

# IMPROVING PERFORMANCE AND THERMOTOLERANCE STATE OF THERMALLY STRESSED BROILER'S USING SUPPLEMENTED NATURAL ANTIOXIDANTS

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## ABSTRACT

The aim of this experiment was studying the efficacy dietary dried powders' of ecofriendly natural antioxidants of beetroot (Br), grape pomace (Gp) and willow (W) in two concentrations 0.5 and 1 % as an anti-heat-stressor and their impacts on performance and thermo-tolerance state on broiler's housed under thermal-stress averaged (33°C). 630 Ross-308day-old-chicks were used. The treatments were applied after 2 weeks of starter feeding as beginning grower feed then finisher diet until 42d marketing age. The treatments were as follow: T1:Control (basal diet BD); T2 (1% Br+BD); T3 (0.5% Br+BD); T4 (1%GP+BD); T5 (0.5%GP+BD); T6 (1%W+BD); T7 (0.5%W+BD). Complete Random Design (CRD) used on three replicates/treatment; thirty bird/rep. The results indicate that both Br and Gp in both (0.5; 1%) levels improved growth performance except birds in W 0.5% wasn't affected and W 1% decreased significantly than Control. The PEF was significantly higher in each of T2, T3, and T4 groups than Control group. All natural antioxidants dietary supplemented enhanced body antioxidants; prevented oxidation; developed thermo-tolerance and stress expressions than the control group. It can be concluded that the antioxidant property of the eco-friendly additives used in the current experiment promoted the biological condition hence the thermally-stressed broilers' productive performance.

Keywords: productivity, meat chicken, heat exhausted, plant based antioxidants.

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تحسين الاداء الانتاجي و حالة التحمل الحراري لفروج اللحم المجهد حرارياً باستخدام مضادات اكسدة طبيعية مضافة

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## المستخلص

هدفت التجربة دراسة فعالية المساحيق الغذائية المجففة للشمندر (Br) و ثفل العنب (Gp) و الصفصاف (W) بتركيزين 0.5 و 1% كمضادات اكسدة طبيعية صديقة للبيئة لعمليها كمضاد للاجهاد الحراري وتأثيراتهم على الأداء الانتاجي وحالة التحمل الحراري في فروج اللحم المربي تحت الاجهاد حراري بمعدل 33م°. استخدم 630 فرخ لحم سلالة Ross-308 بعمر يوم واحد. تم تغذيتها على البادى لأسبوعين. بدأت التجربة عند التغذية بالنامي فالناهي لغاية 42 يوم عمر التسويق. قسمت المعاملات كالتالي: T1:السيطرة (C:عليقة اساسية خالية من الاضافات)، T2: Br 1%+عليقة الاساسية، T3: Br 0.5%+عليقة الاساسية، T4: Gp 1%+عليقة الاساسية، T5: Gp 0.5%+عليقة الاساسية، T6: W 1%+عليقة الاساسية و T7: W 0.5%+عليقة الاساسية. طبق التصميم العشوائي الكامل بثلاث مكررات/معاملة، ثلاثين طائر/مكرر. تشير النتائج إلى أن كلا من Br و Gp في كلا المستويين 0.5 و 1% قد حسن أداء النمو، باستثناء T7 التي لم تتأثر بالمعاملة و T6 التي انخفضت معنوياً مقارنة بمجموعة السيطرة. معامل الكفاءة الانتاجية كان اعلى معنوياً في كل من المجاميع المعاملة T2، T3، T4 عن السيطرة. جميع مضادات الأكسدة الطبيعية المضافة غذائياً عززت مضادات الأكسدة الجسمية، منعت الأكسدة، طورت تعبيرات التحمل الحراري والاجهاد مقارنة بمجموعة السيطرة. نستنتج أن للإضافات الصديقة للبيئة المستخدمة في التجربة الحالية عززت الحالة البيولوجية، ومن ثم أداء فروج اللحم المجهد حرارياً لخاصيتها المضادة للأكسدة الطبيعية.

الكلمات المفتاحية: الإنتاجية، التحمل الحراري، دجاج اللحم، مضادات اكسدة نباتية.



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## INTRODUCTION

It's not a secret that broiler meat consumption is one of the most sought-after protein sources in the worldwide market over the last few decades due to its inexpensive cost and excellent nutritional content (29). Heat stress (HS) harms animals and human being health and causes massive economic forfeiture in the livestock sector plus public health care (19). As it has been reviewed that most poultry species find comfortable in temperatures ranging from 18 to 22 °C. However, when the temperature rises over this range, the effectiveness of sensible heat removal systems declines, causing most chickens to suffer from heat stress (13). Oxidative damage and impaired antioxidant capacity are the consequence of reactive oxygen species (ROS) formation which increases with thermal stress (3). Malondialdehyde (MDA) symbolizes a lipid peroxidation substance that generally recognized as a measure of oxidative stress in tissues (7). Many studies have linked the reduction of poultry growth performance in heat-stressed birds to oxidant damage (17, 25). Heat shock proteins (HSP) are proteins protect cells and its non-specific expression can be boosted when the body is under stress like extra-thermoneutral degrees (19). Heat stress can enhance HSP70 expression, which can reduce ROS generation and apoptotic expression by boosting the antioxidant enzymes working harder (18). The antioxidant defense system; natural and synthetic antioxidants; antioxidant enzymes can attenuate free radicals created biologically. Recently due to the trend of restricting the usage of synthetic compounds worldwide in considering the increasing concerns about consumer health, environment and animal welfare; hence, researchers' studies forwarded to use the potential natural replacements such as phytobiotics and volatile oils existed in plants or herbs to develop healthier products while preserving safety and sensory properties. Plants containing phytochemical (phenols; flavonoids) compounds preventing and lowering oxidative stress (20, 33). Beetroot (*Beta vulgaris*), sometimes known as red beet, is a root vegetable used in the medicinal system as anti-oxidant, free radical scavenging activity, anti-depressant, anti-

