Poultry Science Journal
ISSN: 2345-6604 (Print), 2345-6566 (Online)
http://psj.gau.ac.ir

Lycopene Supplementation Enhances Growth Performance, Antioxidant Enzymes, Heat Shock Protein 70, and Some Biochemical and Immune Parameters in Broiler Chickens Exposed to Heat Stress

Ahmed Gouda, Mosaad M. El-Monairy, Hussein M.A. Hassan, Yasser A.F. Hamouda & Amani W. Youssef

Department of Animal Production, Agricultural and Biological Research Institute, National Research Centre, P.O. Box 12622, Cairo, Egypt

Poultry Science Journal 2025, 13(1): 127-134

Keywords
Immune
Lycopene
Antioxidant
Biochemical
Broiler performance

Corresponding author
Hussein M.A. Hassan
husseinhma@yahoo.com

Article history
Received: July 28, 2024
Revised: October 03, 2024
Accepted: October 10, 2024

Abstract

Heat stress is one of the major physiological factors in poultry that causes oxidative stress, acid-base imbalance, impaired immune function, reduced feed efficiency, and increased mortality. Therefore, this study aimed to evaluate lycopene supplementation on performance, antioxidant enzyme activity, heat shock protein 70 (HSP70), and some biochemical and immune parameters in broiler chickens. A total of 300 one-day-old broiler chicks were randomly distributed into six groups with five replicates and 10 of each. The first group had a basal diet, while the other groups received a basal diet supplemented with lycopene at different levels: 200, 300, 400, 500, or 600 mg/kg. For 35 days, the birds received unlimited feed and water. The results revealed that supplementation of lycopene improved ($P < 0.01$) the body weight, body weight gain, feed intake, and feed conversion ratio. The greatest improvement in performance was recorded with supplementation of lycopene at 500 mg/kg of diet during the starter period and 400 mg/kg of diet during the grower-finisher period. T3 and T4 levels increased ($P < 0.01$) in the groups supplemented with lycopene at all levels of addition, while creatinine and uric acid levels were unchanged ($P > 0.05$) compared to the control group. Adding lycopene improved ($P < 0.01$) serum lipid levels, HSP70, and the gene expression of the antioxidant enzymes GPX1, SOD1, and CAT. Also, the serum levels of IL-1β, IFNγ, IL10, C3, and lysozyme activities were elevated ($P < 0.01$) in the groups receiving lycopene supplementation. These findings indicated that the addition of lycopene at 400 or 500 mg/kg of diet can improve growth performance, antioxidant enzyme activity, lipid profile, HSP70, IL-1β, IFNγ, IL10, C3, and lysozyme activities in the serum of broilers.

Introduction

Heat stress is a serious problem in the poultry sector that affects both health and performance. Stress due to heat happens when chickens can't regulate their body temperature. Heat stress is caused by a combination of factors, including high environmental temperature, humidity, heat radiation, and airspeed (Lara and Rostagno, 2013; Gouda *et al.*, 2020). Birds are unable to metabolize carotenoids under heat stress, so they need to be supplemented in their diets (Eggersdorfer and Wyss, 2018). Some studies indicate that reducing feed and supplementing various vitamins, minerals, osmolytes, and phytochemicals can mitigate the negative effects of heat stress. Various phytochemicals are added to poultry diets to reduce heat stress (Gouda *et al.*, 2020 and 2021). Lycopene, a natural antioxidant, shows potential as a nutritional supplement for broiler chickens, particularly under heat-stressed conditions. It plays an essential role in poultry production (Nabi *et al.*, 2020). Lycopene, a lipophilic compound, acts as an antioxidant, scavenging free radicals and trapping peroxyl radicals (Srinivasan *et al.*, 2007). Adding lycopene to poultry diets can reduce the effects of heat stress by increasing the activity of some antioxidant enzymes such as superoxide dismutase (SOD), glutathione peroxidase (GSH-Px), and catalase (CAT) (Amer *et al.*, 2020). Tomatoes,

Please cite this article as Ahmed Gouda, Mosaad M. El-Monairy, Hussein M.A. Hassan, Yasser A.F. Hamouda & Amani W. Youssef. 2025. Lycopene Supplementation Enhances Growth Performance, Antioxidant Enzymes, Heat Shock Protein 70, and Some Biochemical and Immune Parameters in Broiler Chickens Exposed to Heat Stress. Poult. Sci. J. 13(1) 127-134.

