EFFECT OF SUPPLEMENTING RESVERATROL AND OLEUROPEIN ANTIOXIDANTS TO DIET IN SOME BIOCHEMICAL TRAITS OF BLOOD AND SOME HISTOLOGICAL TRAITS IN THE SMALL INTESTINE FOR BROILER CHICKENS

Ali Faraon Obayes Aljebory

Babylon Agriculture Directorate

avphdidk@yahoo.com

Dhia Khalil Ibrahim

University of Baghdad ,

College of Agricultural Engineering

alialjebory37@yahoo.com

ABSTRACT

The experiment was conducted in the Poultry Farm of Animal Production Department / College of Agriculture at Al-Qasim green University for a period from 3/2/2020 to 16/3/2020 for (42 days) Atotal of 225 broiler chicks, (ROSS-308) unsexed ,day old, and provided feed and water was given *ad libitum* , The ambient temperature ranged from 18-34°C and the relative humidity was 50-60%, chicks were distributed randomly into 5 treatments with 3 replicates per treatment 15 chick / replicate , the experimental treatments were as follows: (T1). control group without any addition, (T2 and T3) addition of resveratrol(res) at 250 and 500 mg. / Kg concentration diet respectively, (T4 and T5) addition oleuropein(ole) at of 250 and 500 mg / kg concentration diet respectively. Results showed that res and ole led to significant decrease ($p<0.01$) in Heterophil/ Lymphocyte (H / L) in all addition treatments compared with control. significant decrease in the serum concentration uric acid, cholesterol, Low Density Lipoproteins(LDL), alanine amino transferase (ALT) and aspartat amino transferase(AST)with a significant increase ($p<0.05$) in serum total protein, albumin, as well as a significant increase ($p<0.01$) in serum glutathione peroxidase (GSH-Px) in additive treatments compared with control, Adding res and ole led to significant improvement in histological parameters, as the villi length and width and the crypts depth were increased ($p<0.01$).

Key Words, resveratrol, oleuropein, Oxidative Stress, broiler chickens.

ISSN 2072-3875

تأثير اضافة مضادات الاكسدة **resveratrol** و**oleuropein** الى العليقة في بعض صفات الدم الكيموحيوية وبعض الصفات النسيجية في الامعاء الدقيقة لفروج اللحم

علي فرعون عبيس الجبوري
مديرية زراعة بابل
باحث علمي
alialjebory37@yahoo.com

ضياء خليل ابراهيم
جامعة بغداد / كلية علوم الهندسة الزراعة
استاذ
avphdidk@yahoo.com

الخلاصة

اجريت التجربة في حقل الطيور الداجنة التابع لقسم الانتاج الحيواني في كلية الزراعة جامعة القاسم الخضراء للمدة من 2020/2/3 حتى 2020/3/16 ,(42 يوما) استخدم فيها 225 فرخا من فروج لحم روز 308 غير مجنس بعمر يوم واحد , وقدم لها العلف والماء بصورة حرة *ad libitum* وكانت درجة الحرارة داخل القاعة تتراوح من 18-34مº والرطوبة النسبية 50-60 % , وزعت الافراخ عشوائيا على 5 معاملات , 3 مكرر لكل معاملة و15 طير/ مكرر وكما يلي : (T1) مجموعة سيطرة من دون اضافة أي مادة , (T2 وT3) اضافة res بتركيز 250 و500 ملغم / كغم علف على التوالي , (T4 وT5) اضافة ole بتركيز 250 و500 ملغم / كغم علف على التوالي . اظهرت النتائج ان إضافة res و ole ادت الى انخفاض معنوي ($p<0.01$) في (H/L) في معاملات الاضافة مقارنة مع معاملة السيطرة T1 , ولوحظ انخفاض معنوي في تركيز حامض اليوريك والكولسترول و(LDL) وانزيم (ALT) و(AST) مع زيادة معنوية ($P<0,05$) في تركيز البروتين الكلي والالبومين وكذلك زيادة معنوية ($P<0.01$) في تركيز انزيم (GSH-Px), في معاملات الاضافة مقارنة مع معاملة السيطرة T1 , مع تحسن في الصفات النسيجية اذ ارتفع معنويا ($p<0.01$) طول الزغابات وزاد عرضها وزاد عمق الخبايا .

