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# EFFECT OF GRAPE SEED EXTRACT ON SOME PHYSIOLOGICAL CHANGES IN BROILERS UNDER HEAT STRESS

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**ABSTRACT:** The major target of this study was to investigate the physiological changes in broiler chicks during exposure to high ambient temperature and elucidate the protective role of grape seed extract in alleviating these expected changes. One hundred and eighty Hubbard broiler chicks (1d-old) were used in the present work. The brooding temperature was maintained at 34°C (55% RH) for the first 2 days, and then decreased gradually to 24°C (55% RH) until 21 days of age. At day 22, birds were randomly divided into 4 groups with 3 replicates (n=15 in each replicate). Birds in group1 (TN) kept at 24 ± 1C and 55±5% RH and fed on commercial diet as a negative control. Birds in groups 2, 3 and 4 subjected to cyclic heat stress by exposing them to 36±1C and 65±5%RH for 8h (from 10 am to 6 pm) during the period between 22d to 40d of age. Birds in group 2 (HSGSE0) fed on commercial diet without grape seed extract supplementation as a positive control. Birds in groups 3 (HSGSE1) and 4 (HSGSE2) fed on commercial diets supplemented with 100 and 200mg/kg grape seed extract, respectively. Results showed that heat stress significantly increased body temperature (BT), respiratory rate (RR), heterophil/lymphocyte (H/L) ratio, corticosterone (CTC), triglycerides (TG), low density lipoprotein (LDL), high density lipoprotein (HDL) and liver malondialdehyde (MDA) and decreased liver superoxide dismutase (SOD) and glutathione (GSH) compared to TN group. Although BT and RR were not affected by GSE supplementation ( 100 or 200mg/kg GSE), liver SOD and GSH were significantly increased and H:L ratio, CTC, TG, LDL, HDL and liver MDA were significantly decreased by GSE supplementation compared to GSE0 group. In conclusion, the obtained data demonstrate that grape seed extract could relieved some negative effects (stress indicators, lipid parameters and antioxidant enzymes) of broilers under heat stress. However, 200mg/kg was more effective than 100mg/kg.

**Key Words:** Heat stress, grape seed, antioxidant, lipid peroxidation, broilers.

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## INTRODUCTION

High ambient temperature is one of the environmental factors influencing physiological traits and production of poultry (Azad et al., 2010). Ambient temperature over 32 °C causes heat stress in broilers (Cooper and Washburn 1998), which induces behavioral and physiological responses reflected by immunosuppression, high mortality rate and slight growth (Mujahid et al. 2005). Heat stress induces oxidative stress that result in increased reactive oxygen species production (Halliwell and Gutteridge, 1989). Excess levels of reactive oxygen species, reflected by the disturbance of balance between the oxidation and antioxidant defense systems, inducing lipid peroxidation, oxidative damages to biological molecules (Ando et al., 1997) and impaired muscle membrane integrity in breast muscle of broiler chickens (Sandercock, 2001). Oxidative stress as a result to heat stress exhibited more than a two fold increase of malondialdehyde as an indicator for lipid peroxidation, in the skeletal muscle (Mujahid et al., 2009 and Wang et al., 2009) and decreased serum vitamin and mineral concentrations which play an important role in the antioxidant defense system (Sahin et al., 2002, 2009). Lipid peroxidation can be minimized by supplementation of antioxidant vitamins (Puthpongsiriporn et al., 2001 and Franchini et al., 2002) or natural substances that possess antioxidant potential (Sahin et al., 2008 and Tuzcu et al., 2008). Antioxidants play an important role in protecting cells from reactive oxygen species by reducing free radicals and preventing the peroxidation of lipids (Grashorn, 2007 and Nanari et al., 2004).

