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Effect of adding two levels of Resveratrol and Oleuropein to the diet on some biochemical traits of blood and gene expression for heat shock protein (HSP70) in the liver for broiler chickens reared under heat stress conditions.

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Abstract

The experiment was conducted in the poultry field belonging to the Animal Production Department, the college of Agriculture at Al-Qasim Green University for a period from 5/9/2020 until 6/20/2020. used in the experiment 225 broiler Chicks from the Ross-308 strain at the age of one day. He gave her fodder and water freely *adlibitum*, Chicks were distributed randomly to 5 treatments at an average of 3 replicates for each treatment, and for each replicate 15 birds, the birds were exposed to a temperature of 36 ± 1 ° C and the humidity 50-60% Chicks were distributed randomly into 5 treatments at a rate of 3 replicates per treatment and each replicate had 15 birds. and the experiment treatments were divided into 5 treatments (T1). A control group fed on a basic diet without adding any substance, (T2 and T3) were fed on a basic diet with the addition of Res at a concentration of 500 and 1000 mg. / Kg of diet, respectively, (T4 and T5) were fed on a basic diet with the addition of Ole at a concentration of 500 and 1000 mg / kg diet respectively. The results of this experiment showed that the adding of Res and Ole led to a significant decrease ($p < 0.01$) in the percentage of H / L and in the concentration of glucose and LDL. Also, a significant decrease ($p<0.01$) in the concentration of uric acid, ALT and AST enzyme, and a significant increase ($P < 0.01$) in the concentration of total protein, albumin and globulin, and an increase in the concentration of GSH-px enzyme in adding treatments compared with the control treatment T1.Also, a significant decrease ($p<0.01$) of gene expression HSP70 in the liver for the Res and Ole treatments compared with the control treatment T1, and the T3 treatment recorded the best results.

Key words: Resveratrol, Oleuropein, Heat Stress, HSP70, Broiler chickens

Introduction

Heat stress is one of the most important problems facing the poultry industry in the world, which affects birds and leads to physiological changes within the bird's body, disruption of the bird's immune functions, and thus ease of infection (Gharib et al., 2005). This results in lower feed consumption, lower body weight and higher mortality (Sahin et al., 2013). Heat stress is also one of the factors that increase and accelerate the production of free radicals, which leads to the occurrence of oxidative stress (Akbarian et al., 2016) Free radicals stimulate a group of enzymes within the cell, including Bax, Bak and Caspase, which lead to apoptosis (Dashzeveg and Yoshid, 2015; Redza-Dutordoir and Averill-Bates, 2016; Wan et al., 2016). Heat stress also leads to an elevated level of HSPs as a kind of high

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heat response (Liu et al., 2016; Murugesan et al., 2017; Xu et al., 2018).Therefore, the researchers resorted to using many means and food additives to reduce the effects of heat stress and oxidative stress on birds, and among these additives the use of antioxidants, including the compound Resveratrol(3,5,4-tryhydroxytrans stilbene) and Oleuropein 2-(3,4-dihydroxy phenyl). ethanol- (hydroxytyrosol),It is two natural compounds polyphenol, which are antioxidants, antiviruses, ant infections, and antibacterial (Visioli et al., 2002; Deng et al., 2008; Omar, 2010; Lee and Lee, 2010; Durlu-Ozkaya and Ozkaya, 2011; Tan et al., 2012; Aguirr et al., 2014; Francioso et al., 2014; Xia et al., 2017), They activate the nuclear factor Nrf2, which in turn activates the production of enzymatic antioxidants to eliminate free radicals(Ungvari et al., 2010; Zhang et al., 2017; ALhaithloul et al., 2019).They inhibit NF-kB and TNF-α that are activated by oxidative stress (Park et al., 2009; Deng et al., 2011; He et al., 2019; ALhaiyhloul et al., 2019).Res also inhibits Bax, Bak and Caspase enzymes, thus protecting DNA from oxidative damage, preserving the cell and preventing apoptosis (Yan et al., 2012; Yin et al., 2015; Zhang et al., 2018).because of the importance of these two compounds, this study aimed to know the effect of adding two levels of each compound to the diet and its effect on some biochemical blood traits and the gene expression of the heat shock protein HSP70 in the liver of broilers reared under heat stress conditions, as well as to know which level has an influencing role in the studied traits.

