

## Ginger (*Zingiber officinale*) and Thymol Dietary Supplementation Improve the Growth Performance, Immunity and Antioxidant Status in Broilers

<sup>1</sup>Doha E. Zidan, <sup>2</sup>Khaled A. Kahilo, <sup>1</sup>Ali H. El-Far and <sup>1</sup>Kadry M. Sadek

<sup>1</sup>Department of Biochemistry, Faculty of Veterinary Medicine,  
Damanhour University, Damanhour, Egypt

<sup>2</sup>Department of Biochemistry, Faculty of Veterinary Medicine,  
Kafrelsheikh University, Kafrelsheikh, Egypt

**Abstract:** This study was conducted to investigate the effects of dietary supplementation of ginger (*Zingiber officinale*) and thymol on the performance, antioxidant status and immune response in broilers for six weeks. Two hundred and seventy of one-day old broiler chicks were randomly assigned to six dietary groups as follows: control (basal diet), Ginger I (5 g ginger powder/kg diet), Ginger II (10 g ginger powder/kg diet), Ginger III (15 g ginger powder/kg diet), Thymol I (200 mg thymol /kg diet) and Thymol II (400 mg thymol /kg diet). Each group was allocated into three replicates of fifteen birds per replicate. The overall body weight gains of birds treated with feed additives were significantly ( $P < 0.05$ ) increased higher in relation to control one. The birds in Ginger III group gave the best feed conversion ratio (FCR) followed by Ginger I, Thymol I and then the control group. In addition, ginger and thymol were significantly decreased the levels of serum total cholesterol and triacylglycerol. Moreover, supplementation of broilers by ginger and thymol significantly decreased the breast and thigh malondialdehyde (MDA) and significantly increased muscle reduced glutathione (GSH) serum interferon gamma (INF- $\gamma$ ) and interleukin 2 (IL-2) levels.

**Key words:** Ginger • Thymol • Broiler • Performance • Immunity • Antioxidant Status

### INTRODUCTION

Antimicrobial compounds produced by microorganisms have been used in animal rations as growth promoters for many years [1]. Antibiotics have been used widely to prevent poultry diseases and for the improvement of meat and egg production [2]. However, use of antibiotics is restricted due to drug resistance in bacteria, drug residue in carcass and also alteration of natural gut microflora [3]. Consequently, the use of natural promoters such as probiotic, prebiotic, symbiotic, enzymes, organic acids, oligosaccharides, phytogetic and other feed additives, to enhance the growth and performance of broiler is the deal for poultry feeding [4].

Ginger (*Zingiber officinale*) is a member of the *Zingiberaceae* family that used as a common spice in most of the Asian countries [5]. Traditionally, ginger has been used as herbal medicine to treat vomiting, pain and indigestion and possesses an anticancer, anticlotting, anti-inflammatory and analgesic activities [6, 7].

Ginger can be used as potential alternative for common artificial growth promoters like antibiotics [8]. Preliminary research indicates that nine compounds found in ginger may bind to serotonin receptors which may influence gastrointestinal function [3]. Research conducted *in-vitro* tests show that ginger extract might control the quantity of free radicals by neutralization [9, 10]. The characteristic odor and flavor of ginger is caused by a mixture of zingerone, shogaol and gingerol [11, 12].

Thymol, [5-methyl-2-(1-methylethyl) phenol], is the main component of thyme essential oil that has a strong antioxidant activity [13]. A dietary supply of thymol to aging rats showed a beneficial effect on the antioxidative enzymes superoxide dismutase (SOD) and glutathione peroxidase (GSH) [14]. Thymol has been reported to stimulate digestive secretions such as salivary amylase in humans and bile acids, gastric, pancreatic enzymes (i.e. lipase, amylase and proteases) and intestinal mucosa in rats [15].

This study was conducted to investigate the effect of ginger and thymol as feed additives on performance, antioxidant status and immunity in broilers.