Moreover, Benzie, 2003 reported that, antioxidant enzymes have the capacity to break down free-radical reactions using a chain reaction mechanism. The living organism can synthesize some of these antioxidants, whereas others need to be provided by the diet (Strain and Benzie, 1999). Recent studies have showed the importance of plant materials by-products that are particularly rich in polyphenols and have a wide range of biological activities. The inclusion of grape flavonoids causes a diminution of tissue lipid peroxidation in kidney, liver, and lung of rats (Preuss et al., 2001 and Rodrigo et al., 2005). Yilmaz and Toledo, 2004, reported that flavonoids have the capacity to act as powerful antioxidants by scavenging free radicals and terminating oxidative reactions. Murray (1995) recorded that grape seed extract contains the most beneficial groups of plant flavonoids, proanthocyanidins oligomers. It contains large quantities of monomeric phenolic compounds such as (+)-catechins, (–)-epicathechin and (–)-epicatechin-3-O-gallate, and dimeric, trimeric, and tetrameric proanthocyanidins (Saito et al., 1998). Recent investigations showed that grape seed extract decreased the incidence of free radical-induced lipid peroxidation and enhanced the antioxidant status in aged rats (Balu et al., 2005). It increases antioxidant capacity in chicken and turkey meat (Mitsumoto et al., 2005 and Mielnick et al., 2006). Grape seed extract contain 92 to 95% proanthocyanidins oligomers (Murray,1995). The activity of proanthocyanidins oligomers is approximately fifty times greater than that of vitamin C and vitamin E, in terms of antioxidant action (Shi et al. 2003). proanthocyanidins might trap reactive oxygen species in plasma and interstitial fluid of the arterial wall, thereby inhibit the oxidation of LDL and show an antiatherosclerotic activity (Yamakoshi et al., 1999). Proanthocyanidins are potent antioxidants and exert many health-promoting effects (Singh et al. 2004). Grape seed proanthocyanidins extract has been shown to have a cholesterol-lowering effect anticancer effect as well as antibacterial, antiviral, and antifungal

activities (Shimada et al., 1999; Yamakoshi et al., 1999; Cos et al., 2003). Masaru et al. (2004) suggest that Grape seed proanthocyanidins extract can increase bone quality and bone strength of rat mandibles in the growth period.

Knowledge about the effect of grape seed polyphenols on lipid peroxidation and antioxidant status in heat-stressed broilers are limited. Therefore, the main goal of this study was to investigate the effect of grape seed extract on respiratory rate, body temperature, heterophil/lymphocyte ratio, corticosterone level, lipid perxidation and some antioxidant activities of broilers maintained at high ambient temperature.

## Materials and Methods

### Birds and experimental design:

One hundred eighty 1-d-old broiler chickens (Hubbard) were obtained from a local hatchery and reared in an environmentally controlled room. The brooding temperature was maintained at 34°C (55% RH) for the first 2 days, and then decreased gradually to 24°C (55% RH) until 21 days of age. The chicks were kept in floor pens with fresh wood shaving. The birds received a commercial starter diet (21 % CP and 3000 kcal ME/ kg.) until 21 days of age, after which a commercial grower diet (19 % CP and 3100 kcal ME/ kg.) was provided until the end of the experiment. Birds had ad-libitum feed and water throughout the experimental period. Birds were raised with continuous lighting. At day 22, birds were randomly divided into 4 groups with 3 replicates (n=15 in each replicate). Birds in group1 (TN) kept at 24 ± 1C and 55±5% RH and fed on commercial diet as a negative control. Birds in groups 2, 3 and 4 subjected to cyclic heat stress by exposing them to 36±1C and 65±5%RH for 8h (from 10 am to 6 pm) during the period between 22d to 40d of age. Birds in group 2 (HSGSE0) fed on commercial diet without grape seed extract supplementation as a positive control. Birds in groups 3 (HSGSE1) and 4 (HSGSE2) fed on commercial diets supplemented with 100 and 200mg/kg grape seed extract, respectively.