microbial, anti-fungal, anti-inflammatory, boosts the energy, diuretic, expectorant and carminative (12, 30) due to its ample antioxidant capacity and apoptosis properties because it is rich in exclusive phyto-constituents, phenolic acids and betalains that is a nutritive dye in addition to the minerals, nutrients, sugars and vitamins; hence beetroot is an excellent dietary supplement in perfecting poultry performance (8, 36). Grape pomace (GP) is a by-product of grape industry composes about 20% of the total weight of grapes processed. It includes of skin, seeds, and stems (21). Gp is high in phenolic compounds such catechins, epicatechin and epicatechin-3-O-gallate, resveratrol and procyanidins (40). Investigations declared that GP has dramatically substantial antioxidant action increases the expression of antioxidant enzyme (SOD, GSH-Px, CAT) activity in serum and hepatocytes; an antibacterial; stimulates growth, improves immunity, alleviates heat-induced liver damage, reduces lipidemia and prevents lipid peroxidation as it used dietary in broiler chicks (11, 16). Willow tree is an old herbal medicine for pain; fever; antioxidant and neuroprotective; inflammation effects. While salicin is the primary active ingredient in willow, important phyto-compounds participate synergistically to the therapeutic benefits of willow bark. In numerous in vitro investigations, *Salix* species have been shown to have substantial antioxidant activity, as well as, an anti-inflammatory impact. The bioactive substances content of willow plays an essential role in poultry improvement (14, 32). This study was aimed to study the effectiveness of ecofriendly antioxidants in plants (Br and W) and by-product (grape pomace) in 0.5 and 1% levels as feed additives to study their impacts in improving the productive performance and ameliorating thermo-tolerance of broilers reared under heat-stress condition.

## MATERIALS AND METHODS

**Experimental treatments** this experiment achieved at Poultry Research House at Girdarash Farm; Animal Resource Department; Science of Agricultural Engineering College; Salahaddin University – Erbil/Iraq; from 19<sup>th</sup> /June/2021 till 1<sup>st</sup> /August/ 2021. A total of 630 day-old Ross-

308 chicks were distributed and housed, randomly, into 21 floor pens of 200 × 150 × 100 cm<sup>3</sup> (length; width; height, respectively) dimensions. 30 birds/pen; a pen represents one replicate (rep.) of a treatment (treat.). The experiment lasted 6 weeks, and consisted of three feed phases (starter was from 0 to 14 days of age; grower was from 15<sup>th</sup> to 28<sup>th</sup> days of age; finisher was from 29<sup>th</sup> to 42<sup>nd</sup> days of age). Basal diets formulation and composition of the experiment are showed in Table (1).

**Table 1. The formulation of basal diet used during the experiment.**

Ingredients (g/Kg)	Starter (0-14) days	Grower (15-28) days	Finisher (29-42) days
Soybean 46%	325	300	235
Corn 8%	300	320	370
Wheat 12%	93	100	100
Wheat Flour	220	214	225
DCP	5	5	4
Premix 2.5%	25	25	25
Soybean oil	14	20	25
Lysine	0.5	0.3	0.25
Methionine	0.5	0.3	0.25
Choline chloride	0.4	0.4	0.4
Salt	0.5	0.5	0.5
Enzyme	0.5	0.5	0.5
Limestone	14.6	13	13.1
Anti-toxin	1	1	1
Total	1000	1000	1000
Analyzed chemical composition (%)			
Crude Protein	22	21	18.5
Ether Extract	3.1	4.4	5
Crude Fat	2.3	2.3	2.2
Starch	40.8	41.35	44.8
Ash	5.2	5.4	5
Metabolic Energy (Kcal/Kg)	2900	3000	3100

The first 2 weeks of age the chicks were raised upon Ross broiler management program guide (2018) means standard environmental factors with starter phase diet. As starting the grower phase at the 15<sup>th</sup> day of age thereby finisher feed at 29<sup>th</sup> day of age until marketing 42 d; the chicks divided according to the natural antioxidants supplemented on seven dietary treatments (3 rep./treat; 30 birds/rep.), as follow: **T1: Control** (C: fed Basal Diet BD with no additives); **T2 (1% Br)**: fed 1% of beetroot + Basal diet; **T3 (0.5 % Br)**: fed 0.5% of beetroot + Basal diet; **T4 (1% GP)**: fed 1% of grape pomace + Basal diet; **T5 (0.5% GP)**: fed 0.5% of grape pomace + Basal diet; **T6 (1% W)**: 1% of Willow + Basal diet; **T7 (0.5% W)**: fed 0.5% Willow + Basal

diet. The birds were supplied with *ad libitum* feed and water during the overall rearing period.

The broilers were raised under continuous heat stress as in-house temperature was (32-34°C) with average of 33 °C during daytime and about (26-30 °C) as average (28 °C) after mid-night hours, during the overall rearing period. An intermittent lighting program of (5D: 19L) hours was applied.

**Antioxidant property of beetroot, grape pomace and willow :** Table (2) illustrates the amount of phenol compounds measured according the Folin-Cipcaltue colorimetric assay and Gallic acid (5). The amount of total flavonoid; total flavonol were determined according Aluminum chloride colorimetric assay (5). The antioxidant activity was recorded as percentage compared to the constant yellow color of beta carotene (31). The antioxidant property of the plant's powder was conducted at laboratories of Urmia University; Faculty of Veterinary Medicine.