Materials and methods

The experiment was conducted in the poultry farm belonging to the Animal Production Department, College of Agriculture, Al-Qasim Green University for the period from 9/5/2020 until 6/20/2020. . used in the experiment 225 broiler Chicks from the Ross-308 strain at the age of one day and the average weight of the chick was 38 g Chicks were placed in cages (1 x 1.5 m).The chicks were raised on a bed of sawdust He gave her fodder and water freely *adlibitum* , Cylindrical feeders were used to provide fodder, and upside-down plastic manholes were used to provide water , and followed the continuous lighting system (24 hours/day) during the first three days, after which the chicks were shown up to 23 lighting hours,_ and one hour of darkness, The average temperature inside the hall was throughout the experiment period and according to the times, as at 9.00 (33 ± 1) m and at 15.00 (36 ± 1) m and at 21.00 (33 ± 1) c, and at 3.00 (28 ± 1) m and humidity 55-65%, Chicks were distributed randomly into 5 treatments, at an average of 3 replicates for each treatment, and for each replicate 15 birds. The diets were prepared weekly. Two materials, Res - Trans processed from the American company Asquared Nutrition, with a purity of 100% and Ole material, and supplied by the Chinese company CO., LTD CHANGSHA VIGORUS - TECHCO with 98% purity, were added to the feeds in the field weekly to maintain their availability. The chicks were fed on three diets as in Table (1) and the treatments were: T1: The chicks were fed on a basic diet without adding any substance (control treatment). T2, T3: The chicks were fed on a basic diets with Res added to it at a concentration of 500 and 1000 mg / kg diet respectively. T4, T5: The chicks were fed on a stander diet added to it with Ole at a concentration of 500 and 1000 mg / kg diet respectively.

Estimate the number of heterophyll cells (H) and lymphocytes (L) and the percentage of cells (H / L)

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The heterophil (H) and lymphocyte (L) cells were counted at the age of 42 days by taking blood from the Wing vein (6 birds from each treatment, 3 males and 3 females) and blood smears were made on glass slides directly from the bird by placing a drop of blood on the slide. With the capillary tube, the blood was then spread on the slide by another glass slide placed on the blood drop and drawn over the first slide at an angle of 45 degrees. After the blood dried (about 6-10 minutes), it was stained with Wright-Giemsa dye according to the method of shen and patterson (1983) and the count was performed Under the microscope, according to the Campbell method (1988), the L / H ratio was calculated by dividing the heterophyll cells by the lymphocytes.

Table 1. The percentages of the components of the diets used in the study and their calculated chemical composition

Components%	Types of diet		
	Starter 1-10 day	growth 11-22 day	Final 23-42 day
yellow corn	47.7	51.1	40
Wheat	10	10	23.7
soybean meal (1)	33	29.2	24.8
Animal Protein Concentrate (2)	5	5	5
oil	2	2.8	4.6
Limestone	1.1	1	1
Table salt	0.3	0.2	0.2
A mixture of vitamins and minerals	0.2	0.2	0.2
Dicalcium phosphate DCP	0.7	0.5	0.5
Total	100	100	100
Calculated chemical composition (3)			
Metabolizable Energy (kcal / kg feed)	3000.5	3093	3203.9
Crude protein (%)	23	21.5	20
Methionine (%)	0.5	0.48	0.46
Lysine (%)	1.3	1.2	1.1
Calcium (%)	0.92	0.83	0.82
Available phosphorous (%)	0.47	0.43	0.41

(1) The soybean meal used from an Argentinian source contains 48% crude protein and 2230 kilocalories / kg as representative energy. (2) The protein concentrate used is animal (Al Wafi), Dutch origin imported from Al Muwafak company that contains 40% crude protein 5% Raw fat, 2% crude fiber, 6.5% calcium, 4% available phosphorus, 3.85% lysine, 3.70% methaionine, 4% methionine + cysteine, 2.3% sodium, 2100 kcal / kg Metabolizable energy and contains a mixture of vitamins and trace minerals to secure the needs of the bird . Phytebase: 15,000 enzyme units / kg concentrate, 5,000 mg / kg choline chloride concentrate. (3) Chemical composition calculated according to NRC, 1994.

