropyl-2-methyl phenol) are the main phenolic components in thyme**,** which act as potent antioxidant scavengers (Hoffman-Pennesi and Wu, 2010). Cross *et al.* (2007) reported the antibacterial, anticoccidial, and antifungal activities of thyme, as well improving the general health of broilers. The active principles of essential oils act as a digestibility enhancer, balancing the gut microbial ecosystem and stimulating the secretion of endogenous digestive enzymes and thus improving growth performance in poultry (Cross *et al.*, 2007; Ayoub *et al.*, 2011; Barakat *et al.*, 2016; El-Far *et al.*, 2016a). Consequently, thyme can be used as an easily available source of natural antioxidants and antibiotics in food products and drugs. For this reason, the current study was conducted to investigate the antioxidant and immunostimulant potential of thyme that reflects the health status and performance of broiler chickens.

2. Material and Methods

2.1. Birds, accommodation, and management

The present study is affirmed by the Ethics of Animal Experiments Committee, Damanhour University, Egypt. Whereas, one hundred and twenty Cobb of one-day-old broiler chicks were incubated and randomly allocated into four equal groups at the first week of age. Each group was subdivided into three replicates (10 birds per replicate). The housing of chicks was done in a clean well-ventilated room, which adjusted according to age by electric heaters. The birds were vaccinated by Hitchner IB (7^{th} day), Gumbro (14^{th} day) and Gumbro and clone (21^{st} day) by eye drop.

2.2. Diet and experimental design

The chicks were fed on the two phases feeding programs from 1^{st} to 21^{st} days on the starter and from 22^{nd} to 35^{th} days on grower diets. The control diet composition was represented in Table 1 and analyzed according to AOAC (2005). The diet was formulated to meet the requirements of NRC (1994). Thyme was obtained from a local market, washed, ground, and mixed with the ration at the concentration of 0.0, 0.5, 1 and 1.5%: these were allocated as Control, Thyme I, Thyme II and Thyme III, respectively. All birds were accessed water *ad libitum*.

2.3. Gas chromatography–mass spectrometry (GC-MS) analysis

The fine powder of thyme was activated by microwave at 50°C for 5 min and extracted with *n*-hexane by a dilution factor of 1: 3 (*v*: *v*). 10 µl of thyme *n*-hexane extract was injected in Trace GC Ultra-ISQ mass spectrometer with a direct capillary column TG–5MS (30 m×0.25 mm×0.25 µm). Column temperature, 60°C (1 min) to 180°C at 3°C/min; injector temperature, 220°C; detector temperature, 220°C; split ratio, 1:10; carrier gas, helium; flow rate, 1.0 ml/min (Fachini-Queiroz *et al.*, 2012). The mass spectra of the identified components were determined by comparison to the Wiley Registry of mass spectral database 8^{th} edition.

2.4. Serum parameters

The blood samples at the 3^{rd} and 5^{th} weeks were collected from wing vein (*n*=15). Each blood sample was left to coagulate at room temperature and centrifuged at 3000 rpm for 5 min. The collected sera were subjected to determination of total protein, albumin, alanine aminotransferase (ALT, EC 2.6.1.2), creatinine, cholesterol, and triacylglycerol (TAG) following the instructions enclosed in the manufactured kits (Biodiagnostic Co., Cairo, Egypt). Also, serum globulin levels were calculated by subtraction of albumin value from the total protein value of the same sample (Coles, 1986).

2.5. ELISA assays

The serum levels of immunoglobulin A (IgA), immunoglobulin G (IgG), immunoglobulin M (IgM), Interferon-γ (INF-γ), and interleukin-10 (IL-10) were determined by ELISA kits following the instructions enclosed in the manufactured kits (Elabscience Co., Wuhan, China).