DOI: 10.22069/psj.2024.22638.2132

especially dark-red varieties, and their byproducts are rich in carotenoids, including lycopene (Arain *et al*., 2018). Using lycopene in broiler diets has positive effects, especially during crucial periods, such as high temperatures and infection-related conditions (Hosseini-Vashan *et al*., 2016). Previous studies have shown that a commercial meal enriched with lycopene improves yolk color and immunity in hens through production (Rosa *et al*., 2018; Mavrommatis *et al*., 2022). Arain *et al*. (2018) indicated that adding lycopene to poultry diets significantly enhances antioxidant enzymes (GSH-Px and SOD) and lowers MDA levels in muscle and blood. Sahin *et al*. (2016) reported that using 20, 50, or 100 mg of lycopene/kg feed increased SOD and GSH-Px, decreased MDA levels, and activated nuclear transcription factor systems in heat-stressed broilers. Supplementation of heat-stressed broilers with lycopene (200 or 400 mg/kg of diet) improved the cumulative feed intake (FI), body weight (BW), and feed conversion ratio (FCR). Additionally, lycopene improves SOD and GSH-Px enzymes (Arain *et al*., 2018). Mezbani *et al*. (2019) reported that the addition of 100 -200 mg lycopene/kg of broiler diets increased GSH-Px and CAT levels and decreased MDA levels. Wang *et al*. (2022) found that adding 30 mg lycopene/kg of broiler diets improved blood antioxidant status by increasing T-AOC, GSH-Px, and SOD enzymes and decreasing MDA levels. Therefore, this study examined how lycopene supplementation affects heat-stressed broilers' growth performance, antioxidant enzyme, heat shock protein 70, and some biochemical and immune parameters.

Materials and Methods

Ethical statement

The National Research Centre's Medical Research Ethics Committee, Cairo, Egypt, approved the experimental design and protocol of the study (Approval Code 3-0-2).

Birds and experimental design

This study was carried out at the Department of Animal Production, National Research Centre, during the summer season (from July to August 2023). A total of 300-day-old broiler chicks (Cobb-500) were purchased from a commercial hatchery and distributed among six experimental groups using a fully randomized design, with five replicates per group and ten birds per replicate. Group 1 (control group) received a corn-soybean meal basal diet without the addition of lycopene, whereas the other experimental groups received the basal diet supplemented with 200, 300, 400, 500, or 600 mg of lycopene/kg diet. Lycopene was prepared according to the methods of Amer *et al.* (2020). The spectrophotometric determination of lycopene from tomatoes was made according to Sadler *et al.* (1990), with a slight modification in the extraction with hexane/ethanol/acetone and absorbance measurement at 472 nm. The NRC (1994) guidelines were followed for the formulation of the basal experimental diets (Table 1).

Table 1. Formulation and composition of the basal diet

Ingredients%	Feeding period	
	(1–21 d)	(22–35 d)
Corn	54.0	58.93
Soybean meal, 44%	34.12	30.25
Corn gluten, 60%	6.1	4.90
Soy oil	1.0	1.18
Limestone	1.65	1.60
Monocalcium phosphate	1.65	1.65
Common salt	0.45	0.45
Premix*	0.30	0.30
DL-methionine, 98%	0.15	0.16
Lysine, HCl, 78%	0.30	0.30
Na_2co_3	0.28	0.28
Nutrients (calculated %)		
ME_n (kcal/kg)	2900	2950
Crude Protein %	23.02	21.00
Calcium %	0.99	0.96
Available phosphorus%	0.45	0.45
Lysine %	1.34	1.24
Methionine %	0.52	0.50

* Minerals and Vitamins provided per kg of diet = Zn (53.0 mg); Fe (67.8 mg); Cu (12 mg); I (0.053 mg); Mn (30.2 mg); Se (0.09 mg); vitamin A (4,550 IU); vitamin E (7.5 IU); vitamin D3 (450 IU); vitamin K (0.752 mg); riboflavin (3.75 mg); pantothenic acid (3 mg); niacin (15.2 mg); vitamin B12 (0.006 mg); biotin (0.152 mg); folic acid (0.376 mg); thiamine (1.07 mg); pyridoxine (3.78 mg); choline (1575 mg).

Table 2. Indoor temperature and humidity levels, including the temperature- humidity index

Week	Minimum temperature (°F)	Maximum temperature (°F)	RH (%)	THI min.	THI max.
1	85.6	92.5	70.0	81.1	86.8
2	86.4	95.2	69.0	81.6	88.9
3	86.5	94.6	75.2	81.5	89.6
4	86.8	95.8	75.6	81.7	90.7
5	87.3	96.2	76.5	82.4	91.3

*THI was calculated according to the following formula: THI = T_d – [0.55 -(0.55 * RH/100)]* (T_d - 58). Where THI is the temperature–humidity index, Td: is the dry bulb temperature in degrees Fahrenheit, and RH is the relative humidity (%). °F-degree Fahrenheit = degree Celsius °C * 1.8 + 32, min = minimum, max = maximum

During the 35-day experiment, all birds were given free access to fresh water and feed, and housed under similar sanitary, management, and environmental conditions. During the experiment, the temperature and humidity of the birds' houses were measured three times per day, and the average weekly findings showed a range of minimum and maximum ambient temperatures (85.6-96.2°F), relative humidity (69-76.5%), and temperature-humidity index (THI=81.1-91.3; Table 2). THI calculations showed that the experimental birds were kept under heat stress, which was also previously reported (Jarraud, 2008).