Biochemical traits of blood

Blood samples were collected at the end of the sixth week of the bird's agen by taking 6 birds (3 males and 3 females) from each treatment (2 birds per replicate). Blood was collected from each bird from the wing vein in an empty 5 ml tube It did not contain an anticoagulant. After that, it was placed in a centrifuge at a speed of 3000 rpm for 15 minutes for the purpose of separating the blood and obtaining the serum. After that, the serum was kept in tubes with tight covers in the freezer at a temperature of -20 ° C until laboratory tests were conducted. The analyzes included estimating the concentration of glucose, low-density lipoproteins (LDL), total protein, albumin, globulin, uric acid,

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GSH-px glutathione peroxidase, ALT and AST. The analyzes were performed in the laboratory of the Consulting Office of the College of Science at the University of Babylon using device Fujifilm Dry chemistry of Japanese origin. As for measuring LDL concentration according to the equation referred to by Grundy et, al (2004):

LDL concentration (mg / 100 mL blood) = total cholesterol concentration - HDL concentration - vLDL concentration

As for the concentration of globulin, it was calculated by the following equation:

Globulin concentration (g / L) = total protein concentration (g / L) - albumin concentration (g / L)

As for the GSH-px enzyme, it was estimated using a measuring kit (Kit) from the German company Roche, according to the method of Rotruch and others (1973).

Gene expression of the heat shock protein HSP70 in the liver

Sample collection

18 birds (9 males and 9 females) were slaughtered from each treatment at the end of the experiment, and then immediately a portion of liver tissue was taken and the sample was cut with a scalpel and the sample was placed in a tube containing an RNA solution later (Lader, 2001) for 24 hours at room temperature, after which the samples were placed. Under freezing, at a temperature of -20 ° C, until a gene expression measurement is performed in Al-Fadhel Company for Training and Development in Babylon province

RNA extraction

RNA was extracted from liver tissue using a kit provided by Genaid-Korea and according to the steps indicated by the supplier in the Kit. After the extraction process was completed, the RNA was kept at a temperature of - 20 ° C. Then, measure the quantity and purity of the RAN using a {Nanodrop (scan drop biometra, Germany)} device.

Choosing the primer

A primer was identified, which was supplied by the Korean company Bioneer, Table (2), and this primer was matched with the genetic sequence of the gene from the NCBI site, as the presence of the primer in the series was confirmed and its location was determined.

Table 2 Genetic sequence of the HSP70 gene and the GAPDH gene.

Gene name	primer	size	References
HSP70	F 5'-ATGAGCACAAGCAGAAAGAG-3'	95bp	Belal et al,2018
	R 5'-TCCCTGGTACAGTTTTGTGA-3'		
GAPDH	F 5'-AGAACATCATCCCAGCGTCC -3'	130bp	
	R 5'-CGGCAGGTCAGGTCAACAAC-3'		

HSP70: heat shock protein70, GAPDH: Glyceraldehyde-3-phosphate dehydrogenase

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Supplementary RNA synthesis (cDNA synthesis)

cDNA was synthesized from RNA samples with Real-Time PCR assay, It used an Accupower Rockscript RT Premix kit provided by the Korean company Bioneer and converted all RNA samples to cDNA by preparing a PreMix reaction for quantitative real-time reverse cloning. This step was performed according to the instructions of the company via , Add 1 µl of RNA, add 1 µl of primer (Oligo poly dT), add 18 µl of water (dd.water) to the Master Max tube. The work of centrifugation (c.f) / 5 seconds after which the samples were placed in the PCR device, according to the conditions shown in Table (3).

Table 3 :Set a standard PCR device for the manufacture of complementary cDNA synthesis

Stages	Temperature (C) °)	Time (seconds: minutes)	The number of cycles
Annealing	**37**	**10:00**	**1**
Elongation	**42**	**30:00**	**1**
Denaturation	**95**	**5:00**	**1**

References: Cheon et al. (1999) with some necessary adjustments.