**Table 2. The polyphenolic (mg/g), poly flavonoid (mg/g), flavenols (mg/g) and the antioxidant activity (%) of the supplemented natural antioxidants.**

	Beetroot	Grape pomace	Willow
Phenol (mg/g)	285.82	281.64	274.09
Flavonoid (mg/g)	145.67	131.56	127.70
Flavenol (mg/g)	95.44	92.30	88.82
Antioxidant activity (%)	74.63	70.8	68.63

### Productive performance

Body weight (BW) and feed intake (FI) were measured on a weekly basis to calculate body weight gain (BWG) and feed conversion ratio (FCR). The number of dead birds recorded daily. The overall (15-42) and (1-42) days BW, BWG, FI, FCR and mortality percentage were calculated. The production efficiency factor (PEF) calculated for the 42 days of age according to (27), the formula was as follow:

$$\frac{\text{Livability (\%)} \times \text{Live Weight (Kg)}}{\text{Age (days)} \times \text{FCR}} \times 100$$

At 42<sup>nd</sup> day of the experiment, samples of blood and liver were collected from two birds per replicate (6 birds/treatment). The serum separated using a centrifuge (3000 rpm for 10 minutes) then have been frozen at -20° C till

the needed parameters evaluated. In addition, at the same time liver samples that eviscerated have been frozen at (-80 °C) in liquid nitrogen and transported to the laboratory as fast as to estimate the required parameters (1). All tests performed on liver and serum samples have been conducted at laboratories of Urmia University; Faculty of Veterinary Medicine.

#### **Liver enzymes, Free fatty acids and antioxidant activity evaluation**

Estimating the liver enzyme activity of Aspartate transaminase (AST); Alanine transaminase (ALT). Alkaline phosphatase (ALP) enzymes activity in liver were assayed using a commercial kit (Parsazmoon, Iran). Based on the kit instruction and using spectrophotometer (StatFax, USA). While, the activity of antioxidant enzymes in the serum were also estimated which are represented by glutathione peroxidase (GPx) enzyme was measured using commercial kit (Padgin Teb, Iran), based on kit instruction. Liver content of Free fatty acids (FFA), GPx and Superoxide Dismutase (SOD) was estimated in liver samples; in measuring these parameters a commercial kit (Padgin Teb, Iran) was used based on instruction and using spectrophotometer (StatFax, USA).

**Oxidation statue** determined through the level of Malondialdehyde (MDA) in serum and liver; the assay of measurements done according to the method of Abubakar (2).

**Thermo-tolerance** measured by evaluation the level of HSPs in serum and Heterophil/Lymphocyte ratio (H/L ratio).

**Heat shock protein 70 and 90 (HSP 70 and 90)** the expression levels of HSP 70 and 90 in serum samples were analyzed according the method of Singh (37).

**Leukocytes determination** in order to determine H/L ratio, counts of heterophils and lymphocytes were made under a microscope after Wright-Giemsa staining of slides (100x) (9).

#### **Statistical analysis**

Completely Randomized Design (CRD) were applied, the collected data were analyzed by using SAS program (33), Duncan's multiple range (10) used for comparison between means among the treatments, to determine the impacts of ecofriendly natural antioxidants have been dietary added on the overall

performance, oxidative state and thermo-tolerance in broiler.

#### **RESULTS AND DISCUSSION**

The obtained results showed that the natural antioxidants groups fed beetroot (T2 and T3) and grape pomace (T4 and T5) powders added improved significantly the overall (15-42 d) BW and BWG than control group with superiority to 0.5 % than 1% concentration; except T6 (W 1%) and T7 (W 0.5%) that were finalized significantly and numerically less BW and BWG than control group, respectively. However, T2, T3, T4 and T5 groups were consumed significantly more feed than control but T6 and T7 groups were ingesting feed as control. In another side, both T2 (1% Br) and T3 (0.5% Br) broilers converted the consumed feed significantly better than each of Control, T4 (1% Gp) and T5 (0.5% Gp). While, the results of both T6 (1% W) and T7 (0.5% W) had significantly higher FCR as compared to control and to other treated groups. The significant efficient groups in productive performance were each of T2, T3 and T4 in PEF results as they compared to control (**Table 3 A**). The obtained results showed that the natural antioxidants groups fed beetroot (T2 and T3) and grape pomace (T4 and T5) powders added improved significantly the overall (1-42 d) productive performance as BW, BWG, FCR and PEF (not including T5 group) than control group with superiority to 0.5 % than 1% concentration; except T6 (W 1%) was less significantly and T7 (W 0.5%) was non-significant than control group in finalized efficiency (**Table 3 B**). The weight and gain improvement herein related to the significantly additional feed consumed, utilized and converted to weight gain by the broilers and vis versa in the mentioned groups because stressed birds decrease feed intake. As known that PEF is the outcome of an equation that put live weight, livability, and FCR at marketing age (42 d) together; the mentioned results related to the value of the traits that considered in the used equation to evaluate the efficiency of production. Our results contracted with (19, 39) in control group; in Br treats (35); in use of Gp (15, 28); Panaite (26) in W sets. Thermal stress is known to increase oxidative damage in broilers (17, 25) resulting in decreased growth performance in heat-

stressed birds (19, 39) as subsequent of high temperatures impair the digestive and absorptive functions of the gastrointestinal system, particularly the gut and liver tissues, which obstructs the correct operation of poultry organs (6). The improved growth of broiler chicks, observed herein, could be due to the natural anti-oxidative substances, can boost broiler performance by minimizing the harmful effects of oxidation (20, 33). Furthermore, the same or the negative results to control group obtained may related to polyphenolic antioxidants can exhibit

antioxidant or mild pro-oxidant effects based on the circumstances and doses (26, 38). The amount of phenolic chemicals included is variable, but However, the bioavailability in the digestive system is what ultimately determines the nutritional value and biological effects; this is because the physicochemical properties of the phenolic component in the plants are what ultimately determines how well they are absorbed that demonstrated good benefits at high; mild; low dosages of inclusion (22).