Growth performance

At 21 and 35 days of age, the birds were weighed individually to measure body weight (BW) and body weight gain (BWG). Feed intake (FI) for each replicate was recorded weekly and the feed conversion ratio (FCR) was calculated.

Collection of samples and biochemical analysis

To induce heat shock protein 70 (HSP70) before sampling, all birds were exposed to acute heat at 40°C for two hours at the end of the trial. After a 12-hour fast, five birds were randomly chosen for sample collection from each group. Blood samples were taken from the aforementioned birds in tubes devoid of anticoagulant to evaluate biochemical parameters. After the samples were allowed to coagulate at room temperature, they were centrifuged for 13 minutes at 3000 ×g. After that, the supernatants (serum samples) were transferred to Eppendorf tubes and stored at –20°C for further examination. Following blood collection, six birds from each treatment group were slaughtered in accordance with the institutional committee's advice, and some liver tissue was removed, vacuum-packed, and kept at -20°C to determine HSP70. Using readily available diagnostic kits (Byk-Sangtec Diagnostica, Dietzenbach, Germany; Immunite2000, DPC, LA), the amounts of triiodothyronine (T3) and thyroxin (T4) in the serum samples were measured in accordance with the manufacturer's instructions. The plasma total cholesterol, triglyceride, and high-density lipoprotein cholesterol (HDL-c) levels were measured using colorimetric diagnostic kits (Egyptian Company for Biotechnology, Cairo, Egypt) in accordance with the methods of Allain *et al*. (1974), McGowan *et al*. (1983), and Vassault *et al*. (1986). The low-density lipoprotein cholesterol (LDL-c) content was calculated following the formula LDL-c = (Total cholesterol/1.19 + Triglycerides/1.9 - HDL-c /1.1–38). Very low-density lipoprotein cholesterol (VLDL-c) was measured in accordance with Griffin and Whitehead (1982) by using the turbidimetric method. Serum creatinine and uric acid levels were measured in accordance with an automatic biochemical analyzer (Robotnik Prietest ECO Ambernath (W), Thane, India) Henry (1974) and Sanders *et al*. (1980).

Hepatic heat shock protein 70 (HSP70) and the gene expression of antioxidant enzymes

Livers were collected from the slaughtered birds and immediately stored in liquid nitrogen at -70°C for HSP70 and subsequent RNA analysis. The enzyme-linked immunoassay for HSP70 in liver tissue was performed following the methods of Anderson *et al*. (1993). Total RNA was extracted from the tissues using the RNX-Plus reagent (Sinaclon Bioscience, Tehran, Iran). After chloroform was added to the homogenate, it was centrifuged. Total RNA was extracted, rinsed with ethanol (75%), and resuspended in diethylpyrocarbonate (DEPC)-treated water. DNase (Sinaclon Bioscience, Tehran, Iran) was used to remove residual DNA. RNA was then measured and quantified spectrophotometrically, and only RNA with an absorbance ratio (A260/A280) greater than 1.9 was used for cDNA synthesis. Using a Prime-Script RT Reagent Kit (TaKaRa Bio, Inc., Japan), total RNA was reverse transcribed into cDNA. At a temperature of 85°C for 5 seconds, the reverse transcription mixture was exposed to stop the activity of the reverse transcriptase, denatured and subsequently stored at –20°C. The quantitative real-time PCR technique (RT–PCR cycler; Rotor Gene Q 6000, Qiagen, USA, with three replicates for each sample of ventricles) is used to determine both superoxide dismutase1 (SOD1), catalase (CAT), glutathione peroxidase (GPX), and β-actin (used as an endogenous standard to ensure that the input load of cDNA was consistent among all samples) and transcripts using SYBR Premix Ex Taq II (Tli Rnase H Plus) (TaKaRa Bio, Inc., Japan). Specific primers for SOD1, CAT, GPX1 and β-actin were designed

with Primer-Blast (www.ncbi.nlm.nih.gov/tools/primerblast/index.cgi? LINK_LOC= BlastHome), as listed in Table 3. 1 µL of cDNA was added to10 µL of SYBR Premix Ex Taq II Mix and 0.5 µmol/L of each specific primer in a total volume of 20 µL, where the thermal profile was 95°C for 30 s; 40 cycles of 94°C for 40s, 64°C for 35s and 72°C for 30s. Fluorescence was measured at the end of each stage, and data obtained from gene expression were normalized to that of β-actin and analyzed using LinRegPCR software version 2012.0 (Amsterdam, Netherlands) to determine the threshold cycle number and reaction efficiency (Ruijter *et al*., 2009). The relative transcript levels and the fold changes in transcript abundance were calculated using the efficiency-adjusted Paffl methodology (Dorak, 2006).