## MATERIALS AND METHODS

**Birds, Diets and Managements:** Two hundred and seventy of unsexed one-day old broiler chicks were obtained from local commercial hatchery were allocated into six groups, each of three replicates consisting of fifteen chicks per replicate and reared on the floor for six weeks. The control corn-soybean based diet was prepared based on NRC [16] recommendations as illustrated in Table 1. For preparing other treatments, the control diet was blended with ginger and thymol to prepare diets for Ginger I, Ginger II, Ginger III and Thymol I, Thymol II groups at the concentrations of 5 g, 10 g, 15 g, 200 mg and 400 mg/kg diet, respectively. No antibiotic were used all over the experiment. Feed and water were provided *ad libitum* during the whole periods.

Table 1: The ingredient percentages and calculated composition analysis of the experimental starter and grower diets (as fed basis)

Ingredients	Starter diet	Grower diet
Corn	52.55	60.27
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	0.39	0.23
Vitamins and minerals premix	0.3	0.3
NaCl	0.5	0.5
Total	100	100
Calculated and analyzed 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 %	0.8	0.58
Calcium %	1.0	0.9
Av. (P) %	0.45	0.40
NaCl	0.15	0.15

SBM= Soybean meal, ME = Metabolizable Energy (Kcal/ kg diet), 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 Heropharm 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.

Birds were housed on the floor where the temperature was maintained at 32 °C during the 1<sup>st</sup> week and then was reduced by 2 °C per week until 24 °C was reached and this temperature was maintained until the end of the experiment. Relative humidity of the room was about 70-80 %.

All of the chicks were vaccinated against Newcastle and infectious bursal disease at 7<sup>th</sup> and 14<sup>th</sup> days of age, respectively. Moreover, chicks were vaccinated again by both vaccines at 21<sup>st</sup> days of age.

**Preparation of Ginger Extract:** The fine powder of ginger roots were extract by methanol according to the method of Shyamala [17] with some modifications. Briefly, 15 g of dried ginger roots were extracted with 100 mL of methanol for 24 h with occasional shaking. The extract was filtered and evaporated to dryness in vacuum.

### Gas Chromatography-mass Spectrometry (GC-MS)

**Analysis:** The chemical composition of ginger roots was performed using Trace GC Ultra-ISQ mass spectrometer with a direct capillary column TG-5MS (30 m×0.25 mm×0.25 µm film thickness). The column oven temperature was initially held at 60 °C and then increased by 5 °C per minute to 280 °C. The injector and detector temperatures were kept at 250 °C. Helium was used as a carrier gas at a constant flow rate of 1 ml/minute for 51.10 minutes. The solvent delay was 2 minute and diluted samples of 1 µl were injected automatically using auto-sampler AS3000 coupled with GC in the splitless mode. EI mass spectra were collected at 70 eV ionization voltages over the range of m/z 50–650 in full scan mode. The ion source and quadrupole temperatures were set at 200 and 150 °C. The components were identified by comparison of their retention times and mass spectra with those of NIST 11 mass spectral database.

**Performance Parameters:** Body weight gain (BWG) and feed intake (FI) of chicks were recorded weekly during the experiment. The chicks were inspected daily and the dead birds were removed following registration of the date and body weights. The feed conversion ratio (FCR) was calculated as the BWG (g) per FI (g) [18]. When calculating FCR, the body weights of dead birds were also considered.

**Biochemical Analysis:** Blood samples were collected from the wing vein at 14<sup>th</sup>, 24<sup>th</sup> and 42<sup>nd</sup> days of experiment. The collected samples were centrifuged at 3000 RPM for 5 minutes. Collected serum samples were subjected to determination of total cholesterol [19], triacylglycerols (TAG) [20], alanine transaminase (ALT) and aspartate

transaminase (AST) [21], creatinine [22] and uric acid [23]. Moreover, interferon gamma (INF- $\gamma$ ) and interleukin 2 (IL-2) were determined by ELISA kits manufactured by Elabscience Co.

At the end of the experimental period, five birds of each replicate were sacrificed and samples of 10 g each were taken from breast and thigh muscles. Muscle samples were homogenized and centrifuged at 3000 RPM for 15 minutes. The clear supernatants were subjected to determination of MDA [24] and GSH [25].