**Table 3. Impact of supplemented natural antioxidant powders on overall A. (15-42d) B. (1-42d) productive performance in thermally stressed broilers.**

**A. (15-42d)**

Traits	T1 Control	T2 (1% Br)	T3 (0.5% Br)	T4 (1% GP)	T5 (0.5% GP)	T6 (1% W)	T7 (0.5% W)	SEM
Weight (g)	2516.25 <sup>d</sup>	2652.57 <sup>b</sup>	2800.76 <sup>a</sup>	2578.62 <sup>c</sup>	2625.50 <sup>b</sup>	2446.45 <sup>e</sup>	2495.36 <sup>d</sup>	24.97
Weight gain (g)	2059.81 <sup>d</sup>	2183.45 <sup>b</sup>	2343.43 <sup>a</sup>	2118.84 <sup>c</sup>	2165.28 <sup>b</sup>	1979.56 <sup>e</sup>	2031.03 <sup>d</sup>	25.30
Feed intake (g/bird)	2996.26 <sup>c</sup>	3101.14 <sup>b</sup>	3072.96 <sup>b</sup>	3069.60 <sup>b</sup>	3148.92 <sup>a</sup>	3020.37 <sup>c</sup>	2986.99 <sup>c</sup>	13.01
FCR (g feed/g gain)	1.455 <sup>c</sup>	1.420 <sup>d</sup>	1.311 <sup>e</sup>	1.449 <sup>c</sup>	1.454 <sup>c</sup>	1.526 <sup>a</sup>	1.471 <sup>b</sup>	0.014
Livability (%)	98.810	98.810	97.620	100.000	97.620	100.000	100.000	0.44
PEF	412.36 <sup>d</sup>	454.29 <sup>b</sup>	528.14 <sup>a</sup>	434.97 <sup>bc</sup>	419.84 <sup>cd</sup>	375.38 <sup>e</sup>	411.70 <sup>d</sup>	10.21

**B. (1-42d)**

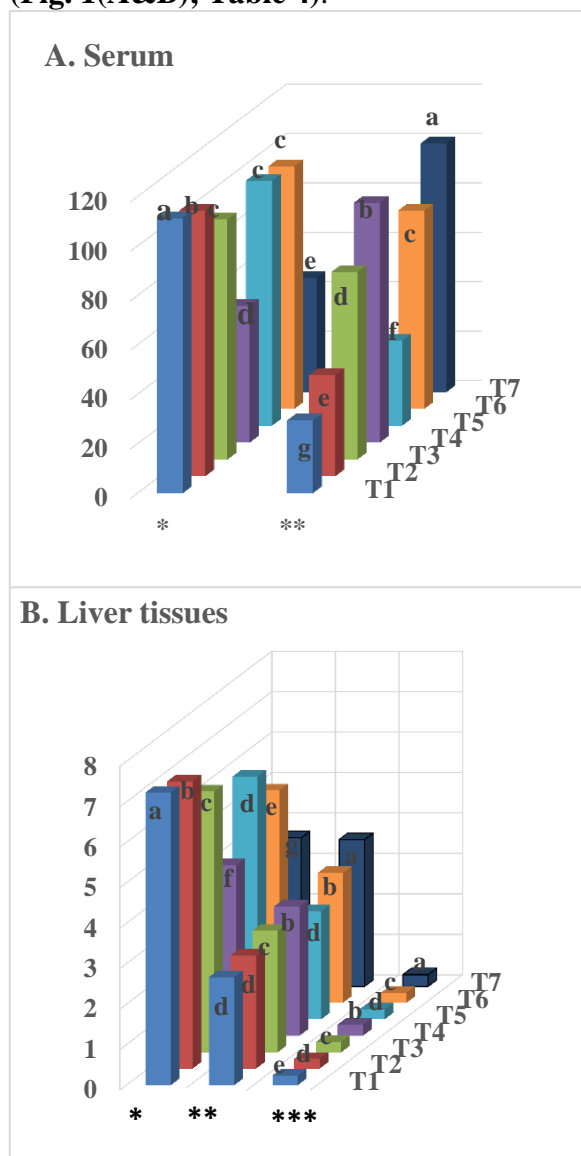
Traits	T1 Control	T2 (1% Br)	T3 (0.5% Br)	T4 (1% GP)	T5 (0.5% GP)	T6 (1% W)	T7 (0.5% W)	SEM
Weight (g)	2516.25 <sup>d</sup>	2652.57 <sup>b</sup>	2800.76 <sup>a</sup>	2578.62 <sup>c</sup>	2625.50 <sup>b</sup>	2446.45 <sup>e</sup>	2495.36 <sup>d</sup>	24.97
Weight gain (g)	2479.99 <sup>d</sup>	2617.23 <sup>b</sup>	2765.38 <sup>a</sup>	2543.28 <sup>c</sup>	2590.54 <sup>b</sup>	2410.61 <sup>e</sup>	2460.19 <sup>d</sup>	25.01
Feed intake (g/bird)	3408.63 <sup>c</sup>	3513.39 <sup>b</sup>	3485.07 <sup>b</sup>	3481.73 <sup>b</sup>	3561.54 <sup>a</sup>	3433.11 <sup>c</sup>	3398.87 <sup>c</sup>	13.13
FCR (g feed/g gain)	1.374 <sup>bc</sup>	1.342 <sup>d</sup>	1.260 <sup>e</sup>	1.369 <sup>c</sup>	1.375 <sup>bc</sup>	1.424 <sup>a</sup>	1.382 <sup>b</sup>	0.01
Livability (%)	98.81	98.81	97.62	100.0	97.62	100.0	100.0	0.23
PEF	397.31 <sup>c</sup>	427.15 <sup>b</sup>	492.70 <sup>a</sup>	444.46 <sup>b</sup>	299.20 <sup>e</sup>	336.94 <sup>d</sup>	440.22 <sup>b</sup>	14.08

T1: Control (basal diet BD); T2 (1% Br + BD); T3 (0.5% Br + BD); T4 (1%GP + BD); T5 (0.5%GP +BD); T6 (1%W + BD); T7 (0.5%W + BD). a, b, c within rows with different superscripts meant differ significantly at ( $P \leq 0.05$ ). The same superscripts among the treatments mean insignificant.