### Preparation of grape seed extract.

Grape fruits were purchased from local market. Seeds were removed from the grapes, air dried in shade for one week and milled to obtain fine powder. The seeds powder was macerated in 75% ethanol for 72 hr at room temperature. The ethanolic extract was evaporated (Rotary Evaporator) to eliminate ethanol and obtain GSE as a lyophilized powder (yield 25-30%) according to Sarkaki et al. (2013).

### Blood and liver samples:

At 40 days of age, Blood samples were collected into heparinized test tubes and kept on ice. Plasma was obtained after centrifugation at 3500rpm for 15 min then stored at -20 ºC for further analysis. Nine birds per treatment group (3 chickens per replication) were killed by decapitation. Liver was removed, chopped into small pieces, immediately frozen in liquid nitrogen and stored at -80 °C until analysis. The homogenate of liver was prepared for the assays of superoxide dismutase (SOD), glutathione (GSH), and malondialdehyde (MDA).

### Measurements:

Body temperature (BT) and respiratory rate (RR) of the broilers were determined using the method described by Boulahson et al. (1995). Body temperature (BT) was monitored using a thermo-code electric gauge. The respiration rate (RR) was measured by counting the breaths/min by observing abdominal movement for one minute. The account and differential of white blood cells were determined according to the procedure outlined by Schalm et al. (1975). The heterophil/lymphocyte ratio was calculated. Plasma samples were subjected to biochemical analysis using commercial

diagnostic kits. Plasma was submitted for determination of corticosterone (CTC), triglycerides (TG), low density lipoprotein (LDL) and high density lipoprotein (HDL). Malondialdehyde (MDA) was measured by the method described by Ohkawa et al. (1979). Superoxide dismutase (SOD) activity was measured based on ability of SOD to inhibit the reduction of nitroblue tetrazolum superoxide (Worthington, 1993). One unit of SOD is defined as the amount of sample resulting in 50% inhibition of nitroblue tetrazolum reduction. The glutathione (GSH) concentration was measured by the methods of Simons and Johnson (1978).

**Statistical analysis:**

The general linear models procedure of the SAS (1988) was used. Significant differences among means were determined by Duncan's multiple- range tests (Duncan, 1955).

## RESULTS

**BT, RR, H:L ratio and CTC:**

Data of body temperature, respiratory rate, heterophil/lymphocyte ratio and corticosterone as affected by grape seed extract supplementation under high ambient temperature are presented in Table (1). Birds kept under high ambient temperature had greater BT, RR, H:L ratio and CTC than those of reared under TN conditions. Pretreatment of HS-treated birds with grape seed extract caused partial recovery of H:L ratio and CTC. Under heat stress, birds which received 100 and 200 GSE had lower CTC by 25.35% and 43.87%, respectively, compared to GSE0 group. H:L ratio decreased significantly with birds received 100 GSE and 200 GSE by 18.46% and 38.46%, respectively compared to GSE0 birds. Body temperature and respiratory rate were not significantly affected by GSE supplementation.

**Plasma Lipid parameters:**

Table (2) summaries The effect of heat stress and grape seed extract supplementation on TG, LDL and HDL values of broilers. Heat stress treatment without any supplementations leads to 45.10%, 63.08% and 27.38% increase in levels of TG, LDL and HDL, respectively as compared with control group. The level of 100 and 200 GSE supplementation had a significant and dynamic impact on plasma lipid parameters in broilers. Under heat stress conditions, plasma TG, LDL and HDL decreased significantly in birds received 100 and 200 GSE by (7.69% and 31.41%), (21.70% and 36.44%) and (3.17% and 19.87%), respectively, compared to the GSE0.

**Liver lipid peroxidation and antioxidant indicators:**

Lipid peroxidation and antioxidant indicators activities of birds as influenced by heat stress and grape seed extract supplementation are illustrated in Table (3). Exposure to heat stress without any supplementation resulted in increase of MDA by 92.65 % and reduction in the values of SOD and GSH by 30.23% and 34.01%, respectively, as compared with control group. However, pretreatment of HS-treated birds with 100 and 200 grape seed extract resulted in the recovery of MDA by 18.07% and 41.22%, respectively, and increased SOD by 22.84% and 26.21%, respectively, as compared with GSE0 ones. The same trend was noticed with GSH (19.75% and 46.61%, respectively).