Then the polymerase chain reaction was examined for quantitative real-time reverse cloning (Quantitative Real-Time PCR (qRT-PCR)), qRT-PCR assay was performed for cDNA samples using cyntol syber green master mix rissia count and Rotorgen Q, Qiagene, Germany) equipped. From the Korean company Pioneer, according to the steps described by Cheon (et al., 1999) by adding cDNA with a volume of 2 µl, a syber of 8 µl, and refined distilled water 7.5 µl, and an upper and lower head of 1 µl each with the addition of MgCl with a volume of 0.5 µl with the addition of some necessary adjustments and depending on the circumstances. Shown in Table (4),This assay uses the SYBR® Green stain in the qRT-PCR Detector PreMix kit which is designed to amplify the cDNA of the HSP70 gene using the primer and the GAPDH gene as a standardized conservative gene to quantify the number of copies produced from PCR compared to the copy number of the standard curve of the genome. Output from qPCR, The cyber green dye in the kit binds to the target gene and the conservative gene, after which the fluorescent signals are recorded in the Thermocycler Real Time PCR. The data were analyzed using (Fold change $=\mathbf{2^{-\Delta\Delta CT}}$) and according method to Livak and Schmittgen (2001).

Table 4: Set a standard qRT-PCR device for the HSP70 gene

Stages	Temperature (C) °)	Time (seconds: minutes)	The number of cycles
Initial denaturation	**94**	**10:00**	**1**
denaturation	**94**	**00:15**	**40**
Annealing/ Extension	**60**	**00:30**	

References: Cheon et al. (1999) with some necessary adjustments

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statistical analysis

Statistical Analysis System -SAS (2012) was used in data analysis to study the effect of different treatments on studied traits according to a complete random design (CRD). The significant differences between the averages were compared with the Duncan (1955) polynomial test.

Results and discussion

H% heterophyll cells,% L lymphocytes, and H / L%

Table (5) shows the effect of my material Res and Ole added to broiler feed on the diet of H and L cells and the H / L ratio of broilers raised under heat stress conditions at 42 days of age. Whereas, it is noticed that there is a significant decrease ($p<0.01$) in the percentage of H cells to the adding treatments compared with the control treatment T1, and the percentage of H cells in treatment T3 decreased significantly compared with the T4 and T5. With regard to L cells, we note that there was a significant superiority ($p<0.01$) for the Res and Ole adding treatments on the control treatment. Treatment T3 significantly excelled on treatments T2, T4 and T5, and treatment T2 excelled on treatment T5, and there were no significant differences between T2 and T4 and between the T4 and T5. As for the percentage of H / L cells, we note that there was a significant decrease ($p<0.01$) for the adding treatments compared with the control treatment, and the two treatments T2 and T3 decreased significantly compared with treatment T5, and there were no significant differences between the two treatments T2 and T4 and between the two treatments T2 and T3 and between the two treatments T4 and T5. The decrease in the percentage of H / L in the blood of birds with the addition of Res and Ole treatments compared with the control treatment T1 is due to the role of Res, which works to inhibit the secretion of the hormone corticosterone from cells of the adrenal cortex by inhibiting cytochrome P450c21-hydroxylase (Supornsilchai et al., 2005).The hormone corticosterone stimulates the release of H cells from the bone marrow and analyzes the L cells, so the H / L percentage is disturbed (Feizi et al., 2012; Duangjinda et al., 2017; Jaiswal et al., 2017; Xu et al., 2018). Ge et al. (2016) stated that Res regulates the overactivity of the hypothalamic-pituitary-adrenal cortex and thus reduces the secretion of the hormone corticosterone. Res and Ole also work to eliminate free radicals and thus inactivate Caspase enzymes that divide the DNA strip and thus protect the lymphocyte from death.Res and Ole also activate Nrf2, which in turn increases the activity of antioxidant enzyme to eliminate free radicals (Ungvari et al., 2010; Hasko and Pacher, 2010; Deng et al., 2011; Yi et al. 2011; Sun et al., 2017; Zhang et al., 2019; ALhaithloul et al., 2019).

Table 5. The effect of adding two levels of Res and Ole to the diet on the percentage of heterophyll cells (H), the percentage of lymphocytes (L)) and the ratio (L / H) of broilers reared under heat stress conditions at the age of 6 weeks.

Treatments	mean ± standard error		
	Heterophyll cells (H)%	Lymphocytes cell (L%)	H / L%
T1	30.23 ±0.31 a	64.47 ±0.47 d	0.468 ±0.06 a
T2	23.46 ±0.49 bc	72.33 ±0.63 b	0.324 ±0.08 dc
T3	22.01 ±0.55 c	74.56 ±0.57a	0.295 ±0.01 d

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T4	**25.13 ±1.08 b**	**71.16 ±0.68 bc**	**0.353 ±0.01 cb**
T5	**25.53 ±0.63 b**	**69.76 ±0.73 c**	**0.365 ±0.01 b**
level of significance	**	**	**

The averages carrying different letters within the same column differ significantly between them. ** (P <0.01).