Table 3. Primers used for quantitative real-time PCR analysis of chicken mRNA

Primer name	Sequence	PCR product, bp	Accession No.
GPX1	F:5"-GCTGTTCGCCTTCCTGAGAG-3" R:5"-GTTCCAGGAGACGTCGTTGC-3"	118	NM_001277853.1
SOD1	F:5"-CACTGCATCATTGGCCGTACCA-3" R:5"-GCTTGCACACGGAAGAGCAAGT-3"	224	NM_205064.1
CAT	F:5"-TGGCGGTAGGAGTCTGGTCT-3" R:5"-GTCCCGTCCGTCAGCCATTT-3"	112	NM_001031215.1
β-actin	F:5"-AGCGAACGCCCCCAAAGTTCT-3" R:5"-AGCTGGGCTGTTGCCTTCACA-3"	139	NM_205518.1

bp = base pair; GPX1 = glutathione peroxidase 1; SOD1 = superoxide dismutase 1; CAT = catalase.

Immune indices

Specific ELISA kits (MyBioSource Co. of CAT.NO. MBS701683 (USA) was used to quantify interleukin 10 (IL-10), interleukin 1β **(**IL-1β**)** (Cat. No. MBS2024496), interferon gamma (IFN-γ) (Cat. No. MBS700243) and complement 3 (C3) (Life Span Biosciences, Inc., Seattle, WA, USA). The serum lysozyme activity was determined according to Lie *et al*. (1986).

Analytical statistics

One-way analysis of variance (ANOVA) was used to analyze the data using SPSS software (version 20.0 for Windows, SPSS, Inc., Chicago, IL). The significance of the variations between the mean values was examined using Duncan's multiple-range test. For differences, $P < 0.05$ was chosen as the significance level.

Results

Growth performance

Compared with those of the control group, BW, BWG, FI, and FCR in the experimental group significantly improved ($P < 0.01$) with the addition of dietary lycopene at all levels during the experimental period (Table 4). Notably, the improvement increased with increasing lycopene levels up to 500 mg/kg feed. The greatest improvement in the FCR was recorded with 500 mg of lycopene supplementation during the starter period and with 400 mg of lycopene supplementation during the grower-finisher period.

Table 4. Effect of lycopene supplementation on growth performance

Item	Lycopene (mg/kg feed)						*SEM*	*P*
	Control	200	300	400	500	600		
1-21 d								
BW(g)	559^{d}	636^{c}	655^{b}	663^{b}	685^{a}	680^{a}	5.186	0.001
BWG (g)	516.6^{d}	593.8^{c}	612.4^{b}	620.6^{b}	642.8^{a}	637.4^{a}	5.295	0.001
FI (g)	747^{d}	817^{c}	824^{bc}	836^{b}	827^{a}	860^{a}	5.759	0.001
FCR	1.45^{a}	1.38^{b}	1.35^{b}	1.35^{b}	1.29^{c}	1.35^{b}	0.017	0.014
22-35 d								
BW(g)	1689^{d}	1841^{c}	1877^{b}	1933^{a}	1967^{a}	1950^{a}	11.175	0.001
BWG (g)	1130^{c}	1205^{b}	1222^{b}	1270^{a}	1282^{a}	1270^{a}	10.094	0.001
FI (g)	2352^{d}	2374^{c}	2407^{b}	2411^{b}	2485^{a}	2470^{a}	5.393	0.001
FCR	2.08^{a}	1.97^{b}	1.97^{b}	1.90^{c}	1.94^{bc}	1.94^{bc}	0.016	0.001
1-35 d								
BW(g)	1689^{d}	1841^{c}	1877^{b}	1933^{a}	1967^{a}	1950^{a}	11.175	0.001
BWG (g)	1647^{d}	1799^{c}	1834^{b}	1891^{a}	1925^{a}	1907^{a}	11.249	0.001
FI (g)	3099^{d}	3191^{c}	3231^{b}	3247^{b}	3352^{a}	3330^{a}	8.281	0.001
FCR	1.88^{a}	1.77^{b}	1.76^{b}	1.72^{c}	1.74^{bc}	1.75^{bc}	0.011	0.001

$^{a-d}$Within a row, different superscript letters indicate significant differences ($P < 0.05$).

Thyroid and kidney functions

Similarly, compared with those in the control group, the levels of the T3 and T4 thyroid hormones in the lycopene-supplemented group increased ($P < 0.01$) at all levels studied. Creatinine and uric acid levels were not significantly altered (P>0.05) by lycopene supplementation (Table 5).

Table 5. Effect of lycopene supplementation on thyroid and kidney functions

Parameters	Lycopene (mg/kg feed)						*SEM*	*P*
	Control	200	300	400	500	600		
T_3 (ng/mL)	3.778^{d}	4.318^{c}	4.806^{bc}	5.150^{ab}	5.648^{a}	5.310^{ab}	0.176	0.001
T_4 (ng/mL)	19.86^{c}	22.41^{b}	24.41^{a}	24.74^{a}	26.05^{a}	25.69^{a}	0.599	0.001
Creatinine (mg/dL)	0.238	0.242	0.244	0.246	0.252	0.252	0.0187	0.993
Uric Acid (mg/dL)	3.084	3.190	3.324	3.442	3.586	3.504	0.2197	0.583

[a-d]Within a row, different superscript letters indicate significant differences ($P < 0.05$).

