Study the effects of adding crushed of Grape seeds *(Vitis vinifera)* in the diet to reduce the effect of heat stress on some physiological parameters broiler roosters (*Hubbard flex*).

Sura Safi Abeais AL-Kafajii

Department of Animal production, College of Agriculture, University of Kerbala, Iraq.
www.drsura97@yahoo.com

Abstract

Targeted research study the effect of adding crushed seed of grape *(Vitis vinifera*) on some biochemical parameters of broiler roosters (Hubbard flex). Thirty roosters, Twenty-sex weeks age, were randomly divided into three equal groups. First group (control) fed on the standard provender along the experimental period (10 weeks), second & third groups (T_1& T_2) fed on standard provender supplemented with 2.5 & 5 g/kg feed, respectively. At the end of experiment, blood samples were obtained from wing vein for estimation Total protein, albumin, globulin, cholesterol, testosterone, Follicle-stimulating Hormone (FSH) & Luteinizing Hormone (LH) concentrations in blood serum. The control treatment showed significant decrease in all physiological traits such as the level of total protein , globulin, testosterone, FSH & LH when compared with the poultry was consumed grape seeds in the diet from summer season, the above changes indicate the ability of heat stress in the induction of oxidative stress in broiler roosters. On the other hand, The result showed that crushed seed of grape supplementation in the two treated groups caused a significant increase ($P<0.05$) in total protein , globulin & cholesterol in two treated groups compared with control. Also, the elevation in albumin didn't reach the significant level ($P>0.05$) in the comparison between the three groups.. The result of testosterone, FSH & LH showed a significant increment ($P<0.05$) in two treated groups compared with control.

Key words: grape seed**,** biochemical parameters, heat stress, Broiler.

دراسة تأثير اضافة مسحوق بذور العنب *(Vitis vinifera)* الى العليقة للتقليل من تأثير الاجهاد الحراري في بعض المعايير الفسلجية لذكور فروج اللحم (هبرد فلكس).

سرى صافي عبيس

قسم الانتاج الحيواني/ كلية الزراعة /جامعة كربلاء/كربلاء/العراق

المستخلص

تهدف الدراسة الحالية لمعرفة التأثيرات الايجابية لإضافة مسحوق بذور العنب إلى الغذاء في بعض الجوانب الفسلجية والتناسلية لديكة سلالة فروج اللحم (هبرد فلكس). استخدم 30 ديكاً من سلالة فروج اللحم هبرد فلكس بعمر 26 أسبوعاً، وقسمت عشوائياً إلى ثلاث مجموعات بواقع 10 طيور لكل مجموعة : مجموعة السيطرة (C) تناولت العليقة الأساسية خالية من أي إضافة، ومجموعتي المعاملة الأولى والثانية (T_1 و T_2) تناولت العليقة الأساسية مضافاً إليها مسحوق بذور العنب بقيمة 2.5 و 5 غم/ كغم علف، على التوالي. وفي نهاية مدة التجربة، أخذت نماذج دم من الوريد الجناحي لغرض الحصول على مصل الدم وقياس مستوى تركيز البروتين الكلي ، الكلوبيولين ،الالبومين ، الكولسيترول، هرمون الشحمون الخصوي, testosterone ، الهرمون المحفز للجريبات FSH والهرمون اللوتيني LH. إذ أظهرت معاملة سيطرة انخفاضاً معنويًا في جميع المعايير الفسلجية مثل انخفاض معنوي في مستوى البروتين الكلي والكلوبيولين ومستوى الهرمونات في بلازما الدم مقارنة مع الطيور التي غذيت على مسحوق بذور العنب خلال الأجواء الحارة مما يدل على قابلية الإجهاد الحراري في إحداث الإجهاد التأكسدي في ديكة سلالة اللحم. من جانب اخر، أظهرت النتائج وجود إرتفاع معنوي ($P<0.05$) في تركيز البروتين الكلي والكلوبيولين والكوليسترول في مجموعتي المعاملة مقارنة بمجموعة السيطرة، واشارت النتائج الى حدوث ارتفاع في مستوى تركيز الالبومين الا انه لم يصل لمستوى المعنوية ($P>0.05$) .كما أشارت النتائج إلى ارتفاع مستوى تركيز هرمون الشحمون الخصوي testosterone ، الهرمون المحفز للجريبات FSH والهرمون اللوتيني LH في مصل الدم معنوياً ($P<0.05$) مجموعتي المعاملة مقارنة بمجموعة السيطرة.