كلمات مفتاحية : oleuropein, resveratrol , الاجهاد التأكسدي , فروج اللحم

البحث مستل من اطروحة دكتوراه للباحث الاول

Introduction

Broiler breeds are characterized by rapid growth and high metabolism, and thus require large amounts of oxygen and produce high amounts of energy. The process of energy production in mitochondria during cellular respiration is accompanied by an increase in the production of free radicals, which leads to the state of oxidation, which is one of the natural processes that They occur inside the body(1) and(2) and these free radicals are removed by the antioxidants present inside the body, but when there are large numbers of free radicals that exceed the capacity of the antioxidants, oxidative stress occurs(3). As these free radicals affect the cells of the body and cause damage to all biological molecules such as DNA, proteins and fats, which leads to cell damage and pathological complications(4) . Therefore, researchers resorted to using a number of means and food additives to reduce the effects of oxidative stress on birds, including the use of natural antioxidants, including resveratrol and oleuropein, which are two natural polyphenol compounds that have antioxidants, viruses, infections and bacteria(5) ,(6)and (7), they activate nuclear factor erythroid 2-related factor-2(Nrf2) which in turn activates the production of antioxidants enzymatic to eliminate free radicals (8and(9) , as They increase the formation of Nitric Oxide(NO) and

ISSN 2072-3875

citrulline(10) and (11), and inhibit nuclear factor kappa (NF-kB) and tumor necrosis factor alpha (TNF-α) that are activated by oxidative stress. (12), (13),(14) and (9), also res reduces the incidence of apoptosis(15) and (16). Because of the effective role of these two compounds in resisting the harmful effects of oxidative stress, and our use of different concentrations from previous studies, this experiment aimed to know the effect of adding two levels of each compound to the diet on the biochemical blood characteristics and some histological traits in the small intestine of broilers.

Materials and methods

The experiment was conducted for a period from 3/2/2020 to 3/16/2020 (42 days). Atotal of 225, day old, unsexed broiler chicks, ROSS-308, were brought from Al-Anwar Hatchery, Babil Governorate, with the average weight 42 g/chick. The chicks were placed in pens of an area (1 x 1.5 m) and reread on a bed of sawdust thick 4-6cm. feed and water was given *ad-libitum*. lighting program was 24 hours during the first 3 days, then the chicks were shown up to 23 hours of lighting and an hour of darkness started at one o'clock pm until the end of the period, The ambient temperature inside the room ranged from 18-34°C is and the relative humidity was 50-60%, . The feed was mixed weekly in the family feed factory ,Trans- resveratrol prepared from the American company aSquared nutrition with a purity of 100% and oleuropein material from the Chinese company CHANGSHA VIGORUS – TECHCO CO,LTD with 98% purity, were added to the feeds in the field weekly to maintain their availability.Chicks were fed on three experimental diets as shown in Table (1) Chicks were fed on three experimental diets, which are the starter diet for a period of 1-10 days, the growth diet for a period of 11-22 days, and the final diet for a period of 23-42 days, and the treatments were as follows, The experimental treatments were as follows: (T1), control group without any addition, (T2 and T3), adding res at 250 and 500 mg/ Kg concentration diet respectively, (T4 and T5) adding ole at of 250 and 500 mg / kg concentration diet respectively.

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

Components%	**Starter1-10 day**	**growth 11-22 day**	**Finisher 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
Limeston	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

ISSN 2072-3875

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
methionine + cysteine (%)	0.86	0.82	0.77
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 (17)NRC,1994.

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

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 (18) and the count was performed under the microscope, according to the (19), the L / H was calculated by dividing the heterophyll cells by the lymphocytes.

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, cholesterol, triglycerides, high-density lipoproteins (HDL), Low-Density Lipoproteins (LDL), very low-density lipoproteins (VLDL), total protein, albumin, globulin, uric acid, GSH-px enzyme., ALT and AST, the analyzes were carried out in the laboratory of the Consulting Office of

ISSN 2072-3875

the College of Science at the University of Babylon using a Japanese-origin Fujifilm Dry-chemistry device. As for the measurement of LDL concentration, it was done according to the equation referred to by (20):

LDL concentration (mg/100ml of blood) = total cholesterol concentration - HDL concentration - VLDL concentration.

As for the concentration of VLDL, it was estimated according to the following equation and based on the method of (21): VLDL concentration = triglycerides \ 5.

As for the globulin concentration, it was calculated by the following equation: Globulin concentration (g/dL) = total protein concentration (g/dL) - albumin concentration (dL g/) As for the enzyme GSH-Px, it was estimated using a measuring kit (Kit) from the German company Roche, according to the method of (22).