Antioxidant enzymes activity measured by evaluation each of GPx; SOD in liver tissues and GPx in serum. The results displayed that thermally stressed birds in control group possessed the least levels of Glutathione antioxidant enzymes in both liver tissues and serum than all other heat stressed broilers in groups that their diets supplemented with ecofriendly natural antioxidants existed in the powders of beetroot; grape pomace; willow by di concentrations 1 and 0.5 %. However, SOD

level in both T2 and T5 had SOD level that non significantly differed than control birds (**Fig. 1(A&B)**). Furthermore, thermally stressed birds in treatments their diets fortified with natural antioxidants existed in the powders of beetroot; grape pomace; willow by both levels 1 and 0.5 % improved the oxidative statue via recorded a significant decrease of MDA in serum and liver tissues; hepatic total lipids and free fatty acids (F.F.A). While the Control group owned significantly

the highest total lipid, MDA and free fatty acids in the liver as compared to all treated groups that were added natural antioxidants (**Fig. 1(A&B); Table 4**).



**Figure 1. Impact of supplemented natural antioxidant powders on antioxidant enzyme activity and oxidation state in A. Serum (\*MDA (n mol/mg); \*\* GPx (U/g)); B. Liver tissues (\*MDA (n mol/mg); \*\* SOD (U/mg); \*\*\* GPx (U/g)) in thermally stressed broilers at 42 days of age.**

Our results of GPx, SOD, and MDA agree with the results of (19, 23, 39) they noted that in heat-stressed broilers, antioxidant enzymes and free radical scavenging decrease. Thus, oxidative damage in broiler livers disrupts lipid metabolism. Furthermore, dietary adding antioxidants in high efficacy controlling fat oxidation and inhibiting lipid peroxidation in heat stress chicken's livers.

The reported enhancement of the antioxidant status and reducing MDA level by using antioxidants confirmed the previous results of using antioxidant phytochemicals as beetroot (23, 36) this accomplished our results of beetroot groups. Furthermore, dietary willow bark powder may reduce broiler liver oxidative stress (6). In addition, Panaite et al. (26) when they added willow powder to feed, they find out significantly increased GPx concentrations in chicken liver tissue which endorsed our finding. While, their obtaining's were disagreed with our results of SOD activity in liver tissues. Our results and the results of (15, 23) are harmonized that Gp dietary inclusion promote antioxidant enzymes (GPx and SOD) activity.

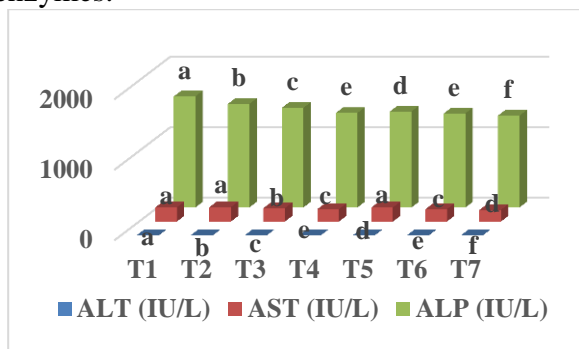
**Table 4. Impact of supplemented natural antioxidant powders on liver tissues lipid profile in thermally stressed broilers at 42 days of age.**

Traits Treatments	Total lipid (mg/100g)	Free fatty acid (%)
T1 (Control)	452.129 <sup>a</sup>	5.367 <sup>a</sup>
T2 (Br 1%)	439.557 <sup>b</sup>	5.154 <sup>b</sup>
T3 (Br 0.5%)	405.629 <sup>d</sup>	4.956 <sup>c</sup>
T4 (Gp 1%)	362.857 <sup>f</sup>	4.749 <sup>de</sup>
T5 (Gp 0.5%)	433.729 <sup>c</sup>	4.891 <sup>cd</sup>
T6 (S 1%)	399.529 <sup>e</sup>	4.727 <sup>e</sup>
T7 (S 0.5%)	312.986 <sup>g</sup>	4.517 <sup>f</sup>
SEM	6.564	0.042

T1: Control (basal diet BD); T2 (1% Br + BD); T3 (0.5% Br + BD); T4 (1%GP + BD); T5 (0.5%GP +BD); T6 (1%W + BD); T7 (0.5%W + BD). a, b, c, d, e, f, g within columns with different superscripts meant differ significantly at ( $P \leq 0.01$ ) & ( $P \leq 0.05$ ).

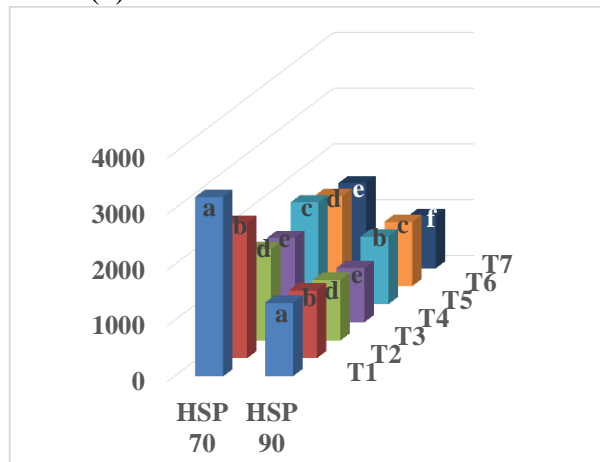
The liver is an organ that has a considerable impact on both avian development and thermoregulation because of its susceptibility to heat stress and its byproducts that left in liver tissues that cause autophagy dysfunction of liver (39). Important serological indexes reflecting liver injury are liver enzymes represented in ALT, AST, and ALP were at the highest levels in the control group which was significantly in contrast to the treated groups, except T2 and T5 were similar to control in AST level (**Fig. 2**). This result is parallel with those of Liu et al. (19) they stated

that broilers subjected to continuous heat stress had significantly enhanced serum liver enzymes.



**Figure 2. Impact of supplemented natural antioxidant powders on liver enzymes in liver tissues in thermally stressed broilers at 42 days of age.**

HSP are cell defense chaperons their overexpression is associated with a variety of stress responses, including heat stress. In this experiment, the higher HSP70 and HSP90 levels were been in the serum of broilers in control group (**Fig. 3**). Our results agree with Liu et al. (19) and reduced HSP expression in supplemented broilers, indicating enhanced thermos-tolerance in these birds under heat stress (4).