Introduction

With the frequent occurrence of extreme weather globally (7), the damage of heat stress (HS) to poultry has become increasingly evident, resulting in serious economic losses (30). Because the skin of birds has no sweat glands and is coated with feathers, their heat generation, cooling, and body temperature regulation are more difficult compared to mammals. When birds are in an environment with a temperature higher than their appropriate temperature, their thermoregulatory mechanism cannot cope with the high temperature change, resulting in HS (30). Heat stress can cause birds wheezing and can cause long-time alternating acid and base poisoning in the animal’s body, lead to respiratory mucosal congestion, and increase heart rate. This results in an increased blood flow at the body surface and muscle but a reduced blood supply to the internal organs and gonads, affecting the uptake and utilization of nutrients in the body of poultry(29).

Studies have shown that HS-induced wheezing can lead to an increased respiratory rate; reduced blood volume in the gastrointestinal tract, liver, and kidney; a decline in feed conversion rate (FCR); and raised mortality (27). Furthermore, it causes a decrease in growth and productivity performance and the deterioration of the immune response due to increased formation of free radicals (Reactive Oxygen Species) ROS and that cause oxidative stress on cells through the occurrence of oxidative damage to proteins and genetic material (DNA) (21), also it causes Lipid peroxidation of DNA and the long chain polyunsaturated fatty acids in the sperm cell membrane is the primary cause of infertility (3, 11). Several studies have shown that the increase in oxidative damage that occurs in the chicken is associated with an increased high ambient temperatures (5, 20).

The Researches for new bio-efficient antioxidants has particularly focused on natural antioxidants to respect the consumer concerns over safety and toxicity. Grape seeds (GS) considered waste product of the grape industry. These wastes contain many components as resveratrol (a naturally occurring polyphenol) and anthocyanidins, which play beneficial effects including anti-oxidant, anti-inflammatory, chemopreventive and anticancer activities (17).

Grape seeds and by-products of wine/grape juice processing provide an abundant source of flavonoids, in which the most abundant classes include the flavan-3-ols. (28, 29). The Phytochemicals analysis of the plant showed that *(Vitis vinifera)* is contained vitamin B6, E, C, A, calcium, magnesium, potassium, tocopherol and iron, as well as it contain phenolic acids, stilbene derivatives, flavan-3-ols, flavonols, and anthocyanidins (8).

The antioxidant compounds present in grape have been identified as phenolic acids (benzoic and hydroxycinnamic acids), flavan-3-ols (catechin and epicatechin), flavonols (quercetin and myricetin), and anthocyanidins (8). (29) reported that the antioxidant potential of grape seed is twenty and fifty fold greater than vitamins E and C, respectively arising from increased levels of polyphenols, proanthocyanidins and oligomers of flavan-3-ol units, especially catechin and epicatechin present in GS (32).

Materials and Methods

Grape *(Vitis vinifera)* were obtained from local market of plants, the seed were separated from the plant , dried by oven at 30°C for 2-3 hours, grinded in an electrical blender into a fine powder which was packed in air-tight bags plastic till use for basal diet supplementation. All experiments of this study were carried out at College of Agriculture/ University of Kerbala, during the period 24/6/2015 to 24/2/2016. A thirty roosters of broiler (Hubbard flex), Twenty-sex weeks age, were randomly divided into three groups of ten animals in each group. The experimental diets were control (C) fed on the standard provender, along the experimental period (10 weeks), second group (T_1) fed on standard provender supplemented with 2.5 g/Kg feed, third group (T_2) fed on standard provender supplemented with 5 g/kg feed.