2.6. Preparation of muscle tissue homogenate

Twenty-four hours after the end of the experimental period, the broilers of control and experimental groups (*n*= 15) were sacrificed under anesthesia with an intramuscular injection of sodium

pentobarbital (50 mg/kg BW), and then muscle samples from left breast and left thigh of each bird were immediately dissected and soaked in ice-cold saline 0.9%. They were homogenized using a motor-driven Teflon and glass Potter-Elvehjem homogenizer in 0.1 M Tris-HCl buffer of pH 7.4 containing 5 mM β-mercaptoethanol (1:4 *w*/*v*). The homogenates were centrifuged at 105,000 ×g for 60 min at 4°C; the supernatants were divided into aliquots then stored at -20°C for further evaluation of oxidative stress and antioxidant status.

2.7. Determination of oxidative stress parameters

The aliquots of muscle homogenates were utilized for the colorimetric assessment of malondialdehyde (MDA) and reduced glutathione (GSH) contents, as well the total superoxide dismutase (T.SOD) and glutathione S-transferase (GST) activities.

2.1.1. Determination of lipid peroxidation

Malondialdehyde is the main aldehyde by-product of lipid peroxidation in biological systems. It was analyzed after the incubation of supernatants with thiobarbituric acid at 95°C for 30 min (pH 3.6) to form thiobarbituric acid-reactive substances (TBARS), a pink colored compound. MDA levels were measured at 532 nm and expressed as nmol MDA/mg protein (Ohkawa *et al.*, 1979).

2.7.2. Determination of reduced glutathione levels

Reduced glutathione assay was based on the reductive cleavage of DTNB [5, 5′-dithiobis (2-nitrobenzoic acid)] by compounds containing sulfhydryl groups and development of a yellow color(Sedlak and Lindsay, 1968). The quantity of reduced chromogen is directly proportional to the GSH content. The absorbance was recorded at 412 nm and expressed as µmol GSH/mg protein.

2.7.3. Determination of the total superoxide dismutase activity

The reduction of nitro blue tetrazolium with NADH-mediated by phenazine methosulfate (PMS) under aerobic conditions was inhibited upon addition of superoxide dismutase. This observation indicated the involvement of superoxide anion radical in the reduction of nitro blue tetrazolium, the radical being generated in the reoxidation of reduced PMS. This assay was determined at 560 nm and represented as U/mg protein (Nishikimi *et al.*, 1972).

Table 1. The starter and grower diet's ingredients percentage and calculated composition (as fed basis)

Ingredients	Starter diet	Grower diet
Corn	52.87	60.47
SBM (CP 44%)	34.26	29.31
Corn gluten (CP 60%)	5.5	3.0
Corn oil	3.3	3.26
Limestone	1.35	1.53
Dicalcium phosphate	1.74	1.47
L-Lysine	0.11	0.13
Dl-methionine	0.17	0.13
Vitamins and minerals premix	0.3	0.3
NaCl	0.4	0.4
Total	100	100
Composition		
ME (Kcal/Kg diet)	3061.2	3119.35
CP %	23.0	20.0
Calorie/protein ratio	133.1	155.97
Lysine %	1.3	1.16
Methionine %	0.58	0.48
Calcium %	1.0	0.9
Av. (P) %	0.45	0.40
NaCl	0.15	0.15

SBM= Soybean meal, ME = Metabolizable Energy, CP = crude protein, Av. (P) = Available phosphorous

*L-lysine 99% feed grade

**Dl-methionine 99% feed grade China

***Vitamin and mineral premix (Hero mix) produced by Hero pharm and composed (per 3 kg) of vitamin A 12000000 IU, vitamin D3 2500000 IU, vitamin E 10000 mg, vitamin K3 2000 mg, vitamin B1 1000 mg, vitamin B2 5000 mg, vitamin B6 1500 mg, vitamin B12 10 mg, niacin 30000 mg, biotin 50 mg, folic acid 1000 mg, pantothenic acid 10000 mg, manganese 60000 mg, zinc 50000 mg, iron 30000 mg, copper 4000 mg, iodine 300 mg, selenium 100 mg, and cobalt 100 mg.