Prepare histological sections

6 Birds (3 males and 3 females) were slaughtered for each treatment at the end of 6 weeks of age. Then, samples of histological sections were taken directly from the jejunum portion of the small intestine to ensure that intestinal tissue was not damaged. Where two sections of each (2 cm) length, one of them longitudinal and the other transverse, were taken from the second part of the small intestine (jejunum).The intestinal contents were removed and the samples taken with distilled water were washed well and I attended the histological clips according to what he mentioned (23), all the histological sections were examined using the Compound microscope. The measurements were recorded using the Ocular micrometer with a magnification power (100 X) after calibrating it with the stage micrometer. The villi length, villi width, and depth of the villi were estimated, noting that the villi length extends from the villi apex to the villi's association with the villi, and the villi width was estimated by taking an average of two readings from the base and middle of the villi, while the depth of the crypt is the distance from The villus base to the end of crypt (24)All measurements were made for (10) readings for each measurement and the average was taken.

Statistical Analysis

(25) 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 (26) polynomial test.

Results and discussion

Heterophil cells (H) % and lymphocytes (L) % and the ratio between them

It is evident from Table 2 the effect of the antioxidants res and ole added to the diet on the percentage of ((H cells and the percentage of L cells)) and (H/L), as it is noted that there are no significant differences in the percentage (H) between the addition treatments and the control treatment T1, while A significant increase ($p<0.01$) was observed in the percentage of (L) cells for the res and ole addition treatments compared with the control group T1, and no significant differences were observed between the treatments T2, T3, T4 and T5. with regard to H/L, a significant decrease ($p<0.01$) was observed for birds of treatments T2, T3, T4 and T5

ISSN 2072-3875

compared with to control group T1 and there were no significant differences between the addition treatments. The reason for the decrease in H/L when adding antioxidants such as phenolic substances, including res and ole is due to the fact that these substances inhibit the nuclear factor NF-kB as well as caspase enzymes in the cell, which leads to the protection of DNA in the lymphatic cell from fragmentation, and also activates the factor NrF2, which activates the production of enzymatic antioxidants to eliminate free radicals.(27), (13) , (28), (16) and (9). this explains why lymphocyte counts are high in res and ole addition treatments and then H/L decreases.

Table 2. Effect of adding the resveratrol and oleuropein antioxidants to the diet on the percentage of heterophil cells (H), lymphocytes (L) and (H/L) of broilers at 6 weeks of age. (mean ± standard error).

Treatments	Heterophil cells (H)%	Lymphocytes(% L%)	H/L
T1	20.75 ±1.43	61.36 ±2.11 [b]	0.338 ±0.12 [a]
T2	19.15 ±0.93	70.08 ±0.86 [a]	0.273 ±0.18 [b]
T3	20.01 ±0.55	67.67 ±1.43 [a]	0.295 ±0.21 [b]
T4	18.98 ±0.31	68.14 ±0.87 [a]	0.278 ±0.13 [b]
T5	19.10 ±0.82	67.26 ±2.18 [a]	0.283 ±0.02 [b]
Levelof Significant	N.S	**	**

**(p <0.01), N.S, Not significant .
T1, control treatment (without addition), T2,T3 addition of resveratrol at a concentration of 250 ,500 mg / kg of diet, T4,T5 addition of oleuropein at a concentration of 250 , 500 mg / kg of diet.

biochemical traits of blood

Table 3 shows the effect of adding the res and ole antioxidants to the diet on the concentration of total protein, albumin, globulin and uric acid for broilers at 6 weeks of age. It was noticed that there were no significant differences between the res and ole addition treatments and the control treatment T1 in globulin concentration. In the concentration of total protein, a significant superiority (P<0.05) was observed in favor of treatments T2, T3 and T5 over the control treatment T1, and no significant differences appeared between treatments T1 and T4, as well as between treatments T2, T3, T4 and T5. As for the albumin concentration, the results showed that the res and ole addition treatments were significantly (P<0.05) superior to the control treatment T1 and there were no significant differences between the addition treatments. As for the concentration of uric acid, a significant decrease in the concentration of uric acid was observed for the addition treatments T2, T3, T4 and T5 compared with the control group T1, and there were no significant differences between all the addition treatments. It is clear from Table 4 the effect of adding res and ole to the diet on the concentration of glucose and AST, ALT and GSH-px enzymes at the age of 6 weeks . GSH-px note significant decreased (P<0.05) and(p<0.01) in the concentrations of ALT and AST enzymes, respectively, in favor of the res and ole addition treatments compared with the control group T1, and no significant differences were observed between the treatments T2, T3, T4 and T5. Regarding the concentration of GSH-px enzyme, it was observed that a significant (p<0.01) of the treatments T2, T3, T4 and T5 was significantly superior to the control treatment T1,