Serum lipid profile

Compared with those in the control group, supplemental dietary lycopene at all concentrations improved ($P < 0.01$) the serum total cholesterol, HDL-c, LDL-c, VLDL-c and triglyceride levels (Table 6).

Table 6. Effect of lycopene supplementation on the serum lipid profile

Parameters (mmol/l)	Lycopene (mg/kg feed)						*SEM*	*P*
	Control	200	300	400	500	600		
Total cholesterol	3.306^{a}	3.158^{b}	3.142^{bc}	3.102^{bcd}	3.040^{d}	3.072^{cd}	0.264	0.001
HDL-c	2.112^{c}	2.224^{b}	2.302^{ab}	2.304^{ab}	2.342^{a}	2.324^{ab}	0.323	0.003
LDL-c	0.926^{a}	0.674^{b}	0.590^{bc}	0.548^{bcd}	0.456^{d}	0.496^{cd}	0.418	0.001
VLDL-c	0.268^{a}	0.260^{ab}	0.250^{ab}	0.250^{ab}	0.242^{b}	0.252^{ab}	0.062	0.097
Triglycerides	1.326^{a}	1.280^{ab}	1.252^{bc}	1.236^{bc}	1.200^{c}	1.208^{c}	0.183	0.005

[a-d] Within a row, different superscript letters indicate significant differences ($P < 0.05$).

Hepatic heat shock protein 70 and the gene expression of antioxidant enzymes

Compared with those in the control group, the HSP70 levels and the gene expression of several antioxidant enzymes, such as GPX1, SOD1 and CAT, increased in the group fed diets supplemented with lycopene ($P < 0.01$) (Table 7). Note that this significant improvement increased with increasing lycopene levels up to 500 mg/kg diet.

Table 7: Effect of lycopene supplementation on hepatic heat shock protein 70 and the gene expression of antioxidant enzymes

Parameters	Lycopene (mg/kg feed)						*SEM*	*P*
	Control	200	300	400	500	600		
HSP70, ng/mg	3.79^{d}	4.55^{c}	4.71^{c}	5.41^{b}	6.26^{a}	6.05^{a}	0.174	0.001
GPX_1,U/mL	0.0046^{d}	0.0272^{c}	0.0548^{b}	0.065^{ab}	0.078^{a}	0.067^{ab}	0.004	0.001
SOD_1, U/mL	0.003^{d}	0.283^{c}	0.394^{b}	0.408^{b}	0.524^{a}	0.428^{b}	0.022	0.001
CAT, U/mL	0.002^{d}	0.023^{c}	0.044^{b}	0.051^{b}	0.067^{a}	0.056^{ab}	0.005	0.001

[a-d]Within a row, different superscript letters indicate significant differences ($P < 0.05$).

Immune indices

The serum levels of IL-1β, IFNγ, IL-10, C3 and lysozyme activity were elevated ($P < 0.01$) in the groups supplemented with lycopene. The greatest increase ($P < 0.01$) occurred when lycopene was supplemented at 500 mg/kg in the diet (Table 8).

Table 8: Effect of lycopene supplementation on immune status

Parameters	Lycopene (mg/kg feed)						*SEM*	*P*
	Control	200	300	400	500	600		
IL-1β, µg/mL	143.2^{c}	148.6^{c}	156.2^{b}	164.4^{a}	170.4^{a}	168.2^{a}	2.209	0.001
IFNγ, pg/mL	6.6^{d}	7.14^{cd}	7.62^{c}	8.54^{b}	10.52^{a}	10.04^{a}	0.190	0.001
IL10, pg/mL	1.66^{d}	2.44^{c}	2.94^{b}	3.38^{b}	4.08^{a}	3.40^{b}	0.165	0.001
Serum C3, g/L	1.26^{d}	1.35^{cd}	1.39^{bc}	1.45^{abc}	1.55^{a}	1.50^{ab}	0.039	0.004
Lysozyme, µg/mL	131.8^{c}	140.2^{c}	158.4^{b}	166.8^{b}	190.4^{a}	182.2^{a}	3.509	0.001

[a-d]Within a row, different superscript letters indicate significant differences ($P < 0.05$).

Discussion

Enhancing performance, maintaining health, and boosting the immune system are the key goals of broiler production. Excessive heat causes oxidative stress in chickens, which raises their risk of pathological disorders and reduces their production efficiency (Mujahid *et al*., 2007; Gouda *et al*., 2020). Free radicals and peroxides, which are normally created within cells during normal metabolism, are known as reactive oxygen species (ROS). Physiological detoxification systems reduce excess ROS produced by cells. When the transcription factor Nrf2 is activated under these conditions, more antioxidant molecules are synthesized, leading to an increase in reactive oxygen species (ROS) generation within the cell. The overabundance of free radicals generated during oxidative stress can harm DNA, lipids, and proteins within cells. In poultry, oxidative stress is linked to lower growth rates, serious health conditions, biological destruction, and losses in revenue (Surai *et al*., 2019; Gouda *et al*., 2021). Oxidative stress, such as excessive ROS generation or decreased antioxidant defense, occurs when there is an imbalance between these systems (Mishra and Jha, 2019; Gouda *et al*., 2020).