2.7.4. Determination of the glutathione S-transferase activity

The activity of GST was measured according to the method of Vessey and Boyer (1984). This assay was based on monitoring the rate of enzyme–catalyzed conjugation of the CDNB [1-chloro-2,4-dinitrobenzene] with GSH. GST activity was measured as the increase in absorbance at 340 nm and represented as l mol CDNB/min/mg protein (A€=9.6/mM/cm).

2.8. Determination of tissue protein

Protein concentrations in muscle homogenates were determined using bovine serum albumin as the standard according to the method of Bradford (1976).

2.9.Performance parameters

The basal diets of starter and grower phases were formulated according to the recommendation of National Research Council Nutrient Requirements for Broiler Chickens (NRC, 1994). Performance parameters including the final body weight, feed intake, feed conversion ratio (FCR) (Lambert *et al.*, 1936) and protein efficiency ratio (McDonald *et al.*, 1987) were determined throughout the whole experimental period.

2.10Statistical analyses

The obtained data were analyzed by one-way analysis of variance (ANOVA), with Duncan's multiple range tests for significant between means using SPSS software package v.20. The data of ELISA assays and antioxidant status were analyzed by One-way ANOVA, Tukey's multiple range tests by Graphpad prism 5. All declarations of significance depended on $p < 0.05$.

3. RESULTS

The data presented in Table (2) and illustrated in Fig. (1) were shown the chemical composition of thyme powder using the GC–MS analysis, which revealed the identification of eleven different components; isocaryophyllene (33.73%), eugenyl acetate (15.85%), eugenol (12.56%), isoeugenol (11.72%), δ-cadinene (5.48%), α-copaene (5.09%), α-humulene (3.90 %), caryophyllene oxide (3.91%), α-cadinene (3,05%), calamenene (1.52%), 2',3',4'-trimethoxyacetophenone (0.69%), β-cadinene (0.76%), cadina-1(2),4-diene (0.67%), azulene (0.24%), α-cubebene (0.60%), and 10,10-dimethylanthrone (0.23%).

The data in Table (3) explained that, at the 3rd-week, the total protein and globulin levels were significantly ($p<0.05$) increased in the thyme groups compared to control, while serum albumin, ALT, and creatinine levels had no significant ($p>0.05$) changes. Serum total cholesterol and TAG levels were significantly ($p<0.05$) decreased in the thyme groups in comparison with that of to control values.

The data illustrated in Fig. (2A) represented that dietary thyme supplementation was non-significantly ($p>0.05$) increased the serum IgA at the 3rd and 5th weeks when compared to control. Fig. (2B) indicated that thyme supplementation was significantly increased serum IgG at the 3rd week, in a concentration-dependent manner with non-significant effect at the 5th week when compared to control. The data are shown in Fig. (2C) revealed that, at the 3rd week, thyme-containing diets (0.5 and 1%) was significantly ($p<0.05$) enhanced the serum IgM, while at 1.5%, it was non-significantly ($p>0.05$) increased compare to control. At the 5th week, the IgM levels were significantly increased in Thyme I ($p<0.01$), Thyme II ($p<0.01$), and Thyme III ($p<0.001$) compared to controls.

Table 2. GC-MS analysis of thyme n-hexane extract

	Compound Name	RT (minutes)	Area %
1	α-cubebene	17.10	0.60
2	α-copaene	19.16	5.09
3	Isocaryophyllene	22.14	33.73
4	Eugenol	23.68	12.56
5	Isoeugenol	24.83	11.72
6	α-Humulene	25.38	3.90
7	β-cadinene	25.62	0.76
8	10,10-Dimethylanthrone	25.90	0.23
9	δ-cadinene	26.15	5.48
10	Calamenene	26.85	1.52
11	α-cadinene	27.01	3.05
12	Azulene	27.23	0.24
13	Cadina-1(2),4-diene	27.51	0.67
14	Eugenyl acetate	28.86	15.85
15	Caryophyllene oxide	29.69	3.91
16	2',3',4'-Trimethoxyacetophenone	33.79	0.69