ISSN 2072-3875

and no significant differences were observed between the treatments T2, T3, T4 and T5. It is noted from Table 5 the effect of adding res and ole to the diet on the concentration of cholesterol, triglycerides, HDL, LDL and VLDL concentrations at the age of 6 weeks. It was noted that there were no significant differences between the addition treatments and the control treatment in the concentration of triglycerides, HDL and VLDL, as for the concentration of cholesterol and LDL, a significant decrease was observed for the addition treatments compared with the T1 .

Table 3. The effect of adding the resveratrol and oleuropein antioxidants to the diet on the concentration of (total protein, albumin, globulin, uric acid) in the blood serum of broilers at 6 weeks of age. (mean ± standard error).

Treatments	total protein (gm/100ml serum)	albumin (gm/100ml serum)	globulin (gm/100ml serum)	uric acid (mg/100ml serum)
T1	3.31 ±0.25 [b]	1.21 ±0.23 [b]	2.10 ±0.13	4.81 ±0.86 [a]
T2	4.18 ±0.06 [a]	1.89 ±0.01 [a]	2.29 ±0.06	3.32 ±0.11 [b]
T3	4.40 ±0.17 [a]	1.81 ±0.14 [a]	2.59 ±0.04	3.52 ±0.70 [b]
T4	3.92 ±0.32 [ab]	1.69 ±0.12 [a]	2.23 ±0.26	3.67 ±0.61 [b]
T5	4.17 ±0.06 [a]	1.73 ±0.12 [a]	2.44 ±0.07	3.46 ±0.25 [b]
Level of Significant	*	*	N.S	*

* (P <0.05), N.S, Not significant .
T1, control treatment (without addition), T2,T3 addition of resveratrol at a concentration of 250 ,500 mg / kg of diet, T4,T5 addition of oleuropein at a concentration of 250 , 500 mg / kg of diet.

Table 4. The effect of adding the resveratrol and oleuropein antioxidants to the diet on the concentration of (glucose, ALT enzyme, AST enzyme and GSH-Px enzyme) in the blood serum of broilers at 6 weeks of age. (mean ± standard error).

Treatments	Glucose (mg/100ml serum)	ALT (IU/L)	AST (IU/L)	GSH-px (IU/L)
T1	201.90 ±10.55	6.74 ±1.64 [a]	176.10 ±2.95 [a]	257.15 ±24.30 [b]
T2	189.99 ±5.65	4.63 ±0.53 [b]	149.24 ±3.71 [b]	341.33 ±14.18 [a]
T3	191.88 ±8.94	5.00 ±0.52 [b]	154.95 ±2.83 [b]	321.26 ±8.41 [a]
T4	191.97 ±2.44	4.98 ± 0.66 [b]	151.33 ±2.08 [b]	337.51 ±8.72 [a]
T5	194.89 ±11.71	5.04 ±0.76 [b]	157.52 ±5.71 [b]	327.83 ±25.63 [a]
Level of Significant	N.S	*	**	*

* (P <0.05), **(p <0.01), N.S, Not significant .
T1, control treatment (without addition), T2,T3 addition of resveratrol at a concentration of 250 ,500 mg / kg of diet, T4,T5 addition of oleuropein at a concentration of 250 , 500 mg / kg of diet.

ISSN 2072-3875

Table 5. Effect of adding the resveratrol and oleuropein antioxidants to the diet in the blood lipid profile for broilers at 6 weeks of age (mean ± standard error).