In this study, BWG, FI, and FCR were improved in the groups supplemented with lycopene. A higher improvement in BWG and FCR was observed when 400 and 500 mg of lycopene were supplemented, which was consistent with the results of Mezbani *et al*. (2019), who found that giving chickens 100 mg/kg of lycopene as a supplement from day 21 to day 42 led to greater body weight and a better feed conversion ratio. Increased antioxidant enzyme activity benefits hens by improving their antioxidant capabilities and promoting daily weight gain (Surai, 2000). Lycopene, a lipophilic compound with antioxidant and free radical scavenging properties, improves broiler performance. Dietary supplementation of 5% dried tomato pomace enhanced growth performance (Sahin *et al*., 2016). The addition of antioxidants is a common method used in poultry feeding systems to increase productivity and maintain health. Lycopene, a carotenoid component found primarily in ripe vegetables and fruits, has been proven to be the most promising antioxidant component (Sevcikova *et al*., 2008).

Regarding of lipids profile, in the current study, lycopene supplementation increased HDL-c levels while lowering triglyceride, total cholesterol, and VLDL levels. These findings aligned with the research conducted by Rao and Shen (2002), which revealed a decrease in blood cholesterol levels in birds that were given a diet that contained lycopene. According to Sahin *et al*. (2006), lycopene supplementation increased HDL and lowered LDL levels in Japanese quail blood plasma. When broilers are subjected to relatively high temperatures, their levels of LDL cholesterol and triglycerides are reduced by dietary supplements containing 5% dried tomato pomace (Hosseini-Vashan *et al*., 2016).

Regarding thyroid hormones, the addition of lycopene increased T3 and T4 levels. During heat stress, birds rely on their neuroendocrine system to maintain homeostasis and normal physiological function. Thyroid hormones (T3) and thyroxine (T4) control metabolic rates. Previous studies have shown a reduction in T3 concentrations in heat-stressed birds but varying T4 values (Etches *et al*., 2008). Also, Lycopene has been demonstrated to boost antioxidant enzymes (SOD and GSH-Px) and reduce MDA levels in broiler muscles and serum. Lycopene, a naturally occurring pigment, has been shown to improve health and act as an antioxidant (Lauretani *et al*., 2008). Lycopene is two and ten times more effective than β-carotene and α-tocopherol, respectively, as a potent scavenger of free oxygen radicals due to its high content of conjugated dienes (Palozza *et al*., 2012). When broilers are exposed to heat stress, it modifies gene transcription variables related to stress (Sun *et al*., 2015; Sahin *et al*., 2016; Li *et al*., 2019). Lycopene reduces heat stress by activating antioxidant enzymes like SOD, GSH-Px, and CAT, raising T-AOC and Nrf2, and decreasing MDA and muscle Keap1 expression. According to Arain *et al*. (2018), lycopene successfully increased levels of antioxidant enzymes (GSH-Px, SOD) and considerably decreased MDA content in muscle and blood. In the current study, the serum levels of IL-1β, IFNγ, IL-10, C3 and lysozyme activity were elevated in the groups supplemented with lycopene up to 500 mg/kg of the diet. Lycopene can improve broiler fertility by improving sperm function and decreasing inflammation by regulating IL-1, IL-2, and IL-10 levels during infection. Lycopene has been shown to regulate IFN-c, IL-1, CLDN-1, and ZO-1. Lycopene supplementation can improve the immune response in broiler chickens. For example, lycopene treatment has been reported to increase IL-10 and phagocytic activity (Fararh *et al*., 2019).

Conclusion

The effects of lycopene supplementation on broiler growth performance and antioxidant enzyme activity of the various treatment groups were determined. Lycopene supplementation had a positive impact on the average body weight gain, feed intake, feed conversion ratio, and serum glutathione peroxidase and superoxide dismutase levels. Supplementing broiler diets with 400 or 500 mg/kg lycopene resulted in better improvement in growth performance coupled with increased antioxidant enzyme activity, biochemical parameters, hepatic heat shock protein 70 and the gene expression of antioxidant enzymes.

Acknowledgement
The work presented in this publication was made possible by a national project (13050402) funded by the National Research Centre (NRC).