At the end of experimental period, blood sample were taken from wing vein of 10 animals of each groups, put the blood sample in tubs without anticoagulant, separation of serum was carried out by centrifugation of the blood at 3000 rpm for 10 min. the clear serum was transferred carefully to clean and dry vials and kept in deep freezer -20 until analysis for determination of total serum protein according to (31), After albumin concentration was determined, then globulin concentration was calculated by:

Globulin concentration (g/100ml) = total protein concentration -Albumin concentration.

Serum level of cholesterol was determined according to (13), also the level of testosterone, FSH & LH in serum measured by Radioimmunoassay, All kit procedures were followed as stated in the manufacturer's protocol.

Statistical analysis

All the values are expressed as mean± SD. Data of the experiment were analyzed by using Complete Random Design . Least significant difference (LSD) was carried out to estimate the significance of difference between the treatments. P value less than 0.05 was considered significant (1).

Results

The result of the effect of crushed grape seed on Total serum protein , Albumin & Globulin of broiler roosters were illustrated in table 1. The result indicated that there is a significant increased ($P<0.05$) in the total protein & globulin in two treated groups compared with the control group. while the increment in albumin didn't reach the significant level ($P>0.05$) in the comparison between the three groups.

Table (1) Effect of crushed grape seed on some biochemical parameters of broiler roosters.

Groups / Parameters	C	T_1 (2.5 g/kg feed)	T_2 (5 g/kg feed)
Total protein(g/100ml)	0.064 ±4.07	0.033 ±4.724	0.051 ±5.459
Albumin(g/100ml)	0.028 ±2.102	0.052 ±2.17	0.041 ±2.261
Globulin(g/100ml)	0.052 ±2.018	0.046 ±2.512	0.023 ±3.712
Cholesterol (mg/dl)	1.264 ±147	1.788 ±153	2.33 ±183.2

*Values given as mean ± SD.
* The LSD of total protein = 0.1573.
* The LSD of albumin = Non-significant.
* The LSD of globulin = 0.1295.
* The LSD of cholesterol = 5.6932.

Table 2 showed the effect of crushed grape seed on testosterone, follicle stimulating hormone (FSH) & luteinizing hormone (LH) of broiler roosters. The results are shown that crushed seed of grape supplementation in the two treated groups caused a significant increase ($P<0.05$) of these hormones when compared with control group.

Table(2)Effect of crushed grape seed on the levels of some hormones of broiler roosters.

Groups / Parameters	C	T_1 (2.5 g/kg feed)	T_2 (5 g/kg feed)
Testosterone(ng/ml)	0.103 ±2.749	0.072 ±3.871	0.071 ±4.971
FSH (miu/ml)	0.097 ±3.936	0.048 ±4.447	0.026 ±4.916
LH (miu/ml)	0.041 ±3.608	0.039 ±4.372	0.017±4.956

*Values given as mean ± SD.
* The LSD of testosterone = 0.2578.
* The LSD of FSH = 0.908.
* The LSD of LH = 0.1045.

Discussion

The result of this study revealed that grape seed supplementation to male broiler in a dose of 2.5 & 5 g/kg feed for 10 weeks produced beneficial effects on bird healthy and improvement immunity. the positive effects of grape seed characterized by increased total serum protein , globulin, testosterone , FSH & LH. These effects may be mediated through several mechanisms since the seed itself is a complex mixture of many biologically active compounds (8).

Our result shows the positive effect of crushed grape seed on serum biochemistry of broiler roosters. The increase of total protein & globulin concentration, came in agreement with pervious report using grape seeds extract against Ehrlich Ascites Tumor on blood traits of broiler breeder hens(18). The elevation in total protein & globulin may be due to the biological active compounds of grape seed particularly proanthocyanidins, which have stimulating effects for building proteins and prevent break down it (4). Proanthocyanidins and Flavonoids can reduce oxidative stress by directly scavenging free-radicals, by interfering with free-radical producing mechanisms and by increasing the function of endogenous antioxidant(24). Additionally, *in vitro*, flavonoids may have profound direct effects on a variety of immune and inflammatory cell functions, particularly once cells are activated with inhibitory as well as stimulatory actions(19). on the other hand, grape seed have antioxidant and anti-inflammatory effects (23).