Biochemical traits of blood

We note from Table (6) the effect of adding two levels of Res and Ole to the feed on the concentration of total protein, albumin, globulin, and uric acid for broilers reared under heat stress conditions at 42 days of age. Where, a significant increase ($p<0.01$) was observed in the total protein concentration of the Res adding treatments (T2 and T3) and Ole (T4 and T5) compared with the control treatment T1. With regard to albumin concentration, the addition significant increase ($p> 0.01$) excelled on the adding treatments on the control treatment T1, the treatment T2 excelled on the treatments T3, T4 and T5, and the treatment T3 excelled on T5 .With regard to the concentration of globulin, the agents of Res and Ole were significant increase on the control treatment T1 and the treatment T3 excelled on the treatment T5. As for the concentration of uric acid, we note a significant decrease ($P <0.05$) for the adding treatments compared with the T1 control treatment, and there were no significant differences between the adding treatments. Table (7) shows the effect of adding two levels of Res and Ole to the diet on the concentration of glucose, low-density lipoproteins (LDL), and AST, ALT and GSH-px enzymes for broilers reared under heat stress conditions. Whereas, it is noticed that there is a significant decrease ($p<0.01$) for the treatment of Res and Ole addition compared with the control treatment T1 in glucose concentration. The treatment T3 decreased significantly compared to the treatment T5, and no significant differences were observed between the treatments T2, T3 and T4, as well as between the treatments T2, T4 and T5. With regard to the LDL concentration, the additive treatments recorded a significant decrease ($P <0.01$) compared to the T1 control treatment. A significant decrease was observed for treatment T3 compared to treatments T2, T4 and T5, as well as significantly decreased for treatments T2 and T5 compared with treatment T4. For AST and ALT enzymes, a significant decrease ($P<0.05$) was observed for the Res and Ole adding treatments compared with the T1 control treatment. Non-significant differences were observed between the adding treatments._With regard to the GSH-px enzyme, we note a significant increase ($p<0.01$) in the concentration of the GSH-px enzyme for the addition treatments compared with the control treatments T1. treatment T3 significantly excelled on the treatments T2, T4 and T5, and the two treatments T2 and T5 excelled on treatment T4. The high concentration of total protein, albumin, and globulin and the low concentration of uric acid in the blood serum of the Res Ole treatment birds is due to the role of these two substances in eliminating free radicals as well as activating Nrf2, which works to stimulate the production of antioxidants enzymatic, which in turn work to eliminate free radicals and thus protect RNA for building a specific protein and protecting amino acids from oxidative stress thus maintains the balance of amino acids that are essential for building protein hormones and enzymes and increasing muscle protein synthesis (Hu et al., 2019; Shimao et al., 2019) Res works to regulate the hyperactivity of the hypothalamic-pituitary-adrenal cortex axis and thus the secretion of the hormone corticosterone decreases (Ge et al., 2016).As Supornsilchai et al. (2005) showed that Res works to inhibit the secretion