Table 3. Effect of dietary thyme supplementation on serum total protein, albumin, globulin, ALT, Creatinine, total cholesterol and triacylglycerol

Item	Control	Thyme supplementation		
		Thyme I	Thyme II	Thyme III
3rd week				
Total protein (g/dl)	4.33±0.06^{b}	5.02±0.29^{a}	5.33±0.08^{a}	5.42±0.04^{a}
Albumin (g/dl)	3.46±0.16^{a}	3.4±0.08^{a}	3.35±0.1^{a}	3.41±0.1^{a}
Globulin (g/dl)	0.87±0.2^{b}	1.63±0.24^{a}	1.98±0.09^{a}	2.01±0.1^{a}
ALT(U/L)	17.25±0.63^{a}	16.5±1.04^{a}	15±0.91^{a}	16±0.71^{a}
Creatinine (mg/dl)	0.3±0.03^{a}	0.26±0.07^{a}	0.35±0.03^{a}	0.36±0.04^{a}
Total cholesterol (mg/dl)	160.45±6.62^{a}	121.75±8.25^{b}	115.75±7.79^{b}	120.83±2.28^{b}
TAG (mg/dl)	178.84±7.58^{a}	151.5±10.85^{b}	139.25±8.73^{b}	138.55±4.54^{b}
5th week				
Total protein (g/dl)	4.46±0.06^{b}	4.61±0.15^{b}	4.76±0.11^{ab}	5.15±0.2^{a}
Albumin (g/dl)	3.25±0.11^{a}	3.17±0.12^{a}	3.16±0.11^{a}	3.13±0.09^{a}
Globulin (g/dl)	1.22±0.06^{b}	1.44±0.07^{b}	1.6±0.2^{ab}	2.02±0.22^{a}
ALT (U/L)	18.25±1.03^{a}	18.25±1.03^{a}	15.25±0.75^{a}	15.5±0.87^{a}
Creatinine (mg/dl)	0.32±0.03^{a}	0.32±0.02^{a}	0.37±0.03^{a}	0.36±0.04^{a}
Total cholesterol (mg/dl)	155.07±1.89^{a}	123.67±1.03^{b}	113.5±7.75^{b}	119±7.08^{b}
TAG (mg/dl)	170.74±4.29^{a}	147.75±7.98^{b}	136±2.35^{b}	139±1.78^{b}

Mean values with different letters in the same row differ significantly at P<0.05

Table 4. Effect of thyme dietary supplementation on growth performance of broiler chickens

	Control	Thyme supplementation		
		Thyme I	Thyme II	Thyme III
BW (1st week)	168.86±2.98[a]	173.86±2.28[a]	169.79±2.36[a]	172.83±2.15[a]
FBW (g)	1728.41±27.68[a]	1748.64±30.86[a]	1735.63±37.11[a]	1687.17±27.36[a]
BWG (g)	1559.55±24.82[a]	1574.77±28.7[a]	1565.83±34.81[a]	1514.35±25.43[a]
FCR	1.87±0.03[a]	1.81±0.03[a]	1.83±0.04[a]	1.90±0.03[a]
FI (g)	2897±23.15 [a]	2852±19.88 [a]	2854±16.33 [a]	2882±25.42 [a]
PER	2.61±0.04[a]	2.67±0.04[a]	2.64±0.05[a]	2.61±0.04[a]

Mean values with different letters in the same row differ significantly at P<0.05
BW = body weight; FBW = final body weight; BWG = body weight gain; FI= Feed intake; FCR = feed conversion ratio; PER = protein efficiency ratio