**Statistical Analysis:** Analysis of variance was performed on the data using the general linear model of SAS software (2002). Means were compared using Duncan's multiple range tests. Level of significance used in all results was  $P < 0.05$ .

## RESULTS

The data presented in Figure 1 and illustrated in Table 2 revealed some active components of the methanolic extract of ginger root analyzed by GC-MS.

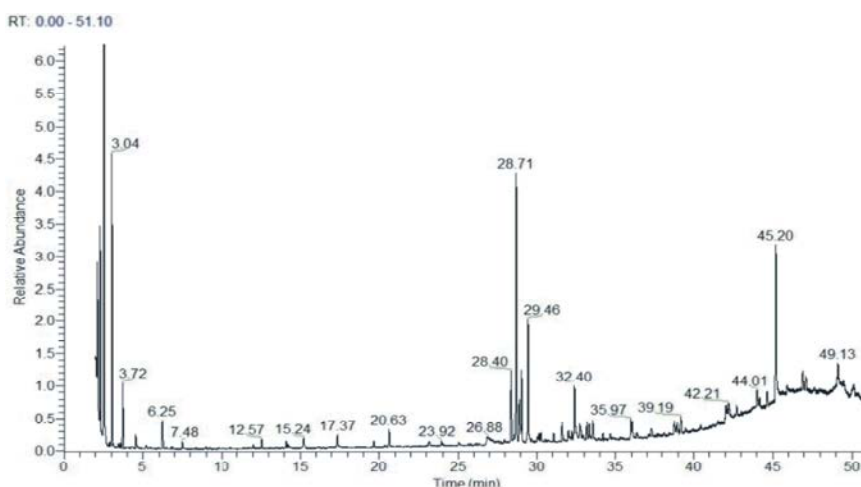


Fig. 1: GC-MS chromatogram of ginger methanolic extract

Table 2: Showed some antioxidant active ingredients of ginger methanolic extract

Peaks	Retention time (minutes)	Name	Area %
1	12.57	Camphene	0.10
2	15.24	$\alpha$ -phellandrene	0.18
3	20.63	Decanal	0.20
4	26.14	Carotene	0.04
5	26.88	Quercetin 7,3',4'-Trimethoxy	0.20
6	28.40	ar-curcumene	0.78
7	28.59	Germacrene D	0.03
8	28.71	Zingiberene	3.01
9	28.91	Farnesene	0.58
10	29.05	Caryophyllene	0.80
11	29.46	$\beta$ -sesquiphellandrene	1.39
12	31.08	Epiglobulol	0.10
13	32.40	Zingerone	0.67
14	31.62	Cubenol	0.27
15	32.22	Diepicedrene-1-oxide	0.10
16	32.74	Squalene	0.24
17	33.97	Rhodopin	0.03
18	35.39	Lycopene	0.02
19	35.97	trans-longipinocarveol	0.21
20	37.28	Lycoxanthin	0.13
21	43.26	Spherodenon	0.02
22	45.20	Gingerol	1.99
23	48.70	Cantaxanthin	0.01
24	49.13	Milbemycin B	0.34

The obtained data cleared the presence of some antioxidant agents in ginger extract as zingiberene, ar-curcumene,  $\alpha$ -phellandrene, gingerol and zingerone. In addition, carotenoids as carotene, lycopene and lycoxanthin of antioxidant and immunostimulant potentials also were identified in ginger extract.

The data illustrated in Table (3) clarify a high significant increased ( $P < 0.01$ ) in feed intake at 1<sup>st</sup> week (Thymol II group) and in 3<sup>rd</sup> and 4<sup>th</sup> weeks (Thymol I and Ginger III groups) and 6<sup>th</sup> week (Ginger I, Ginger III and Thymol I groups). Generally, the highest feed intake was recorded in the Ginger III group (2049 g) and the lowest value was noticed in Thymol II group (1804 g) in comparison to control (1896 g).