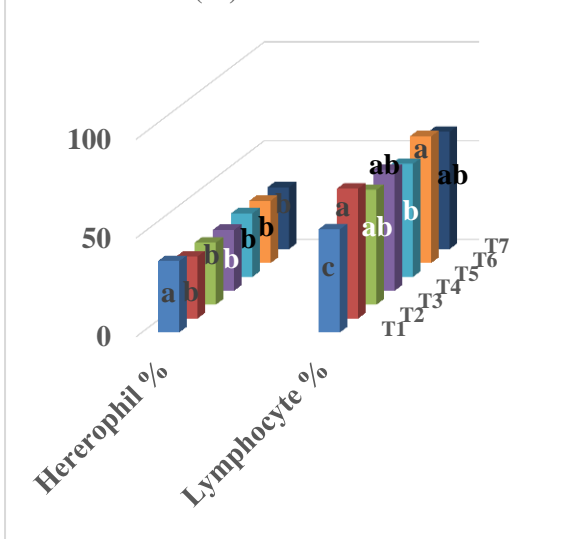


**Figure 3. Impact of supplemented natural antioxidant powders on HSP (relative expression) in serum in thermally stressed broilers at 42 days of age.**

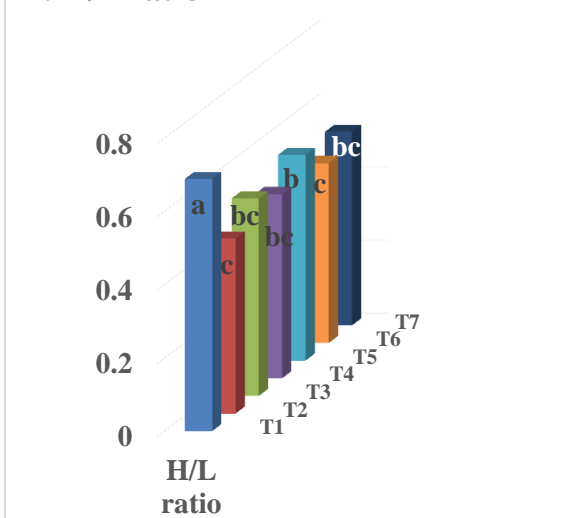
As a result of the obtained reduction in H and H/L values in treated groups compared to control groups, ecofriendly antioxidant treatments are effective ways to reduce the harmful effect of heat stress on chick's immunity; wellbeing, and 1% is the best level for each antioxidant source used in broiler diet (**Fig. 4 A & B**). Our results of H% and H/L ratio in treated as control group is agree with

Muhammad and Al-Hassani (24) and Selim et al. (35) stated that both H. and H/L ratio were significantly less in the blood of the broilers were fed at both levels of 0.5 or 1% aqueous extract of beetroot as natural antioxidant source during summer season from 1 to 40 d of age; this accomplished our results. However, Selim et al. (35) result was disagree with our results of lymphocytes %.

**A. Heterophil (%) and Lymphocyte (%)**



**B. H/L ratio**



**Figure 4. Impact of supplemented natural antioxidant powders on A. percentages of heterophil, lymphocyte and B. H/L ratio in thermally stressed broilers at 42 days of age.**

## CONCLUSION

Clearly can be concluded that the anti-oxidation characteristics of the eco-friendly supplements used in the current experiment (especially beetroot and grape pomace in both 0.5 and 1% levels) improved the productive performance of heat-stressed broilers through



enhancing the evaluated thermos-tolerance expressions as HSP and H/L ratio and decreasing the MDA as the antioxidant levels in the body promoted. More investigations needed to define the perfect level of using the mentioned natural antioxidants to reach the peak of production in stressed thermally broiler is recommended.

#### CONFLICT OF INTEREST

The authors declare that they have no conflicts of interest.

#### DECLARATION OF FUND

The authors declare that they have not received a fund.