Treatments	Cholesterol (mg/100ml serum)	triglycerides (mg/100ml serum)	HDL (mg/100ml serum)	LDL (mg/100ml serum)	VLDL (mg/100ml serum)
T1	151.22 ±10.07[a]	120.55 ±9.05	55.90 ±3.28	71.21 ±11.85 [a]	24.11 ±3.49
T2	120.63 ±6.49 [b]	107.13 ±8.88	55.36 ±2.46	43.85 ±3.43 [b]	21.42 ±0.72
T3	135.71 ±9.09 [b]	111.45 ±4.82	61.70 ±3.16	51.72 ±7.65 [b]	22.29 ±1.95
T4	133.33 ±13.45 [b]	103.55±4.84	57.69 ±3.91	54.93 ±11.00 [b]	20.71 ±2.42
T5	126.26 ±10.54 [b]	109.65 ±7.17	60.07 ±5.54	44.26 ±10.76 [b]	21.93 ±1.47
Level of Significant	*	N.S	N.S	*	N.S

* (P <0.05), N.S, Not significant .
T1, control treatment (without addition), T2,T3 addition of resveratrol at a concentration of 250 ,500 mg / kg of diet,
T4,T5 addition of oleuropein at a concentration of 250 , 500 mg / kg of diet.

The addition of antioxidants, including res and ole, which activate the NrF2, which activates the production of the enzymatic antioxidants CAT, SOD, and GSH-px. Amino acids from oxidation, thus maintaining the balance of amino acids, which here essential for building proteins for hormones and enzymes as well as increasing muscle protein synthesis (4), (29)and (9) In addition res stimulates secretion of growth hormone, which in turn increases protein body production and serum protein protection (30)and(31). With regard to uric acid, it is the final product of protein catabolism(32), noted a decrease in its concentration in the blood serum of birds of addition treatment due to the role of res and ole in protecting protein from oxidation and catabolism. res and ole compared with control treatment T1 because of the role of these two compounds in protecting liver tissue from oxidative damage and reducing ALT and AST excretion, which is well reflected on liver function (33), (14)and(34) . As for the glutathione peroxidase enzyme GSH-px, we note a high concentration of this enzyme in the res and ole addition treatments compared to the control group because these substances activate or increase the replication of nuclear factor erythroid 2-related factor 2 (Nrf2)/antioxidant response element (ARE). It is a key regulator of antioxidant and cellular protective genes, and this factor is present inside the cell and outside the nucleus in the form of a complex Nrf2-Kelch-like ech-associated protein-1 (Keap-1). With the antioxidant response component ARE, Nrf2/ARE thus stimulates or increases the transcription of enzymatic antioxidants, including GSH-px, which in turn acts to eliminate free radicals (27) , (35) , (36) and (9). As for the concentration of cholesterol and low-density lipoproteins (LDL), noted that their decreased concentration blood serum of the birds addition treatment compared with the control treatment, which may be due to the role of antioxidants such as ole compound, this compound possesses antioxidant activity and thus prevents the oxidation of fats and prevents high cholesterol and LDL(37). also, ole prevents LDL oxidation and inhibits 3-hydroxy-3-methlyglutaryl coenzyme-A reductase, which has an important role in cholesterol synthesis(38) as(34) ,

ISSN 2072-3875

showed that adding ole to poultry rations reduces fat concentration in the blood serum. also between (39) that ole reduces cholesterol and triglycerides in serum and liver, and the mechanism of this action may be by inhibiting the absorption of dietary cholesterol in the intestine or by stimulating the formation of bile in the liver as well as stimulating the excretion of cholesterol in the stool (40). With regard to being an antioxidant, res prevents the oxidation of fats and thus lowers cholesterol, LDL and triglycerides in the blood (41). The Res compound also reduces the activity of 3-hydroxy-3-methlyglutaryl coenzyme-A reductase, which contributes to cholesterol synthesis, as well as res increases LDL receptors in hepatocytes(42), which contributes to lowering the level of LDL in the blood.(43) also indicated that res has a role in lowering cholesterol and LDL levels by eliminating free radicals and preventing lipid oxidation, and then Low level of LDL in the blood serum.

Histological traits of the small intestine

showed Table 6 the effect of adding the antioxidants res and ole to the diet on the length, width and depth of the villi of the jejunum part in the small intestine of broilers, the addition treatments were significantly (P<0.01) superior to the control treatment T1 in the length of the villi, and treatment T3 was superior to the treatments T2, T4 and T5, and treatment T5 was superior to T2 and T4, and there were no significant differences between the two treatments T2 and T4, and for the width of the villi, the two treatments T3 and T5 excelled. Significantly on treatments T1, T2 and T4, and there were no significant differences between T3 and T5 and between treatments T1, T2 and T4, With regard to the depth of crypts, we note the superiority of the addition treatments over the control treatment T1, and treatments T3 and T5 outperformed treatments T2 and T4, and there were no significant differences between treatments T3 and T5 and between treatments T2 and T4,The reason for the improvement of some histological properties of the addition of res and ole treatments may be due to the role of these compounds in protecting the cells of the intestine and stimulating the enzymatic antioxidants to eliminate free radicals(44) ,(35), (45) and (9). This was positively reflected on the integrity of the intestine cells, and then improved digestion and absorption in the small intestine.