The data presented in Table (4) indicated that weight gains were highly significantly increased ( $P < 0.01$ ) in 4<sup>th</sup> week and 5<sup>th</sup> week Thymol I group. Also, the weight gains were significantly increased ( $P < 0.05$ ) at 5<sup>th</sup> week of experiment in Ginger III and Ginger I groups. The total weight gains were highly significantly increased ( $P < 0.01$ ) in Ginger III then Thymol I and Ginger I groups when compared to the control group. The data recorded in

Table 3: Effect of dietary supplementation of ginger and thymol on feed intake (g/bird/week) in broilers

	1 <sup>st</sup> week	2 <sup>nd</sup> week	3 <sup>rd</sup> week	4 <sup>th</sup> week	5 <sup>th</sup> week	6 <sup>th</sup> week
Control	143.00±2.97 <sup>b</sup>	355.83±6.52 <sup>a</sup>	804.67±20.47 <sup>b</sup>	1330.00±25.45 <sup>ab</sup>	1628.83±44.49	1896.00±47.74 <sup>bc</sup>
Ginger I	142.50±2.17 <sup>b</sup>	344.17±10.52 <sup>a</sup>	828.83±18.45 <sup>ab</sup>	1333.17±25.33 <sup>ab</sup>	1650.00±36.72	1938.33±36.82 <sup>ab</sup>
Ginger II	142.00±4.18 <sup>b</sup>	336.67±9.53 <sup>a</sup>	811.17±20.74 <sup>ab</sup>	1293.17±36.79 <sup>b</sup>	1651.00±33.56	1904.00±33.38 <sup>bc</sup>
Ginger III	144.50±3.01 <sup>ab</sup>	348.67±6.55 <sup>a</sup>	852.17±18.36 <sup>a</sup>	1354.00±33.27 <sup>a</sup>	1691.83±37.78	2049.83±37.89 <sup>a</sup>
Thymol I	141.17±2.75 <sup>b</sup>	349.67±10.17 <sup>a</sup>	869.33±18.18 <sup>a</sup>	1381.17±23.35 <sup>a</sup>	1704.83±40.01	2008.17±47.11 <sup>ab</sup>
Thymol II	152.50±3.33 <sup>a</sup>	341.67±7.66 <sup>a</sup>	822.67±21.89 <sup>ab</sup>	1262.83±21.42 <sup>b</sup>	1586.67±33.04	1804.33±35.75 <sup>c</sup>

Means within the same column carry different superscripts are significantly different (P<0.05).

Table 4: Effect of dietary supplementation of ginger and thymol on weight gain (g) in broilers

	1 <sup>st</sup> week	2 <sup>nd</sup> week	3 <sup>rd</sup> week	4 <sup>th</sup> week	5 <sup>th</sup> week	6 <sup>th</sup> week	Total WT Gain
Control	212.83±4.00 <sup>a</sup>	267.17±8.56 <sup>c</sup>	298.83±20.86 <sup>c</sup>	448.83±14.26 <sup>b</sup>	435.33±12.92 <sup>a</sup>	416.00±13.9 <sup>ab</sup>	1753.00±44.86 <sup>cd</sup>
Ginger I	201.67±8.62 <sup>b</sup>	288.33±2.53 <sup>b</sup>	316.83±14.82 <sup>bc</sup>	484.67±9.09 <sup>ab</sup>	504.33±9.20 <sup>a</sup>	524.00±9.2 <sup>a</sup>	1795.83±34.81 <sup>ab</sup>
Ginger II	194.67±5.86 <sup>ab</sup>	253.00±2.29 <sup>c</sup>	357.83±13.50 <sup>ab</sup>	474.50±11.61 <sup>ab</sup>	482.00±17.22 <sup>ab</sup>	490.00±12.01 <sup>ab</sup>	1762.00±29.67 <sup>bc</sup>
Ginger III	204.17±4.05 <sup>ab</sup>	358.00±1.41 <sup>a</sup>	367.83±10.85 <sup>a</sup>	483.50±12.75 <sup>ab</sup>	491.83±17.56 <sup>ab</sup>	499.00±13.5 <sup>ab</sup>	1905.33±35.15 <sup>a</sup>
Thymol I	208.50±7.62 <sup>ab</sup>	303.33±11.83 <sup>b</sup>	323.67±20.99 <sup>ab</sup>	519.67±8.64 <sup>a</sup>	511.83±8.23 <sup>a</sup>	522.00±9.14 <sup>a</sup>	1867.00±44.52 <sup>ab</sup>
Thymol II	189.17±4.97 <sup>c</sup>	217.67±6.72 <sup>nd</sup>	323.83±13.05 <sup>ab</sup>	481.00±14.8 <sup>b</sup>	440.17±5.33 <sup>c</sup>	465.00±6.4 <sup>b</sup>	1651.83±32.54 <sup>d</sup>