of the hormone corticosterone from cells of the adrenal cortex by inhibiting cytochrome P450c21-hydroxylase in cells of the adrenal cortex and thus regulates the functioning of the hypothalamic-pituitary-adrenal cortex axis, thus protecting the protein from subduction The effect of the hormone corticosterone, Res also stimulates the secretion of growth hormone, which increases protein production in the body and protects protein in the blood serum (Harvey and Etches, 1997; Liu et al., 2014). Table (7) shows the effect of adding two levels of Res and Ole to diet on glucose concentration, as we note the decrease in its concentration in the blood serum of birds of adding treatments compared with the control treatment T1, Because the adding of Res and Ole works to eliminate free radicals and protect the beta cells of the pancreas from the influence of free radicals, thus preserving the activity of the pancreas in the secretion of the hormone insulin (Rubiolo et al., 2008; Andrikopoulos et al., 2002), Res also reduces blood sugar, as it stimulates glucose entry into cells, promotes the transport of GL4T4 into cells, especially muscle cells, and also enhances GL4T4 production in muscles (Deng et al., 2008; Tan et al., 2012). Res also inhibits secretion,The hormone corticosterone from the adrenal cortex (Supornsilchai et al., 2005) thus maintains the level of blood sugar. Res also acts on the formation of citrulline and in turn stimulates the secretion of the insulin hormone from the pancreas (Allerton et al., 2018).Ole also reduces blood sugar by increasing insulin secretion or stimulating glucose absorption (Gonzalez et al., 1992). With regard to the concentration of low-density lipoprotein LDL, we notice a decrease in its concentration in the serum of addition agents compared with the control treatment T1 and this is due to the role of antioxidants, including the substance Res works to reduce the secretion of the hormone corticosterone (Supornsilchai et al., 2005), In this way, the adipose tissue is not degraded, it also works to eliminate free radicals and prevent fat oxidation, thus reducing LDL and triglycerides in the blood (Gocmen et al., 2011). Res also reduces the activity of the 3-hydroxy-3-methlyglutaryl coenzyme –A reductase enzyme that contributes to In addition to cholesterol synthesis, Res increases LDL receptors in liver cells (Yashiro et al., 2012).Which contributes to lowering the level of LDL in the blood, Feng et al. (2017) also indicated that Res has a role in reducing the level of cholesterol and LDL by eliminating free radicals and preventing fat oxidation and thus lowering the level of LDL in the blood serum. Also, Ole has antioxidant activity to eliminate free radicals, thus providing a protective effect against lipid oxidation and preventing the rise of low-density lipoproteins, LDL (Sarica and Topbas, 2014).Ole also inhibits LDL oxidation and inhibits the 3-hydroxy-3-methlyglutaryl coenzyme –A reductase enzyme, which has an important role in cholesterol synthesis (Sung et al., 2004). Parasei (2014) also showed that adding Ole to poultry diets reduces the fat concentration in the blood serum . Well among the Romani et.al (1999) that Ole works to reduce cholesterol and triglycerides in the blood serum and liver. The mechanism of this action may be by inhibiting the absorption of dietary cholesterol in the intestine or by stimulating the secretion of bile in the liver as well as stimulating the excretion of cholesterol in the stool (Rezar et al, 2015).As for ALT and AST enzymes, we note that their concentration decreased in the blood serum of adding treatments compared with the control treatment T1.This is due to the role of Res and Ole in eliminating free radicals and thus protecting the liver tissue from oxidative damage and thus reducing the secretion of ALT and AST enzymes (Zhang et al., 2019; He et al., 2019; Agah et al., 2019).Parsaei et al. (2014) stated that Ole has a role in inhibiting oxidative stress and protecting liver tissue from oxidative damage.In this methods,

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ALT and AST enzymes are inhibited from the liver cells (Tiot et al., 2001). Res works by inhibiting the secretion of the hormone corticosterone from the adrenal cortex (Supornsilchai et al., 2005).Thus reducing the secretion of ALT and AST enzymes. With regard to the GSH-px enzyme, we notice a higher concentration in the serum of birds with the adding treatments compared with the control treatment T1. This is due to the role of additives in reducing the severity of oxidative stress by eliminating free radicals and stimulating Nrf2 / ARE activity. Thus enhancing the capacity of enzymatic antioxidants, including GSH-px, which in turn eliminates free radicals (Sahin et al., 2010; Cheng et al., 2015; Feng et al., 2017; He et al., 2018; Zhang et al., 2019; ALhaiyhloul et al., 2019).As heat stress affects birds and significantly reduces the activity of antioxidant enzymes, oxidative stress due to heat stress also significantly reduces GSH-px levels due to reduced Nrf2 expression (Sahin et al., 2010; Liu et al., 2014; Wan et al., 2017 ; Zhang et al., 2017; Chen et al., 2018).

Table 6. The effect of adding two levels of Res and Ole to the diet on the concentration of (total protein, albumin, globulin, uric acid) of broilers reared under heat stress conditions at the age of 6 weeks.