#### REFERENCES

1. Abdulla, N.R., A.N. Mohd Zamri, A.B. Sabow, K.Y. Kareem, S. Nurhazirah, F.H. Ling, A.Q. Sazili, and T.C. Loh. 2017. Physico-chemical properties of breast muscle in broiler chickens fed probiotics, antibiotics or antibiotic–probiotic mix. *Journal of Appl. Anim. Res.*, 45(1):64-70.  
<https://doi.org/10.1080/09712119.2015.1124330>.
2. Abubakar, K., M.M. Mailafiya, S.M. Chiroma, A. Danmaigoro, T.Y. Zyoud, E. Abdul Rahim and M.Z. Abu Bakar Zakaria. 2020. Ameliorative effect of curcumin on lead-induced hematological and hepatorenal toxicity in a rat model. *J. Biochem. Mol. Toxicol.*, 34(6):22483.  
<https://doi.org/10.1002/jbt.22483>.
3. Abudoulrahman, K.K., M.A. Mustafa and A.A. Abduljabbar. 2019. The Effect of Heat Stress on Oxidative Stress and Antioxidant Status in Local Quail Hens Supplemented with Onion and Garlic Oils. *Tikrit J. Agric. Sci.*, 19(1):103-110.  
<https://doi.org/10.25130/tjas.19.1.11>.
4. Belal, S.A., D.R. Kang, E.S.R. Cho, G.H. Park and K.S. Shim. 2018. Taurine reduces heat stress by regulating the expression of heat shock proteins in broilers exposed to chronic heat. *Brazilian J. Poult. Sci.*, 20:479-486.  
<https://doi.org/10.1590/1806-9061-2017-0712>.
5. Chaleshtori, R.S., F.S. Chaleshtori and M. Rafieian. 2011. Biological characterization of Iranian walnut (*Juglans regia*) leaves. *Turkish J. Biol.*, 35(5):635-639.  
<https://doi.org/10.3906/biy-1005-1>.
6. Chen, Y., Y. Cheng, C. Wen and Y. Zhou. 2020. Protective effects of dietary mannan oligosaccharide on heat stress–induced hepatic damage in broilers. *Environ. Sci. Poll. Res.*, 27(23):29000-29008.  
<https://doi.org/10.1007/s11356-020-09212-2>.
7. Cheng, K., Z.H. Song, X.C. Zheng, H. Zhang, J.F. Zhang, L.L. Zhang, Y.M. Zhou and T. Wang. 2017. Effects of dietary vitamin E type on the growth performance and antioxidant capacity in cyclophosphamide immunosuppressed broilers. *Poult. Sci.*, 96(5):1159-1166.  
<https://doi.org/10.3382/ps/pew336>.
8. Chhikara, N., K. Kushwaha, P. Sharma, Y. Gat and A. Panghal. 2019. Bioactive compounds of beetroot and utilization in food processing industry: A critical review. *Food Chemistry*. 272:192-200.  
<https://doi.org/10.1016/j.foodchem.2018.08.022>.
9. Cook, F. W. 1959. Staining fixed preparations of chicken blood cells with combination May-Greenwald-Wright-Phloxine B stain. *Avian Dis.*, 3(3):272-290.  
[https://doi.org/10.1016/S0021-9258\(18\)64849-5](https://doi.org/10.1016/S0021-9258(18)64849-5).
10. Duncan, D.B. 1955. Multiple range and multiple F tests. *Biometrics*. 11(1):1-42.  
<https://doi.org/10.2307/3001478>.
11. Emami, N.K., U. Jung, B. Voy and S. Dridi. 2020. Radical response: effects of heat stress-induced oxidative stress on lipid metabolism in the avian liver. *Antioxidants*. 10(1):35.  
<https://doi.org/10.3390/antiox10010035>.
12. Fu, Y., J. Shi, S.Y. Xie, T.Y. Zhang, O.P. Soladoye and R.E. Aluko. 2020. Red beetroot betalains: Perspectives on extraction, processing, and potential health benefits. *Journal of Agricultural and Food Chemistry*. 68(42):11595-11611.  
<https://doi.org/10.1021/acs.jafc.0c04241>.
13. Getachew B. 2021. Review on the Effect of Heat Stress on Poultry Production and Productivities. *Food Sci., Nut. Technol.*, 6(2): 000260.  
<http://doi.org/10.23880/fsnt-16000260>.
14. Gligorić, E., R. Igić, L. Suvajđić and N. Grujić-Letić. 2019. Species of the genus *Salix* L.: Biochemical screening and molecular docking approach to potential acetylcholinesterase inhibitors. *Appl. Sci.*,



9(9):1842.

<https://doi.org/10.3390/app9091842>.

15. Gungor, E., A. Altop and G. Erener. 2021. Effect of raw and fermented grape pomace on the growth performance, antioxidant status, intestinal morphology, and selected bacterial species in broiler chicks. *Animals*. 11(2):364. <https://doi.org/10.3390/ani11020364>.

16. Hosseini-Vashan, S.J., M. Safdari-Rostamabad, A.H. Piray, and H. Sarir. 2020. The growth performance, plasma biochemistry indices, immune system, antioxidant status, and intestinal morphology of heat-stressed broiler chickens fed grape (*Vitis vinifera*) pomace. *Anim. Feed Sci. Technol.*, 259:114343.

<https://doi.org/10.1016/j.anifeedsci.2019.114343>.

17. Kikusato, M., G. Xue, A. Pastor, T.A. Niewold, and M. Toyomizu. 2021. Effects of plant-derived isoquinoline alkaloids on growth performance and intestinal function of broiler chickens under heat stress. *Poult. Sci.*, 100(2):957-963.

<https://doi.org/10.1016/j.psj.2020.11.050>.

18. Liu, L., C. Fu, M. Yan, H. Xie, S. Li, Q. Yu, S. He, and J. He. 2016. Resveratrol modulates intestinal morphology and HSP70/90, NF- $\kappa$ B and EGF expression in the jejunal mucosa of black-boned chickens on exposure to circular heat stress. *Food and Function*. 7(3):1329-1338.

<https://doi.org/10.1039/C5FO01338K>.

19. Liu, Y.L., K.N. Ding, X.L. Shen, H.X. Liu, Y.A. Zhang, Y.Q. Liu, Y.M. He, and L.P. Tang. 2022. Chronic heat stress promotes liver inflammation in broilers via enhancing NF- $\kappa$ B and NLRP3 signaling pathway. *BMC Vet. Res.*, 18(1):1-9.

<https://doi.org/10.1186/s12917-022-03388-0>.

20. Mahbuba, A. M., A. Ab Jabbar, A., and N. A. Mustafa. 2022. The effectiveness of some medicinal plants on body performance, hematological, ileum morphology and immune status of Japanese quail. *Iraqi Journal of Agricultural Sciences*, 53(4):724-731. <https://doi.org/10.36103/ijas.v53i4.1582>.

21. Makri, S., I. Kafantaris, D. Stagos, T. Chamokeridou, K. Petrotos, K. Gerasopoulos, A. Mpesios, N. Goutzourelas, S. Kokkas, P. Goulas and D. Komiotis. 2017. Novel feed including bioactive compounds from winery

wastes improved broilers' redox status in blood and tissues of vital organs. *Food Chem. Toxicol.*, 102:24-31.

<https://doi.org/10.1016/j.fct.2017.01.019>.

22. Martin, K.R. and C.L. Appel. 2009. Polyphenols as dietary supplements: A double-edged sword. *Nut. Diet. Suppl.*, 2:1-12.

<https://doi.org/10.2147/NDS.S6422>.

23. Mohammad, S. D., and S. Y. Al-Sardary. 2024. Natural antioxidants impact on carcass traits, meat quality and economic feasibility in broiler subjected to heat stress. *Anbar J. Agric. Sci.*, 22(1):129-146.