Means within the same column carry different superscripts are significantly different (P<0.05)

Table 5: Effect of dietary supplementation of ginger and thymol on the feed conversion ratio (FCR) in broilers

	1 <sup>st</sup> week	2 <sup>nd</sup> week	3 <sup>rd</sup> week	4 <sup>th</sup> week	5 <sup>th</sup> week	6 <sup>th</sup> week
Control	1.26±0.07 <sup>bc</sup>	1.11±0.03 <sup>ab</sup>	1.72±0.04 <sup>b</sup>	2.72±0.13 <sup>abc</sup>	2.90±0.02 <sup>ab</sup>	1.94±0.04 <sup>bc</sup>
Ginger I	1.40±0.03 <sup>b</sup>	1.23±0.06 <sup>a</sup>	1.35±0.03 <sup>c</sup>	3.55±0.52 <sup>a</sup>	3.11±0.19 <sup>ab</sup>	2.13±0.13 <sup>ab</sup>
Ginger II	1.42±0.06 <sup>b</sup>	1.15±0.03 <sup>ab</sup>	1.63±0.09 <sup>b</sup>	2.22±0.07 <sup>c</sup>	3.25±0.02 <sup>ab</sup>	1.93±0.03 <sup>bc</sup>
Ginger III	2.04±0.06 <sup>a</sup>	1.24±0.05 <sup>a</sup>	2.40±0.03 <sup>a</sup>	2.61±0.11 <sup>bc</sup>	4.22±0.11 <sup>a</sup>	2.50±0.05 <sup>a</sup>
Thymol I	1.25±0.08 <sup>bc</sup>	1.03±0.02 <sup>b</sup>	1.45±0.02 <sup>c</sup>	3.13±0.43 <sup>ab</sup>	3.86±1.24 <sup>a</sup>	2.14±0.29 <sup>ab</sup>
Thymol II	1.20±0.02 <sup>c</sup>	1.14±0.05 <sup>ab</sup>	1.33±0.04 <sup>c</sup>	2.22±0.08 <sup>c</sup>	2.22±0.01 <sup>b</sup>	1.62±0.03 <sup>c</sup>

Means within the same column carry different superscripts are significantly different (P<0.05)

Table 6: Effect of dietary supplementation of ginger and thymol on ALT and AST in broilers

	ALT (U/ml)		AST (U/ml)	
	24 <sup>th</sup> day	42 <sup>nd</sup> day	24 <sup>th</sup> day	42 <sup>nd</sup> day
Control	15.50±1.22 <sup>a</sup>	16.42±0.50 <sup>a</sup>	18.23±1.35 <sup>a</sup>	15.58±1.33 <sup>a</sup>
Ginger I	12.90±0.81 <sup>a</sup>	12.27±1.47 <sup>a</sup>	16.23±0.93 <sup>a</sup>	14.30±0.95 <sup>a</sup>
Ginger II	14.56±1.29 <sup>a</sup>	13.90±1.59 <sup>a</sup>	17.81±2.33 <sup>a</sup>	14.42±0.59 <sup>a</sup>
Ginger III	13.25±1.58 <sup>a</sup>	14.76±1.65 <sup>a</sup>	16.20±0.59 <sup>a</sup>	13.99±0.89 <sup>a</sup>
Thymol I	14.03±1.56 <sup>a</sup>	15.66±0.82 <sup>a</sup>	18.23±0.49 <sup>a</sup>	15.07±0.96 <sup>a</sup>
Thymol II	15.47±1.16 <sup>a</sup>	16.19±0.75 <sup>a</sup>	17.81±1.06 <sup>a</sup>	16.03±1.78 <sup>a</sup>