Table 6. The effect of adding two levels of Res and Ole to the bush on villi length, width and depth of crypts of the jejunum part for broilers at 6th week of age. (mean ± standard error).

Treatments	Villi length(micron)	Width villi(micron)	Depth crypts(micron)
T1	711.66 ±10.13 d	160.66 ±1.85 b	129.44 ±0.88 c
T2	777.00 ±7.23 c	167.33 ±3.84 b	140.46 ±1.76 b
T3	815.33 ±7.51 a	177.66 ±4.25 a	151.00 ±2.08 a
T4	774.33± 2.60 c	164.00 ±3.60 b	136.00 ±1.54 b
T5	797.66 ±4.63 b	180.33 ±1.45 a	151.66 ±1.34 a
Level of Significant	**	**	**

**(p <0.01).T1, control treatment (without addition), T2,T3 addition of resveratrol at a concentration of 250 ,500 mg / kg of diet, T4,T5 addition of oleuropein at a concentration of 250 , 500 mg / kg of diet.

ISSN 2072-3875

Conclusions

The addition of resveratrol and oleuropein reduced the oxidative state in birds and this is shown by improving some indicators, including decrease in the ratio of H/L, uric acid, LDL cholesterol, ALT and AST enzymes an increase concentration GSH-Px enzyme and Histological traits.

REFERENCES

1.VonSchantz,T.,Bensch,S.,Grahn,M., Hasselquist,D.andWittzell,H.(1999). Good genes, oxidative stress and condition-dependent sexual signals. Proc. Biol. Sci .,266(1414):1-12.

2.Murphy,.M.P.(2009). How mitochondria produce reactive oxygen species. Biochem. J.,417(1): 1–13 .

3.Surai,P.F.,Kochish,I.I.,Fisinin,V.I.an d Kidd,M.T.(2019).Antioxidant defence systems and oxidative stress in poultry biology: An update. Antioxidants., 8(7):235. doi: 10.3390/antiox8070235.

4.Hu, R., He, Y., Arowolo, MA., Wu, S.and He, J. (2019).Polyphenols as potential Attenuators of heat stress in poultry production. Antioxidants., 8(3):67-78.'

5.Visioli, F., Poli, A.and Galli, C.(2002) Antioxidant and other biological activityes of phenols from olives and olive oil. Med. Res. Rev., 22(1): 65–75.

6.Durlu-Ozkaya,F.and Ozkaya, M. T. (2011). oleuropein using as an additive for feed and products used for humans.J.Food.Process.Technol ., 2(3).doi:10.4172/2157-7110.1000113.

7.Francioso,A.,Mastromarino,P.,Masci ,A., d'Erme, M.and Mosca, L.(2014) . Chemistry, stability and bioavailability of resveratrol. Med .Chem .,10 (3) :237–245.

8.Zhang,C.,Zhao, X.,Wang, L.,Yang, L., Chen, X.and Geng, Z.(2017). resveratrol beneficially affects meat quality of heat-stressed broilers which is associated with changes in muscle antioxidant status. Anim. Sci. J.,88(10):1569–1577.

9.Alhaithloul,H.A.,Alotaibi,M.F.,Juma h,M.B.,Elgebalya,H.andMahmoud ,A.M.(2019).*Olea europaea* leaf extract upregulates Nrf2/ARE/HO-1 signaling and attenuates cyclophosphamide-induced oxidative stress, inflammation and apoptosis in rat kidney.Bio.Pharm.,111 :676–685.

10.Visioli, F., Bellosta, S.and Galli, C. (1998). oleuropein, the bitter principles of olives, enhances nitric oxide production by mouse macrophages. Life Sci.,62(6): 541–546.

11.Allerton,T.D.,Proctor,D.N.,Stephen s,J.M.,Dugas,T.R.,Spielmann,G.an d Irvin,B.A.(2018). L-Citrulline Supplementation: Impact on Cardio metabolic Health. Nutrients., 10, 921.