References

Allain CC, Poon LS, Chan CS, Richmond W & Fu PC. 1974. Enzymatic determination of total serum cholesterol. Clinical Chemistry, 20: 470–475. DOI:10.1093/clinchem/20.4.470

Amer AA, Asmaa TYK, Ali O, Khalid MM, El-Sayed IH & Zaib URR. 2020. Influence of dietary graded levels of lycopene on the growth performance, muscle cholesterol level and oxidative status of Japanese quail fed high-fat diet. Anais da Academia Brasileira de Ciências, 92: (Suppl. 2). DOI:10.1590/0001-3765202020190065

Anderson R, Wang C, Van Kersen I, Lee K, Welch W, Lavagnini P & Hahn G. 1993. An immunoassay for heat shock protein 73/72: Use of the assay to correlate HSP73/72 levels in mammalian cells with heat response. International Journal of Hyperthermia, 9: 539-552. DOI:10.3109/02656739309005051

Arain MA, Mei Z, Hassan FU, Saeed M, Alagawany M, Shar AH & Rajput IR. 2018. Lycopene: a natural antioxidant for prevention of heat-induced oxidative stress in poultry. World's Poultry Science Journal, 74: 89–100. DOI: 10.1017/S0043933917001040

Dorak M. 2006. Real time PCR (1st Ed.), Taylor & Francis, Oxford, UK. DOI: 10.4324/9780203967317

Eggersdorfer M & Wyss A. 2018. Carotenoids in human nutrition and health: Archives of Biochemistry and Biophysics, 652: 18–26. DOI: 10.1016/j.abb.2018.06.001

Etches RJ, John TM & Gibbins AV. 2008. Poultry Production in Hot Climates. 2nd Ed. CABI; Oxfordshire, UK: Behavioural, physiological, neuroendocrine and molecular responses to heat stress; pages: 31–66.

Fararh KM, El-Aziz AMA and Alhelbawy NA. 2019. Anti-inflammatory, immunomodulatory and anti-oxidant effects of lycopene and vitamin E in *E.coli* infected broilers. International Journal of Advanced Research, 7: 790–800.

Gouda A, Tolba S A & El-Moniary M M. 2021. Zinc and ascorbic acid dietary supplementation affected antioxidant status, heat shock protein 70, and some immunoglobulins in Japanese quail under heat stress conditions. Biological Trace Element Research, 200**:** 2889–2896. DOI: 10.1007/s12011-021-02884-y

Gouda A, Amer S A, Gabr S & Tolba S A. 2020. Effect of dietary supplemental ascorbic acid and folic acid on the growth performance, redox status, and immune status of broiler chickens under heat stress. Tropical Animal Health and Production, 52**:** 2987–2996. DOI: 10.1007/s11250-020-02316-4

Griffin H & Whitehead C. 1982. Plasma lipoprotein concentration as an indicator of fatness in broilers: Development and use of a simple assay for plasma very low density lipoproteins. British Poultry Science, 23: 307–313. DOI: 10.1080/00071688208447962.

Henry R. 1974. Determination of serum creatinine. In Clinical Chemistry: Principles and Techniques, 2nd Ed. Harper and Row Publishers: New York, NY, USA.

Hosseini-Vashan SJ, Golian A & Yaghobfar A. 2016. Growth, immune, antioxidant, and bone responses of heat stress-exposed broilers fed diets supplemented with tomato pomace. International Journal of Biometeorology, 60: 1183–1192. DOI: 10.1007/s00484-015-1112-9

Jarraud M. 2008. Guide to meteorological instruments and methods of observation (WMO-No. 8). World Meteorological Organization. Geneva, Switzerland.

Lara LJ & Rostagno MH. 2013. Impact of heat stress on poultry production. Animals, 3: 356–369. DOI: 10.3390/ani3020356

Lauretani F, Semba RD, Bandinelli S, Dayhoff-Brannigan M, Giacomini V, Corsi AM, Guralnik JM & Ferrucci L. 2008. Low plasma carotenoids and skeletal muscle strength decline over 6 years. Journals of Gerontology Series A: Biological Sciences and Medical Sciences, 63: 376-383. DOI: 10.1093/gerona/63.4.376

Li C, Tan F, Yang J, Yang Y, Gou Y, Li S & Zhao X. 2019. Antioxidant effects of Apocynum venetum tea extracts on d-galactose-induced aging model in mice. Antioxidants, 8: 381. DOI 10.3390/antiox8090381

Lie Ø, Syed M & Solbu H. 1986. Improved agar plate assays of bovine lysozyme and haemolytic complement activity. Acta Veterinaria Scandinavica, 27: 23-32. DOI: 10.1186/bf03548556

Mavrommatis A, Zografaki ME, Marka S, Myrtsi ED, Giamouri E, Christodoulou C, Evergetis E, Iliopoulos V, Koulocheri SD, Moschopoulou G, Simitzis PEA, Pappas C, Flemetakis E, Koutinas A, Haroutounian SA & Tsiplakou E. 2022. Effect of a carotenoid extract from *Citrus reticulata* byproducts on the immune- oxidative status of broilers. Antioxidants, 11:144. DOI: 10.3390%2Fantiox11010144

McGowan M, Artiss JD, Strandbergh DR & Zak BA. 1983. peroxidase-coupled method for the colorimetric determination of serum triglycerides. Clinical Chemistry, 29: 538–542.

Mezbani A, Kavan BP, Kiani A & Masouri B. 2019. Effect of dietary lycopene supplementation on growth performance, blood parameters and antioxidant enzymes status in broiler chickens. Livestock Research for Rural Development, 31: 3101–3112.