Means within the same column carry different superscripts are significantly different (P<0.05)

Table 7: Effect of dietary supplementation of ginger and thymol on creatinine and uric acid in broilers

	Creatinine (mg/dl)		Uric acid (mg/dl)	
	24 <sup>th</sup> day	42 <sup>nd</sup> day	24 <sup>th</sup> day	42 <sup>nd</sup> day
Control	3.39±0.22 <sup>a</sup>	1.59±0.27 <sup>a</sup>	9.14±0.53 <sup>a</sup>	6.42±0.59 <sup>a</sup>
Ginger I	2.43±0.63 <sup>a</sup>	1.14±0.18 <sup>a</sup>	6.63±1.48 <sup>a</sup>	3.64±0.36 <sup>a</sup>
Ginger II	2.74±0.75 <sup>a</sup>	1.61±0.18 <sup>a</sup>	7.11±1.40 <sup>a</sup>	4.63±0.50 <sup>a</sup>
Ginger III	2.12±0.34 <sup>a</sup>	1.46±0.41 <sup>a</sup>	5.75±0.57 <sup>a</sup>	4.36±0.70 <sup>a</sup>
Thymol I	2.70±0.45 <sup>a</sup>	1.14±0.49 <sup>a</sup>	6.29±0.56 <sup>a</sup>	5.84±0.83 <sup>a</sup>
Thymol II	1.97±0.28 <sup>a</sup>	1.10±0.20 <sup>a</sup>	7.31±0.45 <sup>a</sup>	5.99±0.45 <sup>a</sup>

Means within the same column carry different superscripts are significantly different (P<0.05)

Table (5) indicated that feed conversion were highly significantly increased ( $P<0.01$ ) in Ginger I and Thymol I groups at 4<sup>th</sup> week. Also, the feed conversion was significantly increased at 5<sup>th</sup> week of experiment in Ginger III and Thymol I groups. The feed conversion at 6<sup>th</sup> week of experiment were highly significantly increased ( $P<0.01$ ) in Ginger III then Ginger I and Thymol I groups when compared to the control.

The data presented in Table 6 and 7 showed no change in serum ALT, AST, creatinine and uric acid levels in ginger and thymol fed groups in relation to control one.

The data obtained in Table 8 revealed a high significant decrease in total cholesterol and TAG ( $P<0.01$ ) in Ginger III and significantly decreased in Ginger I and II

at 24<sup>th</sup> and 42<sup>nd</sup> days, whereas their levels were significantly decreased in thymol fed groups in comparison to control group.

The levels of serum INF- $\gamma$  were significantly increased in Ginger III, Ginger II, Ginger I, Thymol II and Thymol I, respectively in a dose-dependent manner when compared with control at 14<sup>th</sup>, 24<sup>th</sup> and 42<sup>nd</sup> days. The IL-2 levels were significantly increased in ginger and thymol fed groups in relation to control, whereas Ginger III, Ginger I, Thymol I and Thymol II, respectively (Table, 9).

The lipid peroxidation product, MDA levels in breast and thigh muscles were significantly decreased ( $P<0.05$ ) and the GSH levels were significantly increased ( $P<0.05$ ) in ginger and thymol fed groups when compared with control (Table, 10).

Table 8: Effect of dietary supplementation of ginger and thymol on total cholesterol and triacylglycerol (TAG) in broilers