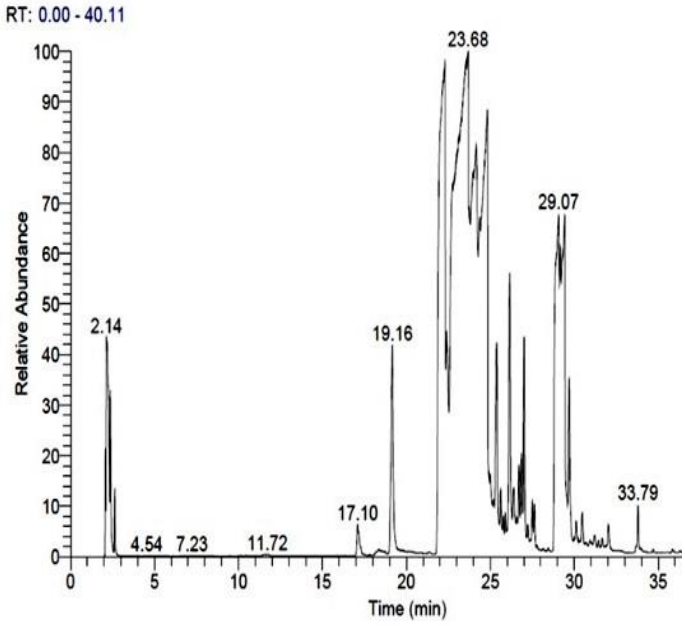

Fig. 1. GC-MS analysis chromatogram of thyme *n*-hexane extract

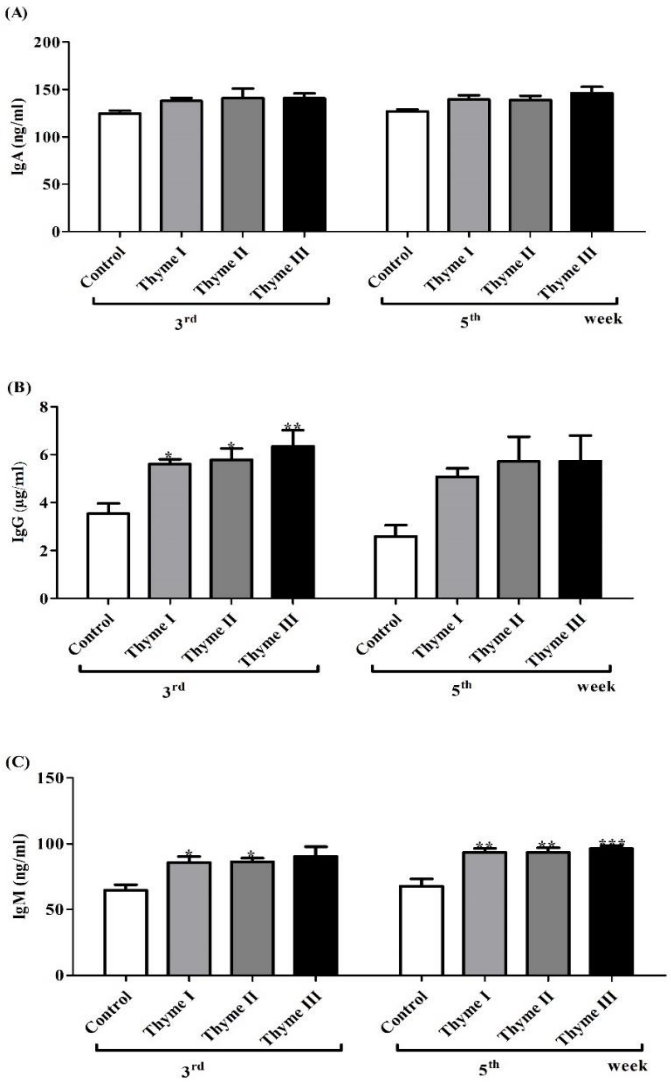

Fig. 2. Represents the effects of thyme on serum (A) IgA, (B) IgG, and (C) IgM. $^{*}p < 0.05$, $^{**}p < 0.01$ and $^{***}p < 0.001$ vs. control.