## DISCUSSION

This study was carried out to investigate the physiological response of broiler chickens to cyclic heat stress and elucidate the possible protective effects of grape seed extract in alleviating the changes in lipid peroxidation and antioxidant indicators in plasma and liver of broilers. Heat stress significantly increased values of BT, RR, H:L ratio and

CTR compared to birds maintained at normal ambient temperature. These results are in agreement with those reported by Lin et al. (2006), Al-Ghamdi (2008) and Attia et al. (2010). They found that there was a significant increase in body temperature and respiration rate due to heat stress. Also, Sandercock et al. (2001) observed that exposure to high ambient temperature, elevated the body temperature. They suggested that, birds try to reduce body temperature by increasing their respiratory rate or panting. Star et al. (2008) and Willemsen et al. (2011) reported that there was a significant increase in corticosterone level due to heat stress, indicating that the chickens were stressed. Heterophil/lymphocyte ratio has been used as a reliable indicator of stress in birds (Gross and Siegel 1983). Moreover, McFarlane and Curtis (1989) and Yalcın et al. (2003) reported that heat exposure increased H/L ratio in broiler chicks, indicating that the birds from the HS group were significantly stressed compared with control birds. Our results showed that, heterophil/lymphocyte ratio and corticosterone level of birds which maintained in the environmental temperature at 36+1C and received grape seed extract at 100 and 200mg/kg diet were lower than the H/L ratio and CTC level of ones which did not receive grape seed extract in their diet. This indicates that grape seed extract supplementation could reduce the effect of heat stress in broilers. These results are in agree with Aengwanich and suttajit (2010) who reported that 300 and 400 mg/kg of polyphenols could reduce the effect of heat stress in broilers that maintained in the environmental temperature at 38+2C. In our study, BT and RR of heat-stressed birds which received grape seed extract on their diet at 0, 100 and 200mg/kg did not differ. These data showed that grape seed extract had no effect on the body temperature or respiratory rate of broilers under heat stress. These results are in accordance with Aengwanich and Suttajit (2010) who reported that polyphenols had no effect on the BT or RR of broilers under heat stress.

Oxidation of LDL is one of the primary mechanisms of lipid abnormalities such as hypertriglyceridemia (Devaraj and Jialal 2000), and hypercholesterolemia (Chen et al. 1997; Nassir et al. 1997) in stressed conditions. Our results showed that exposure to high ambient temperature significantly increased TG, LDL and HDL. Dietary supplementation of grape seed extract significantly decreased TG, LDL and HDL. These results are in harmony with Attia et al. (2010) who noted that chronic heat stress significantly increased plasma triglycerides. Also, Teissedre and Waterhouse (2000) noted a high correlation between the total phenol content and low-density lipoprotein oxidation in human. Furthermore, Akbari and Torki (2013) suggested that the high concentration of antioxidants might decrease lipid peroxidation and therefore reduce the serum concentration of triglycerides.

Our data of lipid peroxidation and antioxidant enzymes of liver showed that exposure to high ambient temperature significantly increased liver MDA and decreased SOD and GSH activities. In this regard, Mujahid et al. (2007) and Tan et al. (2010) indicated that high ambient temperature causes oxidative stress and tissue damage via lipid peroxidation. Furthermore, Sahin et al. (2010) observed that exposure to heat stress increased hepatic MDA level and decreased hepatic SOD and GSH-Px activities. The antioxidant power of polyphenols (i.e., proanthocyanidins, catechins, epicatechin, and procyanidin) found in grape seeds is reported to be 20 times greater than vitamin E and 50 times greater than vitamin C (Shi et al., 2003). In this study, dietary supplementation of grape seed extract significantly decreased MDA and increased SOD and GSH activities. Similar result was obtained by Sahin et al. (2010) who found that, in response to increasing supplemental

green tea polyphenol level, there were linear decreases in hepatic MDA level and linear increases in hepatic SOD and GSH-Px activities. Moreover, Aengwanich and Suttajit (2010) found the same result when they used polyphenol extracted from tamarind seed coat.