	Total cholesterol (mg/dl)		TAG (mg/dl)	
	24 <sup>th</sup> day	42 <sup>nd</sup> day	24 <sup>th</sup> day	42 <sup>nd</sup> day
Control	128.50±16.33 <sup>a</sup>	134.24±5.85 <sup>a</sup>	135.75±7.18 <sup>a</sup>	119.00±7.75 <sup>a</sup>
Ginger I	119.25±5.65 <sup>ab</sup>	98.08±7.50 <sup>ab</sup>	100.40±5.01 <sup>b</sup>	87.44±4.12 <sup>b</sup>
Ginger II	113.75±9.54 <sup>ab</sup>	83.32±6.70 <sup>b</sup>	95.23±6.28 <sup>bc</sup>	86.34±7.79 <sup>b</sup>
Ginger III	66.00±18.20 <sup>b</sup>	62.40±4.54 <sup>c</sup>	79.18±6.17 <sup>c</sup>	75.80±8.09 <sup>c</sup>
Thymol I	141.00±11.20 <sup>a</sup>	113.58±10.90 <sup>ab</sup>	103.83±3.87 <sup>b</sup>	100.90±3.69 <sup>ab</sup>
Thymol II	133.00±10.50 <sup>a</sup>	108.16±3.40 <sup>ab</sup>	125.33±9.39 <sup>ab</sup>	110.14±8.00 <sup>a</sup>

Means within the same column carry different superscripts are significantly different ( $P<0.05$ )

Table 9: Effect of dietary supplementation of ginger and thymol on INF- $\gamma$  (pg/mL) and IL-2 (pg/mL) in broilers

	INF- $\gamma$			IL-2		
	14 <sup>th</sup> day	24 <sup>th</sup> day	42 <sup>nd</sup> day	14 <sup>th</sup> day	24 <sup>th</sup> day	42 <sup>nd</sup> day
Control	124.43±4.53 <sup>c</sup>	130.68±4.2 <sup>c</sup>	152.89±2.50 <sup>b</sup>	142.85±2.34 <sup>b</sup>	147.90±1.69 <sup>d</sup>	142.88±3.17 <sup>c</sup>
Ginger I	141.10±5.73 <sup>b</sup>	163.25±2.72 <sup>ab</sup>	174.04±3.57 <sup>b</sup>	164.19±6.81 <sup>ab</sup>	165.25±3.76 <sup>b</sup>	150.75±6.41 <sup>b</sup>
Ginger II	143.00±4.49 <sup>b</sup>	172.15±1.80 <sup>a</sup>	172.83±3.52 <sup>a</sup>	170±7.95 <sup>a</sup>	164.28±2.63 <sup>b</sup>	163.40±1.61 <sup>b</sup>
Ginger III	160.08±5.81 <sup>ab</sup>	159.00±3.93 <sup>b</sup>	195.00±2.23 <sup>ab</sup>	171.74±5.91 <sup>a</sup>	182.25±2.35 <sup>a</sup>	154.35±3.63 <sup>a</sup>
Thymol I	138.45±4.43 <sup>bc</sup>	153.48±3.82 <sup>b</sup>	166.66±3.56 <sup>b</sup>	169.62±9.90 <sup>a</sup>	160.25±3.58 <sup>bc</sup>	146.32±3.87 <sup>b</sup>
Thymol II	148.13±2.63 <sup>ab</sup>	157.51±2.47 <sup>b</sup>	170.00±1.83 <sup>ab</sup>	146.55±1.62 <sup>b</sup>	151.68±5.66 <sup>cd</sup>	154.80±1.56 <sup>b</sup>

Means within the same column carry different superscripts are significantly different ( $P<0.05$ )

Table 10: Effect of dietary supplementation of ginger and thymol on breast and thigh MDA and GSH in broilers

	MDA (nmol/g tissue)		GSH (mmol/g tissue)	
	Breast	Thigh	Breast	Thigh
Control	18.04±0.02 <sup>b</sup>	17.47±0.29 <sup>a</sup>	0.08±0.01 <sup>b</sup>	0.02±0.02 <sup>b</sup>
Ginger I	11.47±0.50 <sup>a</sup>	11.90±0.87 <sup>d</sup>	0.15±0.01 <sup>a</sup>	0.12±0.05 <sup>a</sup>
Ginger II	12.13±0.04 <sup>a</sup>	14.53±0.03 <sup>bc</sup>	0.15±0.01 <sup>a</sup>	0.14±0.02 <sup>a</sup>
Ginger III	13.45±0.61 <sup>a</sup>	14.30±0.46 <sup>bc</sup>	0.15±0.01 <sup>a</sup>	0.14±0.02 <sup>a</sup>
Thymol I	13.85±0.55 <sup>a</sup>	15.47±0.28 <sup>b</sup>	0.14±0.02 <sup>a</sup>	0.12±0.02 <sup>a</sup>
Thymol II	12.63±0.56 <sup>a</sup>	12.87±0.11 <sup>cd</sup>	0.14±0.01 <sup>a</sup>	0.16±0.08 <sup>a</sup>