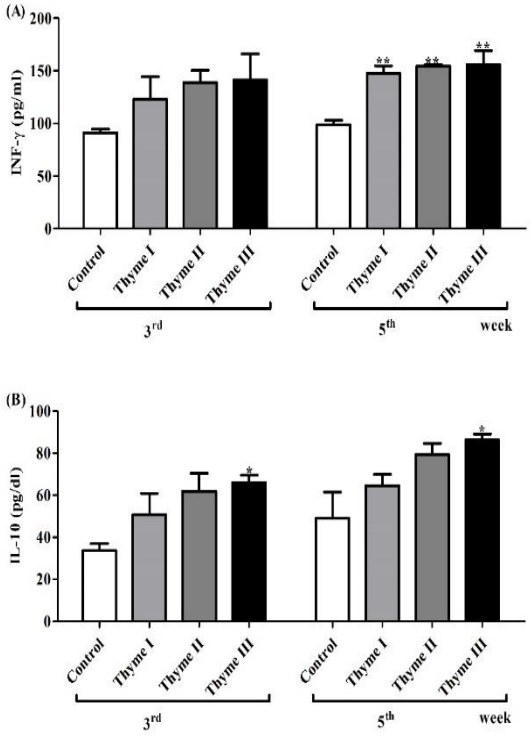

Fig. 3. Represents the effects of thyme on (A) INF-γ and (B) IL-10. $^{*}p < 0.05$ and $^{**}p < 0.01$ vs. control.

Regarding the non-specific immune parameters, the serum INF-γ levels were increased non-significantly ($p>0.05$) at the 3rd week and significantly ($p<0.01$) at the 5th-week upon the dietary inclusion of thyme at different concentrations when compared to control (Fig. 3A). The data illustrated in Fig. (3B) represented that the serum IL-10 levels were increased significantly ($p<0.05$) at the higher concentration (1.5% thyme) and non-significantly ($p>0.05$) at the lower concentrations (0.5, 1% thyme) at the 3rd and 5th weeks when compared to control.

The data are shown in Fig. (4A) revealed that breast muscle contents of MDA were significantly reduced following thyme supplementation, in a concentration-dependent manner with the highest reduction observed at thyme 1.5% ($p< 0.01$) in comparison with control group. The MDA levels in thigh muscles were non-significantly decreased ($p>0.05$) in Thyme 0.5% and 1.5%, while significantly decreased ($p<0.05$) in thyme 1% compared to control.