<https://doi.org/10.32649/ajas.2024.145551.1107>.

24. Muhammad, A.H. and A.S. Al-Hassani. 2022. Effect different levels of turmeric root powder to diet on some traits of broiler exposed to heat stress. *Iraqi Journal of Agricultural Sciences*, 53(4):950-957. <https://doi.org/10.36103/ijas.v53i4.1607>.

25. Nanto-Hara, F., H. Ohtsu, M. Yamazaki, T. Hirakawa, K. Sato and H. Murakami. 2021. Effects of dietary brown rice on the growth performance, systemic oxidative status, and splenic inflammatory responses of broiler chickens under chronic heat stress. *J. Poult. Sci.*, 58(3):154-162.

<https://doi.org/10.2141/jpsa.0200063>.

26. Panaite, T. D., M. Saracila, C. P. Papuc, C. N. Predescu and C. Soica. 2020. Influence of dietary supplementation of *Salix alba* bark on performance, oxidative stress parameters in liver and gut microflora of broilers. *Animals*. 10(6): 958.

<https://doi.org/10.3390/ani10060958>.

27. Pascariu, S. M., I. M. Pop, D. Simeanu, G. Pavel and C. Solcan. 2017. Effects of wine by-products on growth performance, complete blood count and total antioxidant status in broilers. *Brazilian J. Poult. Sci.*, 19:191-202.

<https://doi.org/10.3390/ani10060958>.

28. Pascual, A., M. Pauletto, A. Trocino, M. Birolo, M. Dacasto, M. Giantin, F. Bordignon, C. Ballarin, M. Bortoletti, G. Pillan and G. Xiccato. 2022. Effect of the dietary supplementation with extracts of chestnut wood and grape pomace on performance and jejunum response in female and male broiler chickens at different ages. *J. Anim. Sci., Biotechnol.*, 13(1):1-17.

<https://doi.org/10.1186/s40104-022-00736-w>.

29. Petracci, M., S. Mudalal, F. Soglia and C. Cavani. 2015. Meat quality in fast-growing broiler chickens. *World's Poult. Sci., J.*, 71(2):363-374.  
<https://doi.org/10.1017/S0043933915000367>.
30. Raish, M., A. Ahmad, M.A. Ansari, K. M. Alkharfy, A. Ahad, A. Khan and M.A.A. Hamidaddin. 2019. Beetroot juice alleviates isoproterenol-induced myocardial damage by reducing oxidative stress, inflammation, and apoptosis in rats. *3 Biotech.* 9(4):1-11.  
<https://doi.org/10.1007/s13205-019-1677-9>.
31. Reza, S. C., R. K. Mahmoud, M. Seifollah, S. C. Ali and A. Elham. 2012. Antioxidant and antibacterial activity of the extracts of *Echinophora platyloba* DC. *African J. Pharm. Pharmacol.*, 6(37):2692-2695.  
<https://www.doi.org/10.5897/AJPP12.931>.
32. Righi, F., R. Pitino, C.L. Manuelian, M. Simoni, A. Quarantelli, M. De Marchi and E. Tsipaloku. 2021. Plant feed additives as natural alternatives to the use of synthetic antioxidant vitamins on poultry performances, health, and oxidative status: A review of the literature in the last 20 years. *Antioxidants.* 10(5):659.  
<https://doi.org/10.3390/antiox10050659>.
33. Saracila, M., T. D. Panaite, C. Soica, C. Tabuc, M. Olteanu, C. Predescu, and R. D. Criste. 2019. Use of a hydroalcoholic extract of *Salix alba* bark powder in diets of broilers exposed to high heat stress. *South African J. Anim. Sci.*, 49(5):944-956.  
<https://doi.org/10.4314/sajas.v49i5.18>.
34. SAS, Institute. 2005. SAS User's Guide: Version 9.2 review ed. SAS Institute Inc, Cary, NC.  
<https://doi.org/10.1201/9780429341847>.
35. Selim, N. A., S. F. Youssef, A. F. Abdel-Salam and S. A. Nada. 2013. Evaluations of some natural antioxidant sources in broiler diets: 1-effect on growth, physiological and immunological performance of broiler chicks. *Int. J. Poult. Sci.*, 12:561-571.  
<https://doi.org/10.3923/ijps.2013.561.571>.
36. Sigwela, V., M. De Wit, A. du Toit, G. Osthoff and A. Hugo. 2021. Bioactive betalain extracts from cactus pear fruit pulp, beetroot tubers, and amaranth leaves. *Molecules.* 26(16):5012.  
<https://doi.org/10.3390/molecules26165012>.
37. Singh, K. M., S. Singh, I. Ganguly, R. K. Nachiappan, A. Ganguly, R. Venkataramanan and H. K. Narula. 2017. Association of heat stress protein 90 and 70 gene polymorphism with adaptability traits in Indian sheep (*Ovis aries*). *Cell Stress and Chaperones.* 22(5):675-684.  
<https://doi.org/10.1007/s12192-017-0770-4>.
38. Surai, P.F. 2020. Antioxidants in poultry nutrition and reproduction: an update. *Antioxidants.* 9(2):105.  
<https://doi.org/10.3390/antiox9020105>.
39. Tang, L. P., Y. L. Liu, J. X. Zhang, K. N. Ding, M. H. Lu and Y. M. He. 2022. Heat stress in broilers of liver injury Effects of heat stress on oxidative stress and autophagy in liver of broilers. *Poult. Sci.*, 10:(10)102085.  
<https://doi.org/10.1016/j.psj.2022.102085>.
40. Yang, J., J. Huang, C. Shen, W. Cheng, P. Yu, L. Wang, F. Tang, S. Guo, Q. Yang and J. Zhang. 2018. Resveratrol treatment in different time-attenuated neuronal apoptosis after oxygen and glucose deprivation/reoxygenation via enhancing the activation of Nrf-2 signaling pathway in vitro. *Cell Transplantation.* 27(12):1789-1797.  
<https://doi.org/10.1177/0963689718780930>.