In conclusion, it could be recommended that exposure to high ambient temperature significantly increased body temperature, respiratory rate, stress indicators, lipid level, lipid peroxidation and decreased antioxidant indicators in liver of broilers. Although the two levels of Grape seed extract used in this study had no impact on body temperature or respiratory rate of the chicks, grape seed extract supplementation partially relieved the negative effects of heat stress on stress indicators, lipids profile, lipids peroxidation and activity of some antioxidant enzymes in broilers. Dietary supplementation of 200mg/kg proofed to be more effective than 100mg/kg in this regard.

**Table (1):** Effect of heat stress and grape seed extract supplementation on body temperature (BT), respiratory rate (RR), H:L ratio and plasma corticosterone (CTC) level.

| | TN | HSGSE 0 | HSGSE 1 | HSGSE 2 | SEM | Significant |
|---|---|---|---|---|---|---|
| BT, °C | $40.32^{b}$ | $42.35^{a}$ | $42.28^{a}$ | $42.15^{a}$ | 0.21 | * |
| RR, breath/min | $50^{b}$ | $122^{a}$ | $118^{a}$ | $115^{a}$ | 3.95 | *** |
| H:L ratio | $0.38^{c}$ | $0.65^{a}$ | $0.53^{b}$ | $0.40^{c}$ | 0.02 | ** |
| CTC, ng/ml | $12.01^{c}$ | $28.13^{a}$ | $21.00^{b}$ | $15.79^{c}$ | 2.44 | ** |

Means within row for each item having different superscript differ significantly * ($p \leq .05$), ** ($p \leq .01$), *** ($p \leq .001$)

**Table (2):** Effect of heat stress and grape seed extract supplementation on plasma lipids profile.

| | TN | HSGSE 0 | HSGSE 1 | HSGSE2 | SEM | Significant |
|---|---|---|---|---|---|---|
| TG (mg/dl) | $104.11^{c}$ | $151.06^{a}$ | $139.45^{b}$ | $103.62^{c}$ | 4.79 | *** |
| LDL (mg/dl) | $38.79^{c}$ | $63.26^{a}$ | $49.53^{b}$ | $40.21^{c}$ | 3.55 | *** |
| HDL (mg/dl) | $25.49^{b}$ | $32.47^{a}$ | $31.44^{a}$ | $26.02^{b}$ | 2.77 | ** |

Means within row for each item having different superscript differ significantly ** ($p \leq .01$), *** ($p \leq .001$)

**Table (3):** Effect of heat stress and grape seed extract supplementation on lipid peroxidation and some antioxidant enzymes in liver.

| | TN | HSGSE 0 | HSGSE 1 | HSGSE 2 | SEM | Significant |
|---|---|---|---|---|---|---|
| MDA (nmol/g protein) | 2.04$^{c}$ | 3.93$^{a}$ | 3.22$^{b}$ | 2.31$^{c}$ | 0.27 | *** |
| SOD (U/mg protein) | 176.05$^{a}$ | 122.83$^{c}$ | 159.18$^{b}$ | 166.47$^{ab}$ | 5.47 | *** |
| GSH(µg/mg protein) | 4.91$^{a}$ | 3.24$^{c}$ | 3.88$^{b}$ | 4.75$^{a}$ | 0.19 | *** |

Means within row for each item having different superscript differ significantly *** ($p \leq .001$)