Treatments	mean ± standard error (weeks)			
	Total protein (g/ 100 mL serum)	albums (g / 100 mL serum)	Globulin (g/ 100 mL serum)	Uric acid (mg / 100 serum
T1	2.31 ±0.06 d	1.12 ±0.02 d	1.19 ±0.04 c	6.67 ±9.38 a
T2	3.65 ±0.02 a	1.47 ±0.01 a	2.18 ±0.01 ab	4.52 ±0.66 b
T3	3.61 ±0.05 ab	1.40 ±0.08 b	2.21 ±0.01 a	4.60 ±0.18 b
T4	3.53 ±0.02 b	1.36 ±0.08 bc	2.17 ±0.03 ab	4.80 ±0.28 b
T5	3.43 ±0.02 c	1.32 ±0.02 c	2.11 ±0.02 b	5.06 ±0.50 b
level of significance	**	**	**	*

The averages carrying different letters within the same column differ significantly between them. * (P <0.05), ** (P <0.01).

Table 7. The effect of adding two levels of Res and Ole to the diet on the concentration of (glucose and low density lipoproteins (LDL), ALT enzyme, AST enzyme and GSH-px enzyme) of broilers reared under heat stress conditions at the age of 6 weeks

Treatments	mean ± standard error (weeks)				
	Glucose (mg / 100ml serum)	LDL (mg / 100 mL serum)	ALT (IU / L)	AST (IU / L)	GSH-px (IU / L)
T1	312.33 ±0.38 a	91.16 ±11.85 a	8.96 ±0.66 a	376.00 ±30.08 a	658.33 ±9.70 d
T2	258.33 ±5.04 bc	55.50 ±0.28 c	6.76 ±0.06 b	272.00 ±5.85 b	877.00 ±8.88 b
T3	245.00 ±8.14 c	47.33 ±1.85 d	6.33 ±1.76 b	237.33 ±34.45 b	956.34 ±3.28 a
T4	265.00±5.50 bc	67.46 ±1.24 b	7.62 ± 0.88 b	304.00 ±9.16 b	841.72 ±7.26 c
T5	270.66 ±4.17 b	60.00 ±2.88 c	7.00 ±0.57 b	299.57 ±38.36 b	892.10 ±7.23 b
level of significance	**	**	*	*	**

The averages carrying different letters within the column differ significantly between them. * (P <0.05), ** (P <0.01)

Gene expression for the heat shock protein HSP70 in the liver

Figure (1) shows the effect of adding two levels of Res and Ole to the diet on the gene expression of the heat shock protein HSP70 in the liver of broilers reared under heat stress at the age of 42 days. The proportion of the expression was calculated and expresse (Fold change**)** Figure (2). As we note the high level of HSP70 gene expression in the liver of the T1 control birds compared with the adding

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treatments, while the gene expression decreased significantly (P <0.01) in the adding treatments. The treatment T3 recorded a significant decrease (P <0.01) in the gene expression HSP70 compared with the treatments T2, T4 and T5 .The reason for the decrease in HSP70 gene expression of the Res and Ole addition treatments is due to its role in alleviating heat and oxidative stress, eliminating free radicals, and inhibiting a group of cellular factors that are activated by heat stress and lead to apoptosis .It works by inhibiting the nuclear factor NF-kB that causes inflammation, as well as inhibiting the production of interleukins IL-1, IL-4, IL-6 and tumor necrosis factor (TNF-α) as well as inhibition of Bax and Bak enzymes and caspase-3 enzymes that work on cell DNA fragmentation and death. The role of Res and Ole in eliminating free radicals and inhibiting factors above with Nrf2 / ARE activation that stimulates the production of enzymatic antioxidants to eliminate free radicals led to a decrease in HSP70 gene expression (Rubiolo et al., 2008; Hasko and Pacher, 2010; Impellizzeri et al., 2011; Deng et al., 2011; Sahin et al., 2011; Yin et al., 2015; Cheng et al., 2015; Sarica et al., 2015; Ryu et al., 2015; Liu et al., 2016; Alhaithloul et al., 2019; He et al., 2019; He et al., 2020), , While we observe an increase in the level of HSP70 gene expression in the liver of control birds due to exposure to heat and oxidative stress, where free radicals stimulate a group of cellular factors that stimulate apoptosis (Hu et al., 2019; Xing et al., 2019),Therefore, HSP70 increased, which in turn works to inhibit apoptotic factors by binding and modifying these factors, including Bax, Bak, Bid, TNFα, Fas, and Apaf-1 enzymes, thus preventing the production of the Apoptosome complex and not activating caspase enzymes that break down cell DNA and die (Takayama and Somero , 2003; Beere, 2004; Ikwegbue et al., 2017).