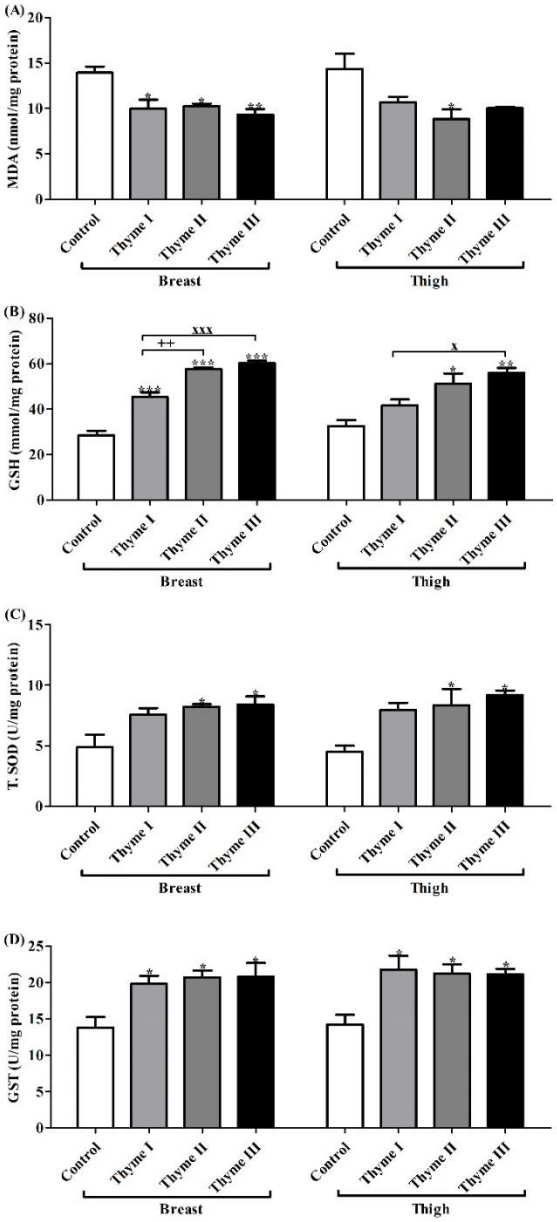

Fig. 4. Represents the effects of thyme on (A) MDA, (B) GSH, (C) T. SOD, and (D) GST. $^{*}p < 0.05$, $^{**}p < 0.01$, and $^{***}p < 0.001$ vs. control; $^{++}p< 0.01$vs Thyme II; $^{x}p < 0.05$ and $^{xxx}p< 0.001$ vs Thyme III.

Results are shown in Fig. (4B) represented that the level of GSH in breast and thigh muscles was significantly ($p<0.05$) enhanced following thyme supplementation as compared to control, with maximum induction observed at the higher concentration. The results are shown in Fig. (5C) explored that the breast and thigh muscles T.SOD

activities were increased significantly ($p<0.05$) in thyme 1 and 1.5%, but non-significantly ($p>0.05$) in thyme 0.5% when compared to control. Fig. (4D) indicated that the breast muscle GST activities were significantly increased ($p<0.05$) in thyme 1 and 1,5%, and non-significantly increased ($p>0.05$) in thyme 0.5% compared to control. The thigh muscle GST activities were significantly increased ($p<0.05$) in the thyme-supplemented broilers compared to control.

Results are shown in Table (4) claimed that dietary thyme supplementation for broiler chickens had no significant changes in performance parameters, only a slight improvement was recorded in thyme 0.5% compared to control and higher concentrations.

4. DISCUSSION

The GC-MS analysis of thyme verified that the isocaryophyllene, major active ingredient of thyme is the highest area percentage (33.73%) on the chromatogram. Caryophyllenes, including *β*-caryophyllene, *α*-caryophyllene, *α*-humulene, and γ-caryophyllene (isocaryophyllene) are sesquiterpenes present in various essential oils such as *Eugeniacaryophyllus*, *Humulus lupulus*, and *Teucrium marum* (Ricci *et al.*, 2005; Jirovetz *et al.*, 2006). Natural bicyclic *β*-caryophyllene and isocaryophyllene are *trans* and *cis* double isomers, respectively, while *α*-humulene is a ring-opened isomer. These molecules possess anti-inflammatory (Cho *et al.*, 2007), anti-carcinogenic (Zheng *et al.*, 1992), and antioxidant (Asbaghian *et al.*, 2011).

Serum total protein which consists of albumin and globulin can effectively reflect the protein metabolism, feed condition, and growth of animals. Globulins are responsible for humoral immunity. In the present study, thyme was significantly increased the serum total protein and globulin levels. These results came in accordance with the findings of El-Ghousein and Al-Beitawi (2009) who found that the serum levels of total protein and globulin were significantly increased by the addition of thyme to broiler chickens. Zhu *et al.* (2016) studied that thyme essential oil markedly increases serum total proteins and globulins at the 21st day.

Serum ALT activity is very low under normal conditions; however, it was increased in the presence of liver damages or an increase in the permeability of liver cells. The obtained data explained that, at the 3rd and 5th weeks, serum ALT activity and creatinine content were non-significantly changed in thyme-treated broilers. It has been reported that the ALT activity had been decreased upon thyme essential oil admin (Zhu *et al.*, 2016), and the addition of thyme to broiler diets (Tollba, 2003). Hence, the supplementation of thyme in broiler's diet has no harmful effect on liver and kidney functions.