The Antioxidative activities of grape seed may be responsible for the significant increase in the testosterone concentrations in the birds supplemented with grape seed at 2.5 & 5 g/kg feed. This is in agreement with the reports of each (9,12) who indicated that oxidative stress reduced the levels of key enzymatic and non-enzymatic antioxidants in Leydig cells, resulted in decline in testosterone secretion. (14) also stated that the natural antioxidant of grape, transresveratrol had increased the serum concentration of testosterone in rats which might have shifted the levels of testosterone in this study also.

Androgenesis in the testes involves multi-critical steps that start with the synthesis of cholesterol, followed by its transport within the steroidogenic testicular tissues and its metabolism to form steroid biosynthesis. Steroidogenic cells (SCs) acquire cholesterol either by de novo synthesis or from the high density lipoproteins (HDL) or low density lipoproteins (LDL that circulate in the blood (10). Scavenger receptor class B member 1 (SRB-1) is a specific receptor for lipoproteins and is located on the surface of SCs. SRB-1 aids LDL and HDL uptake by SCs (10). The increased production of luteinizing hormone (LH) and follicle-stimulating hormone (FSH) allows the synthesis of cholesterol by activating the enzymes of cholesterol biosynthesis, including cholesterol ester hydrolase. Furthermore, LH and FSH production regulates the uptake of cholesterol esters by SCs through the enhancement of the expression of HDL and LDL receptors, which recognize SRB-1 (15). The present study detected a significant increase on the concentration of cholesterol, FSH, LH as a result testosterone hormone (6).

Following cholesterol biosynthesis, StAR transports it to the inner mitochondrial membrane (IMM) (16).In the IMM, pregnenolone is produced by the action of cholesterol on P450scc, which is a major enzyme that regulates steroidogenesis (25). The results of the present study detected a increase the serum levels of LH in two treated group (T1,T2), since LH is responsible for steroidogenesis through StAR activation in SCs in testicular tissues leading to stimulating the leydig cell for increase synthesis and secretion of testosterone (15,22). In agreement with these findings, previous studies have confirmed the role of StAR expression and activity in steroidogenesis (15,22).

Heat stress causes imbalance between oxidant/antioxidant compounds, by decreasing the level and activity of the cellular antioxidant enzymes such as catalase (CAT), superoxide dismutase(SOD), Glutathion peroxidase (GSH-PX) that may indirect alter the activity of the neuroendocrine system of poultry resulting activation of the hypothalamic-pituitary- adrenal (HPA) axis leading to elevation plasma corticosterone, on other hand, it caused inhibition of the hypothalamic-pituitary-gonad (HPG) axis leading to decreasing the level of FSH, that necessary for expression LH receptors in gonads, in turn, LH decreasing causing inhibition in the synthesis & secretion of testosterone from leydig cells (2). Notably, GS counteracted the heat stress inhibitory effects due to its contents of natural antioxidant potential (polyphenols, proanthocyanidin) that is twenty and fifty fold greater than vitamins E and C, respectively . So that, these natural antioxidant capable to protect the liver , kidney & testes (leydig and sertoli) cells against the oxidative damage produced by heat stress through increase the level of SOD, CAT & GSH-PX, that leading to disturbances in oxidative damage and lipid peroxidation by inhibiting the activity of NADH dehydrogenase & Xanthine oxidase that catalase lipid peroxidation this leading to cell protection from peroxidative injuries of free radicals scavengers, so the leydig and sertoli cells was stimulated under effect LH and FSH, respectively, to release testosterone from leydig cells, and lesser amount of estrogen as well as activin, that stimulated hypothalamus and pituitary glands for secretion of LH &

FSH, from sertoli cells , that may be explain the increase in the level testosterone, FSH& LH (4) .

Our result shows the positive effect of crushed grape seed that agreement with those obtained by (26) who observed increase in the concentration of cholesterol, testosterone, semen volume, concentration and motility in broiler breeders treated grape seed when compared to control (4). Finally, the antioxidant activity of grape seed may also play an important role in cell protection from enhancement of oxidative injuries, also the ability of grape seeds for improvement immunity comes from these important biochemical compounds & Minerals in there components .