Mishra B & Jha R. 2019. Oxidative stress in the poultry gut: Potential challenges and interventions. Frontiers in Veterinary Science, 6: 60. DOI: 10.3389/fvets.2019.00060

Mujahid A, Akiba Y & Toyomizu M. 2007. Acute heat stress induces oxidative stress and decreases adaptation in young white leghorn cockerels by down regulation of avian uncoupling protein. Poultry Science, 86: 364-371. DOI: 10.1093/ps/86.2.364

Nabi F, Arain MA, Rajput N, Alagawany M, Soomro J, Umer M, Soomro F, Wang Z, Ye R & Liu J. 2020. Health benefits of carotenoids and potential application in poultry industry: a review. Journal of Animal Physiology and Animal Nutrition, 104: 1809–1818. DOI: 10.1111/jpn.13375

NRC (National Research Council). 1994. Nutrient Requirements of Poultry. 9th Rev. Ed. National Academy Press, Washington, D.C., 174 pages.

Palozza P, Catalano A, Simone R & Cittadini A. 2012. Lycopene as a guardian of redox signaling. Acta Biochimica Polonica, 59: 21-25. DOI: 10.18388/abp.2012_2163

Rao AV & Shen H. 2002. Effect of low dose lycopene intake or lycopene bioavailability and oxidative stress. Nutrition Research, 22:1125–1131. DOI: 10.1016/S0271-5317(02)00430-X

Rosa AP, Scher A, Sorbara JO, Boemo LS, Forgiarini J & Londero A. 2012. Effects of canthaxanthin on the productive and reproductive performance of broiler breeders. Poultry Science, 91: 660–666. DOI: 10.3382/PS.2011-01582

Ruijter J, Ramakers C, Hoogaars W, Karlen Y, Bakker O, Van den Hoff M & Moorman AFM. 2009. Amplification efficiency: linking baseline and bias in the analysis of quantitative PCR data. Nucleic Acid Research, 37: e45. DOI: 10.1093/nar/gkp045

Sadler G, Davis J & Dezman D. 1990. Rapid extraction of lycopene and β-carotene from reconstituted tomato paste and pink grapefruit homogenates. Journal Food Science, 55: 1460-1461. DOI:10.1111/J.1365-2621.1990.TB03958.X

Sahin K, Onderci M, Sahin N, Gursu MF & Kucuk O. 2006. Effects of lycopene supplementation on antioxidant status, oxidative stress, performance and carcass characteristics in heatstressed Japanese quail. Journal of Thermal Biology, 31: 307–312. DOI: 10.1016/J.jtherbio.2005.12.006

Sahin K, Orhan C, Tuzcu M, Sahin N, Hayirli A, Bilgili S & Kucuk O. 2016. Lycopene activates antioxidant enzymes and nuclear transcription factor systems in heat-stressed broilers. Poultry Science, 95: 1088–1095. DOI: 10.3382/ps/pew012

Sanders G, Pasman A & Hoek F. 1980. Determination of uric acid with uricase and peroxidase. Clinica Chimica Acta, 101: 299–303.

Sevcikova S, Skrivan M & Dlouha G. 2008. The effect of lycopene supplementation on lipid profile and meat quality of broiler chickens. Czech Journal of Animal Science, 53: 431-440. DOI: 10.3390/ANI14020203

Srinivasan M, Sudheer AR, Pillai KR, Kumar PR, Sudhakaran PR, Menon VP. 2007. Lycopene as a natural protector against gamma-radiation induced DNA damage, lipid peroxidation and antioxidant status in primary culture of isolated rat hepatocytes in vitro. Biochimica et Biophysica Acta (BBA)-General Subjects, 1770 (4): 659–665.

Sun B, Chen C & Wang W. 2015. Effects of lycopene supplementation in both maternal and offspring diets on growth performance, antioxidant capacity and biochemical parameters in chicks. Journal of Animal Physiology and Animal Nutrition, 99: 42–7. DOI: 10.1111/jpn.12196

Surai PF. 2000. Effect of selenium and vitamin E content of the maternal diet on the antioxidant system of the yolk and the developing chick. British Poultry Science, 41: 235–43. DOI: 10.1080/713654909

Surai PF, Kochish II, Romanov MN & Griffin DK. 2019. Nutritional modulation of the antioxidant capacities in poultry: the case of vitamin E. Poultry Science, 98: 4030-4041. DOI: 10.3382/ps/pez072

Vassault A, Grafmeyer D, Naudin C, Dumont G, Bailly M, Henny J, Gerhardt M, & Georges P. 1986. Protocole de validation de techniques. Annales de Biologie Clinique, 44: 45.

Wang S, Wu H, Zhu Y, Cui H, Yang J, Lu M, Cheng H, Gu L & Xu L. 2022. Effect of lycopene on the growth performance, antioxidant enzyme activity, and expression of gene in the Keap1-Nrf2 signaling pathway of Arbor Acres broilers. Frontiers in Veterinary Science, 83: 33 – 46. DOI: 10.3389/fvets.2022.833346