12.Park, D.W., Baek, K., Kim, J.R., Lee, J.J., Ryu, S.H., Chin, B.R.and Baek, S.H.(2009). resveratrol inhibits foam cell formation via NADPH oxidase 1-mediated reactive oxygen species and monocyte chemotactic protein-1. Exp. Mol. Med.,41(3): 171–179.

13.Deng,Y.,Alex,D.,Huang,H.Q.,Wang ,N.,Yu,N.,Wang,Y.T.,Leung,G.P.a ndLee,S.M.(2011).Inhibition of TNF-α-mediated endothelial cell-monocyte cell adhesion and adhesion molecules expression by the resveratrol derivative, trans-3,5,4-trimethoxystilbene. Phytother. Res

14.He,S.,Yu,Q.,He,Y.,HuR.,Xia,S.and He,J.(2019).Dietary resveratrol

ISSN 2072-3875

supple-mentation inhibits heat stress induced high activated innate immunity and inflammatory response in spleen of yellow-feather broilers . Poult. Sci., 98(12):6378-6387.

15.Yan, Y., Yang, J,Y., Mou, Y.H., Wang, L.H., Zhou, Y.N., et al. (2012). Differences in the activities of resveratrol and ascorbic acid in protection of ethanolinduced oxidative DNA damage in human peripheral lymphocytes. Food Chem. Toxicol., 50(2): 168-174.

16.Zhang, C., Huang. J.and Kang., X.T(2018). resveratrol Attenuates LPS-induced Apoptosis via Inhibiting NF-κB Activity in Chicken Peripheral Lymphocyte Cultures. Brazili. J. Poult. Sci.,20(4):747-752.

17.National Research Council (NRC) .(1994). Nutrient requirement of poultry gthEdn. National Academy press. Washington. D. C. USA.

18.Shen, P.F.and Patterson, L. T.(1983).Asimplified wright's stain technique for routine avian blood smear staining. Poult. Sci., 62(5) : 923-924.

19.Campbell, T.W. (1988). Avian Hematology and cytology. 1st edition, Ames, I.A. lowa state university press.

20.Grundy,S.M.,Cleeman,J.I.,Merz,C. N.B.,Brewer,H.B.,Clark,L.T.,Hun ninghake,D.B.and Coordinating Committee of the National Cholesterol Education Program. (2004). Implications of recent clinical trials for the national cholesterol education program adult treatment panel III guidelines. J. Am. Coll .Cardiol ., 44(3): 720-732.

21.Friedewald, W. T., Levy, R. I. and Fredrickson, D. S. (1972). Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. Clin chem., 18(6): 499-502.

22.Rotruck,J.T.,Pope, A.L., Ganther, H.E., Swanson, A.B.,Hafeman , D.G.and Hoekstra, W .(1973) **.** Selenium biochemical role as a component of glutathione peroxidase . Science.,179(4073) : 588 –590.

23.Suvarna,S.K.,Layton.C.and Bancroft.J.D.(2007). Bancroft's theory and practice of histological techniques. British Library. Book ISBN: 9780702068874**.**

24.Edens, F.W., Cantor, A.H., Pescatore, A.J.and Pierce, J.L(.2009). Effect of dietary selenium on small intestine villus integrity in Reovirus-challen-ged Broilers. Int. J. Poult. Sci., 8(9): 829- 835.

25.SAS. (2012). Statistical Analysis System, User's Guide. Statistical. Version 9.1th ed. SAS. Inst. Inc. Cary. N.C. USA.

26.Duncan, D.B.(1955). Multiple Rang and Multiple F-test. Biometrics., 11:4-42.

27.Ungvari,Z.,Bagi,Z.,Feher,A.,Recchi a,F.A.,Sonntag,W.E.,Pearson,K.,a bo,R.D.C.and Csiszar,A..(2010). resveratrol confers endothelial protection via activation of the antioxidant transcription factor Nrf2. Am.J. Physiol Heart Circ. Physiol., 299(1): 18–24.

28.Yi, C. O., Jeon, B.T., Shin, H.J., Jeong, E.A., Chang, K.C., Lee, J.E., et al.(2011). resveratrol activates AMPK and suppresses LPS-induced NF-κB-dependent COX-2 activation in RAW 264.7 macrophage cells. Anat. Cell Biol.,44(3):194-203.

29.Shimao,R.,Muroi,H.,Furukawa,K., Toyomizu,M.and Kikusato,M.