Birds fed on dietary thyme at given levels resulted in significant reduction in serum total cholesterol and TAG. It has been also found They were decreased in broilers fed 2% thyme (El-Ghousein and Al-Beitawi, 2009) and in laying hens fed 0.25% thyme (Ali *et al.*, 2007). Furthermore, Al-Kassie (2009) found that feeding thyme oil at the rate of 100 and 200 ppm decreased plasma cholesterol level. (Ali, 2014) found that supplementing broiler diet with 0.5%, 1% or 1.5% thyme leaves powder decreased cholesterol level. The hypocholesterolemic and antihyperlipidemic effect of thyme may be due to the action of thymol and carvacrol on HMG-CoA reductase, the rate-limiting enzyme of cholesterol, which reduced fat absorption from the gut or the lipid catabolism for gluconeogenesis (El-Ghousein and Al-Beitawi, 2009; Abdulkarimi *et al.*, 2016).

The results of the present study revealed that dietary thyme was significantly increased the serum levels of IgG, IgM and INF-γ levels in broilers. While thyme 1.5% supplementation was significantly increased the levels of serum IL-10. Herbs that are rich in flavonoids as thyme extend the activity of vitamin C, act as antioxidants and may, therefore, enhance the immune function (Acamovic and Brooker, 2007). Nadia *et al.* (2008) found that 0.1% thyme-fed to laying hens gave better antibody production response compare to 100 or 200 mg/kg vitamin E which is a potent immunomodulation.

The balance between reactive oxygen species (ROS) and the intrinsic antioxidant defenses decides the cellular antioxidant activity (Burton and Jauniaux, 2011). Lipid peroxidation leads to the formation of various products such as MDA (Droge, 2002). The results of the current study showed that the supplementation of thyme to the broiler's diet reduced MDA levels in breast and thigh muscles. Interestingly, the tissue GSH content and T.SOD, and GST activities were significantly increased improved in breast and thigh muscle. These results agree with the findings of El-Hack and Alagawany (2015) who found that serum

SOD activity and GSH levels were significantly increased in the groups fed diets with thyme. The muscle MDA levels were significantly decreased by the addition of 9 g thyme/kg. It has been suggested that the high biological activity of thyme as a natural antioxidant is attributed to the presence of phenolic hydroxyl groups that serve as a hydrogen donor to the proxy radicals produced in the first stage of lipid oxidation, thus inhibiting the formation of hydroxyl peroxide (Hashemipour *et al.*, 2013).

The obtained data showed that the average body weight, body weight gain, feed intake, feed conversion ratio, and protein efficiency ratio of broilers were non-significantly improved in low thyme concentration of dietary supplementation compared to control and higher concentrations. The results of the present study agree with previous observation that indicated herbs, plant extracts, essential oil and/or the main components of the essential oil that did not affect body weight gain, feed intake or feed efficiency in broilers (Botsoglou *et al.*, 2004; Bampidis *et al.*, 2006; Cross *et al.*, 2007). Ocak *et al.* (2008) found that body weight gain, feed intake or feed conversion ratio were not significantly affected by dietary supplementation of thyme leaves. Hosseini *et al.* (2016) found that body weight, feed consumption and feed conversion ratio were not affected by dietary supplementation of 5 g/kg and 7.5 g/kg ground thyme compared to the control birds.

5. CONCLUSION

From the obtained results, it could be concluded that supplementing thyme leaves powder into broiler diet showed no significant effect on growth performance. Remarkably, it, in a concentration-dependent manner, increased immunity and antioxidant activity in the broiler chickens producing a meat with fewer quantities of MDA.

6. Competing Interests

The authors have no conflict of interest.

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