Means within the same column carry different superscripts are significantly different ( $P<0.05$ )

## DISCUSSION

The GC-MS analysis of ginger methanolic extract revealed the presence of some antioxidant compounds and carotenoids that related to the antioxidant and immunostimulant potentials of ginger. This result was proofed by numerous research investigations that identified zingiberene and ar-curcumene [26], zingerone [11], gingerol [27],  $\beta$ -sesquiphellandrene [28] and carotene [29] in ginger root.

The broiler feeds supplemented by Ginger III gave the best feed conversion ratio which depends on the weight gain and feed intake followed by Ginger I and Thymol I. The improvement of growth performance may be due to the intestinal environment balance and stimulating the immune response. The ginger supplementation significantly increased the body weight and improved the feed conversion compared to birds fed with control diet due to its carminative and tonic effects [30, 31]. At 22<sup>nd</sup> day of age, the broilers receiving 7.5 g/kg of ginger root powder experienced significantly increased body weight and body weight gain compared to the control group [32].

The dietary supplementation of ginger and thymol did not affect the serum activities of ALT and AST and the levels of creatinine and uric acid. There were no significant differences observed in the activities of the serum ALT, AST and creatinine levels, indicating that none of the three dosages of ginger oil given to birds was toxic [33].

The serum levels of total cholesterol and TAG were significantly decreased in ginger fed groups. This result comes in accordance of [34] who's stated that the dietary supplementation of broilers by ginger significantly decreased the abdominal fat pad, serum cholesterol and TAG. Moreover, total cholesterol and serum (low density lipoprotein) LDL-C concentration was significantly ( $P<0.05$ ) decreased in ginger received group [35]. Plasma TAG, total cholesterol and creatinine as well as activities of transaminases were significantly decreased [36]. The supplementation with ginger (1% and 3%) reduced total cholesterol level compared with the control diet [37]. Ginger treatment can reduce total serum cholesterol by inhibition of hydroxyl-methyl-glutaryl-coenzyme-A reductase, either by bile-acid conversion or fecal excretion of cholesterol [38]. The feeding of rats with ginger significantly elevated the activity of hepatic cholesterol 7-alpha-hydroxylase which is a rate limiting enzyme in the biosynthesis of bile acids and stimulates the conversion of cholesterol to bile acids leading to the excretion of cholesterol from the body [39].

Broiler chicks those fed by ginger had a significant decrease in MDA and a significant increase in GSH levels. MDA is formed as an end product of lipid peroxidation and therefore the extent of lipid peroxidation by reactive oxygen species can be monitored by MDA levels [40]. Hence, the reduced serum MDA level in ginger-supplemented as compared with control broilers indicated that lipid peroxidation was reduced by ginger via enhancing anti-oxidative action. All together, these results demonstrated that ginger supplemented at the level of 5 g/kg improved antioxidant status of broiler chickens.

The addition of either ginger or thymol to the diet of broilers significantly increased the serum levels of  $INF-\gamma$  and IL-2 indicating their ability to stimulate immunity [41, 42].

Thymol was recommended as poultry feed additive of antimicrobial potential [43]. Thymol increased the performance of broilers that may be due to its ability to increased digestion. The addition of thymol by a concentration of 100 mg/kg diet improved the performance of broilers as well as apparent ileal digestibility of nutrients due to improved secretion of digestive enzymes [44]. In addition, dietary supplementation of broiler by thymol induced significant decreases in total cholesterol and lipid peroxidation [45, 46].

## CONCLUSION

From this study we can concluded that both ginger and thymol were induce improvements in broiler performance, antioxidant status and immunity in a dose-dependent manner. Further investigation should be conducted to evaluate the effects of ginger and thymol combination and combination with other natural product active principle in broiler productivity.

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