ISSN 2072-3875

(2019). Effects of low-dose oleuropein diet supplementation on the oxidative status of skeletal muscles and plasma hormonal concentration of grow- ing broiler chickens, Briti. Poult. Sci., 60(6):784-789.

30.Harvey,S.andEtches,R.J.(1997).PerspectivesinAvianEndocrinology.J. W. Arrowsmith Ltd, Bristol, UK.

31.Liu,L.,He,J,H.,Xie,H,B.,Yang,Y,S., Li,J,C.andZou,Y,.(2014).resveratrol induces antioxidant and heat shock protein mRNA expression in res-ponse to heat stress in black-boned chickens. Poult.Sci., 93(1) :54–62.

32.Coles,E.H.(1986).Veterinary clinical pathology.4th ed. W.B. saunders. Philadelphia, London, Hong kong.

33.Zhang,C.,Kang,X.,Zhang,T.and Huang,J.(2019).Positive Effects of Resveratrol on Egg-Laying Ability, Egg Quality, and Antioxidant Activity in Hens. J. Appl. Poult. Res., 28(4):1099–1105.

34.Parsaei,S., Amini, Z.and Houshmand, M.(2014). Effects of olive leaf on blood metabolites and humoral immunity response of broiler chickens. Int. J .Adv. Biol. Biom. Res., 2(3):741-751.

35.Haskó, G.and Pacher, P.(2010). Endothelial Nrf2 activation: A new target for resveratrol? Am. J. Physiol. Heart Circ.Physiol., 299(1):10–12.

36.Sahin,K.,Orhan,C.,Akdemir,F.,Tuzcu,M.,Iben,C.andSahin.N.(2011).resveratrol protects quail hepatocytes against heat stress: modulation of the Nrf2 transcription factor and heat shock proteins. J. Anim. Physiol. . Anim. Nutr.,96(1): 66–74.

37.Sarica, S.and Toptas,S.(2014). Effects of dietary oleuropein supplementation on growth performance, serum lipid concentrations and lipid oxidation of Japanese quails. J. Anim. Physiol. Anim., 98(6):1176-1186.

38.Sung, J.H., Choi, S.J., Lee, S.W.,Park, K.H.and Moon, T.W.(2004). Isoflavones found in Korean soybean paste as 3-hydroxy-3-methylglutaryl coenzyme A reductase inhibitors. Biosci. Biotechnol. Biochem., 68(5) : 1051-1058

39.Romani,A.,Mulinacci,N.,Pinelli,P., Vincieri,F.F.andCimato,A.(1999). PolyphenolicOlea Europa-ea L. J. Agric . Food Chem.,47(3) : 964–967.

40.Rezar, V., Levart, A.and Salobir, J.(2015). The effect of olive by products and their extracts on antioxidative status of laying hens and oxidative stability of eggs enriched with n-3 fatty acids. Poljoprivreda., 21(3): 216-219.

41.Gocmen, A.Y.,Burgucu, D.and Gumuslu,S.(2011). Effect of resveratrol on platelet activation in hypercholesterolemic rats: CD40-CD40L system as a potential target. Appl. Physiol. Nutr. Metab., 36(3): 323–330.

42.Yashiro,T.,Nanmoku,M.,Shimizu, M.,Inoue,J.andSato,R.(2012).resveratrol increases the expression and activity of the low density lipoprotein receptor in hepatocytes by the proteolytic activation of the sterol regulatory element-binding proteins. Atherosclerosis ., 220(2): 369–374.

43.Feng,Z.H.,Gong,J.G.,Zhao,G.X.,Lin,X.,Liu,Y.C.andK.W.Ma(2017).Effects of dietary supplementation of resveratrol on performance, egg quality,yolk cholesterol and antioxidant enzyme activity of laying hens, Br. Poult. Sci.,58(5):544-549.

44.Rubiolo,J.A.,Mithieux,G.andVega,F.V.(2008). resveratrol protects primary rat hepatocytes against oxidative stress damage:Activation

ISSN 2072-3875

of the Nrf2 transcription factor and augmented activities of antioxidant enzymes. Eur. J. Pharmacol., 591(3): 66-72.

45. Andrikopoulos, N.K., Kaliora, A.C., Assimopoulou, A.N.and Papageorbgiou,V.P.(2002)**.**Inhibitory activity of minor polyphenolic and nonpolyphenolic constituents of olive oil against in vitro low-density lipoprotein oxidation.J. Med. Food.,5(1):1–7.

ISSN 2072-3875