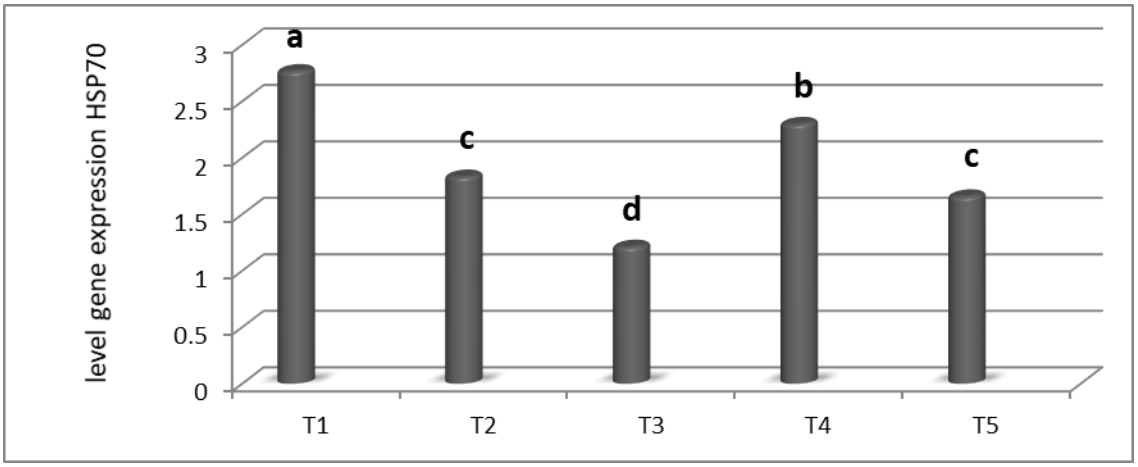

Figure 1. The effect of adding two levels of Res and Ole to the diet on the gene expression of the heat shock protein HSP70 in the liver of broilers exposed to heat stress at 42 days of age.

The different letters on the columns indicate significant differences between the parameters (P<0.01). and the similar letters indicate that there are no significant differences between the treatments.

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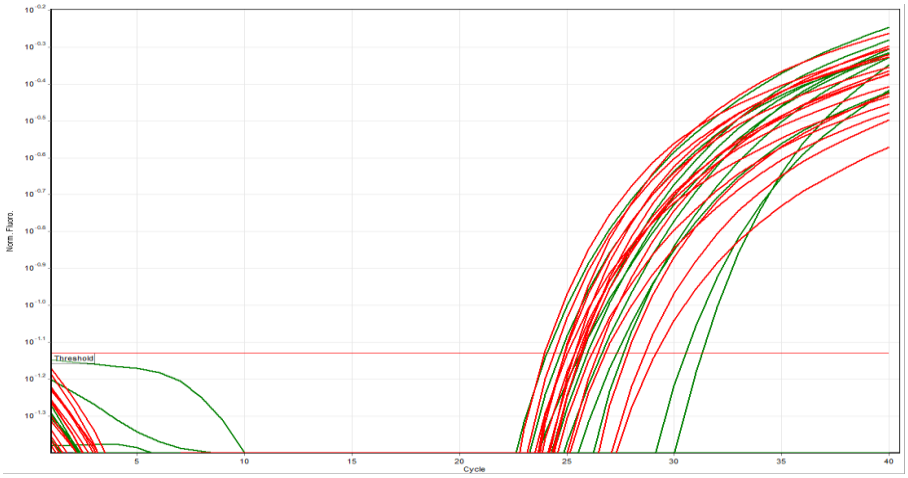

Figure 2. curve of HSP70 gene amplification with GAPDH in broiler livers exposed to heat stress with PCR technique. The red samples were HSP70 and GAPDH green.

Conclusions

The addition of Res and Ole improved most of the physiological traits of broilers reared under heat stress conditions by reducing heat stress and oxidative stress on birds.Addition of Res and Ole reduced HSP70 gene expression in the liver due to their antioxidant role. Res 1000 mg / kg was more effective. In general, Res efficacy was better than Ole in improving some physiological traits and gene expression of HSP70 in the liver of broilers reared under heat stress conditions.

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