In conclusion, the results presented in current study demonstrated that incorporation of GS as 5 g/kg feed is able to enhance the cell protection from oxidative stress leading to improve the bird healthy, production and immune response of broilers. Future in vitro studies are required in order to outline the direct beneficial effect of GSE on leydig cell function, and its availability for treatment of testicular disfunction.

References

1- الراوي، خاشع محمود وخلف الله، عبد العزيزي محمد (1980). تصميم وتحليل التجارب الزراعية. دار الحكمة للطباعة والنشر، جامعة الموصل-الموصل.

2- محمد، ظافر ثابت ؛ الخيلاني، فراس مزاحم حسين و الضنكي، زياد طارق محمد. (2013). دراسة تأثير إضافة مضادات الأكسدة إلى العليقة للتقليل من تأثير الإجهاد الحراري على الأداء الإنتاجي وحالة مضادات الأكسدة في الدجاج البياض البني مجلة الأنبار للعلوم البيطرية، 6 (1): 96-108.

3- Aitken, R.J.; Clarkson, J.S. & Fishel, S. (1989). Generation of ROS lipid peroxidation and human sperm function. Biol. Reprod., 40: 183 -197.

4- Al-Attar, A. M. (2015). Effect of grape seed oil on diazinon-induced physiological and histopathological alterations in rats. Saudi Journal of Biological Sciences. 22, 284–292.

5- Altan, O.; Pabuccuoğlu, A.; Altan, A.; Konyalioğlu, S. & Bayraktar, H. (2003). Effect of heat stress on oxidative stress, lipid peroxidation and some stress parameters in broilers. Br. Poult. Sci., 44:545-550.

6- Barlow, N.J.; Phillips, S.L.; Wallace, D.G.; Gaido, K.W. &Foster, P.M.(2003). Quantitative changes in gene expression in fetal rat testes following exposure to di(n−butyl) phthalate. Toxicol Sci 73: 431−441.

7- Barnett, A. G.; Hajat, S.; Gasparrini, A.; Rocklov, J.(2012). Cold and heat wave in United States, Environ. Res., 112, 218–224.

8- Caillet, S.; Salmieri, S. & Lacroix, M. (2006). Evaluation of free radical scavenging properties of commercial grape phenol extracts by a fast colorimetric method. Food Chem. 95, 1-8.

9- Cao, L.; Leer Suchetha, S. & Azhar, S. (2004). Aging alters the functional expression of enzymatic antioxidant defense systems in testicular rat Leydig cell. J. Steroid Biochem. Mol. Biol., 88: 61-67.

10- Cao, G.; Zhao, L.; Stangl, H.; Hasegawa, T.; Richardson, J.A.; Parker, K.L. & Hobbs, H. H. (1999). Developmental and hormonal regulation of murine scavenger receptor, class B, type 1. Mol. Endocrinol. 13: 1460−1473.

11- Cecil, H.C. & Bakst, M. R. (1993). In vitro lipid peroxidation of turkey spermatozoa. Poult. Sci.,72: 1370-1378.

12- Chen, Z.; Zhang, J. R.; Zhou, Y. W.; Liang, C.& Jiang, Y. Y. (2015). Effect of heat stress on the pituitary and testicular development of Wenchang chicks. Arch. Anim. Breed., 58, 373–378.

13- Elias, A. & Franey, R. J. (1968). Serum cholesterol mesurment based on ethanol extraction and ferric chloride sulfuric acid . Clinical Chemistry Acta, 2: 225-263.

14- Emilia Juan M.; Pons, e. G.; Munuera, T.; Ballester, J.; Rodriguez – Gil, J & Planas, J. M. (2005). trans - Resveratrol a natural antioxidant from grapes increases sperm output in healthy rats. J. Nutr., 135: 757-760.

15- Fauser, B.C.J.M. (1999). Molecular biology of steroidogenesis.In: Molecular Biology in Reproductive Medicine. Fauser, B.C.J.M.; Rutherford, A.J.; Strauss, J.F. & Van Steirteghem, A. (eds.). 1st edition. Parthenon Publishing, New York, pp234−251.