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## الملخص العربي

## تأثير مستخلص بذر العنب على التغييرات الفسيولوجية لدجاج التسمين المعرض للاجهاد الحرارى

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الهدف الرئيسى من هذه الدراسة هو بحث التغييرات الفسيولوجية لكتاكيت دجاج التسمين المعرضة لدرجات الحرارة المرتفعة، وتوضيح الدور الوقائى لمستخلص بذر العنب فى تخفيف هذه التغيرات المتوقعة. استخدم فى هذه الدراسة١٨٠ كتكوت تسمين هبرد (عمر يوم واحد). حضنت جميع الكتاكيت على درجة حرارة ٣٤°م، ٥٥% رطوبة خلال اليومين الاوليين، ثم انخفضت تدريجيا حتى وصلت الى درجة حرارة ٢٤°م، ٥٥% رطوبة فى اليوم ٢١ من العمر. قسمت الطيور فى اليوم ٢٢ من العمر الى ٤ مجاميع، بكل مجموعة ٣ مكررات ( ١٥ كتكوت لكل مكررة). المجموعة الاولى (TN) هى مجموعة المقارنة السالبة، عرضت الطيور الى درجة حرارة ٢٤°م، ٥٥% رطوبة ) طوال فترة التجربة من اليوم ٢٢ وحتى اليوم ٤٠ من العمر، وتغذت على عليقة تجارية. عرضت طيورالمجاميع الثانية والثالثة والرابعة الى درجة حرارة مرتفعة (٣٦°م، ٦٥% رطوبة) لمدة ٨ ساعات يوميا ( من ١٠ صباحا الى ٦ مساءا) طوال فترة التجربة من اليوم ٢٢ وحتى اليوم ٤٠ من العمر. تغذت طيور المجموعة الثانية (HSGSE0) على عليقة تجارية لا تحتوى مستخلص بذر العنب، كمجموعة مقارنة موجبة. تغذت طيور المجموعتين الثالثة (HSGSE1) والرابعة (HSGSE2) على عليقة تجارية تحتوى على ١٠٠، ٢٠٠ملجم/كجم من مستخلص بذر العنب على الترتيب.

أظهرت النتائج زيادة معنوية فى درجة حرارة الجسم الداخلية، ومعدل تنفس الطيور، ونسبةH/L بالدم ومستوى البلازما من الكوتيكوستيرون والتراجلسريد والكوليسترول منخفض الكثافة (LDL) والكوليسترول عالى الكثافة (HDL) ومستوى MDA بالكبد نتيجة تعرض الطيور لدرجات الحرارة المرتفعة، فى حين انخفض مستوى الانزيمات المضادة للأكسدة (SOD & GST) بصورة معنوية مقارنة بالمجموعة الاولى. وبالرغم من عدم تأثر كل من درجة حرارة الجسم الداخلية، ومعدل تنفس الطيور باضافة مستخلص بذر العنب الى العليقة بالتركيزين ١٠٠، ٢٠٠ملجم/كجم، الا انه قد لوحظ زيادة معنوية فى مستوى الانزيمات المضادة للأكسدة (SOD & GST)، كما حدث انخفاض معنوى فى كلا من نسبةH/L بالدم ومستوى البلازما من الكوتيكوستيرون والتراجلسريد والكوليسترول منخفض الكثافة (LDL) والكوليسترول عالى الكثافة (HDL) ومستوى MDA بالكبد مقارنة بالمجموعة الثانية.

الخلاصة: من خلال النتائج المتحصل عليها نستنتج أن استخدام مستخلص بذر العنب يمكن ان يكون له دور وقائى مضاد للتأثيرات السلبية الناتجة عن تعرض الطيور للاجهاد الحرارى مثل عوامل الاجهاد وصورة الدهون فى بلازما الدم وانزيمات الاكسدة فى كبد دجاج التسمين، كما أظهر المستوى ٢٠٠ملجم/كجم تفوقا بالمقارنة بالمستوى ١٠٠ملجم/كجم.