16- Hasegawa, T.; Zhao, L.; Caron K.M.; Majdic, G.; Suzuki, T.; Shizawa, S.; Sasano, H. & Parker, K.L. (2000). Developmental roles of the steroidogenic acute regulatory protein (StAR) as revealed by StAR knockout mice. Mol Endocrinol 14: 1462−1471.

17- Kundu, J.K.&Surh, Y. J. (2008).Cancer chemopreventive and therapeutic potential of resveratrol: mechanistic perspectives. Cancer Lett.,269: 243-61.

18- Mahmoud, E. A. (2015). Anticarcinogenic Effect of Grape Seeds Extract Against Ehrlich Ascites Tumour in Mice. Global Veterinaria 15 (2): 207-214.

19- Middleton, E.J.;Kandaswami,C. &Theoharides, T.C. (2000). The effects of plant flavonoids on mammalian cells:implications for inflammation, heart disease, and cancer. Pharmacological Reviews. 52:673-751.

20- Mujahid, A.; Akiba, Y.; Warden, C. H. & Toyomizu, M. (2007). Sequential changes in superoxide production, anion carriers and substrate oxidation in skeletal muscle mitochondria of heat stressed chickens. FEBS Lett. 581:3461-3467.

21- Mujahid, A.; Pumford, N. R.; Bottje, W.; Nakagawa, K.; Miyazawa, T.; Akiba, Y. & Toyomizu, M. (2007). Mitochondrial oxidative damage in chicken skeletal muscle induced by acute heat stress. Jpn. Poult. Sci., 44:439-445.
22- Murugesan, P.;Muthusamy, T.;Balasubramanian, K. & Arunakaran, J. (2007). Effects of vitamins C and E on steroidogenic enzymes mRNA expression in polychlorinated biphenyl (Aroclor 1254) exposed adult rat Leydig cells. Toxicology 232: 170−182.
23- Nandakumar, V.; Singh, T. & Katiyar, S.K. (2008). Multi-targeted prevention and therapy of cancer by proanthocyanidins. Cancer Lett. 269, 378–387.
24- Nijveldt, R. J.; Van Nood, E.; Van Hoorn, D. E. C.; Boelens, P. G.; Van Norren, K. & Van Leeuwen, P. A. (2001). Flavonoids: a review of probable mechanisms of action and potential applications. America. Journal of Clinical Nutrition. 74:418-425.
25- Omura, T. & Morohashi, K. (1995). Gene regulation of steroidogenesis. J Steroid Biochem Mol Biol 53: 19−25.
26- Priya, K. T.; Elizabeth Manju, D. K.; Thangavel, A. & Leela, V. (2012). dietary supplementation of amla and grape seed on testosterone concentration and semen characteristics in broiler breeders. Tamilnadu J. Veterinary & Animal Sciences. 8 (5) 253-258.
27- Quinteiro-Filho, W. M.; Ribeiro, A.; Ferraz-de-Paula, V.; Pinheiro, M. L.; Sakai, M.; Sá, L. R.; Ferreira, A. J.; & Palermo-Neto, J. (2010). Heat stress impairs performance parameters, induces intestinal injury, and decreases macrophage activity in broiler chickens, Poultry Sci., 89, 1905–1914.
28- Scalbert, A. & Williamson, G. (2000). Dietary intake and bioavailability of polyphenols. J. Nutr. 130, 2073S- 2085S.
29- Shi, J.; YU, J.; Pohorly, P.E. & Kakuda Y.(2003). Polyphenolics in grape seeds-Biochemistry and functionality. J. Med. Food, 6, 291-299.
30- St-Pierre, N. R.; Cobanov, B.; & Schnitkey, G.(2003). Economic losses from heat stress by US livestock industries, J. Dairy Sci., 86, E52–E77.
31- Tietz, N.W. (1982). Fundamentals of Clinical Chemistry. 2nd edition, W.B. Saunders Company, Philadelphia.
32- Yilmaz, Y.& Toledo, R.T. (2004). Major flavonoids in grape seeds and skins: antioxidant capacity of catechin, epicatechin, and gallic acid. J.Agric Food Chem 52, 255-260.