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Effect of Adding Grape Pomace Powder, Grape Oil, and Nano-Powder to the Diet on Physiological Characteristics and Oxidation Indicators of Broiler (308)

Forqan Q Mohammed Ali ^{1*}, Fadhil Rasool Abbas Al-Khafaji ²

¹⁻² Department of Animal Production, Al-Qasim Green University, Iraq

* Corresponding Author: Forqan Q Mohammed Ali

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Abstract

This study aimed to evaluate the effect of adding grape pomace powder, grape seed oil, and grape seed nanopowder to broiler diets on physiological characteristics and oxidative stress markers. The experiment was conducted using 420 one-day-old Ross 308 broiler chicks, randomly assigned to seven treatments with three replicates per treatment. The treatments included the addition of different levels of grape pomace powder, grape seed oil, and grape seed nanopowder, as well as a control treatment. The results showed no significant effect of the feed additives on red and white blood cell counts, hemoglobin concentration, or packed cell volume. However, the treatments containing grape pomace showed a significant decrease in the hematocrit-to-lymphocyte ratio (H/L), indicating improved immune status and reduced physiological stress. The results also showed a significant improvement in biochemical markers, with increased concentrations of total protein, albumin, and globulin, and a significant decrease in serum glucose concentration. Regarding lipid metabolism, a significant decrease was observed in triglyceride, cholesterol, and low-density lipoprotein (LDL and VLDL) levels, along with a significant increase in high-density lipoprotein (HDL), particularly in the treatment that included the moderate addition of grape pomace powder. These effects are attributed to the richness of grape pomace in phenolic compounds and flavonoids with antioxidant activity, which contribute to improved metabolic balance and reduced oxidative stress. The study concludes that the addition of grape pomace and its derivatives, especially at moderate levels, can be used as an effective natural feed additive to improve the health and physiological status of broiler chickens and reduce oxidative stress, thereby enhancing livestock production efficiency.

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Keywords: grape pomace powder, grape oil, nano-powder, physiological characteristics, oxidation indicators, broiler

Introduction

Agriculture currently faces two challenges: ensuring global food security and reducing environmental impact by transforming food production byproducts, which constitute a significant proportion of environmental pollutants, into valuable animal feed (Springmann *et al.*, 2018; Al-Jaryan *et al.*, 2023; Al-Jebory *et al.*, 2023 a&b) ^[2, 3, 4]. Studies have focused on using plant compounds as a source of natural antioxidants instead of synthetic antioxidants, which can have harmful effects, including mutations and the accumulation of toxins in the body (Sen *et al.*, 2010) ^[36]. Grape pomace, a byproduct (seeds, skins, and pulp), is a rich source of flavonoids that act as potent antioxidants by neutralizing free radicals and reducing the effects of oxidative reactions (Gonzalez-Parama *et al.*, 2004). Dietary supplements containing these antioxidants increase vitamin E levels in muscle tissue, improving the oxidative stability of meat during storage (Carreras *et al.*, 2004). Grape seeds, a component of grape pomace, contain an average of 6–20% oil (Iuga *et al.*, 2020). Grape seed oil is used in various fields, including animal feed (Gornas *et al.*, 2019). It is characterized by its antioxidant, antibacterial, and antifungal properties, which help reduce oxidative stress. It is used to lower LDL and raise HDL levels in the blood and has an inhibitory effect on the growth of some pathogens

(Deptic *et al.*, Nerantzis; 2020, *et al.*, 2006). Grape seed oil also possesses high levels of natural antioxidants (Paydar *et al.*, 2001). Furthermore, nanotechnology has emerged as a tool to enhance the efficacy of these additives. Yeo & Kang (2008) ^[41] demonstrated that using nanoparticles to improve the bioavailability of grape compounds can increase their effectiveness in the body by improving intestinal stability and absorption, without requiring an increase in the feed dosage.

Materials

This experiment was conducted at Al-Anwar National Poultry Company in Babylon Governorate for 35 days from 12/12/2024 to 13/1/2025. The objective was to evaluate the effect of adding grape pomace powder, grape seed oil and nano powder to the feed on the performance of broiler chickens. 420 one-day-old, unsexed Ross 308 chicks were randomly distributed into seven treatments with three replicates of 20 chicks each. The experimental treatments were as follows:

- 1- (T1) Control treatment (no supplementation)
- 2- (T2) Addition of 0.5 kg/100 kg of feed to grape pomace powder
- 3- (T3) Addition of 1 kg/100 kg of feed to grape pomace powder
- 4- (T4) Addition of 0.25 L/100 kg of feed to grapeseed oil
- 5- (T5) Addition of 0.5 L/100 kg of feed to grapeseed oil
- 6- (T6) Addition of 1 g/100 kg of feed to nano-grape pomace powder
- 7- (T7) Addition of 3 g/100 kg of feed to nano-grape

pomace powder

The grape pomace was obtained from local markets and dried at a specific temperature. The temperature was maintained at 33-36°C, away from direct sunlight. The seeds were used to extract the oil through cold pressing, where they were compressed in a mechanical press operating solely on pressure without the use of heat. Grape pomace nanopowder was prepared in the laboratories of the Ministry of Science and Technology. Zinc nanoparticles were synthesized from grape pomace extract using the Elumalai method with modifications, following these steps:

10 ml of 20% grape pomace extract was placed in a 1000 ml flask containing distilled water. The flask was then placed on a stirrer equipped with a heating device that operated at varying temperatures. After one minute of stirring, 10 grams of zinc sulfate (ZnSO₄) were gradually added while continuing to stir until a homogeneous mixture was obtained. The solution was then filtered to remove impurities. Sodium hydroxide was added to neutralize the acidity of the mixture (Jefri *et al.*, 2019). The mixture was then transferred to a sealed glass container and placed in an oven at 200°C for two hours to complete the reaction. To achieve separation—that is, to separate the filtered solution from the sediment—the sediment was collected and dried in an oven at 70°C to remove any remaining moisture and obtain red powder nanoparticles (Munir and Begum, 2019) ^[33]. Finally, the nanoparticles were stored in a tightly sealed glass container in a dry, dark place until use.

Table 1: Nutritional Value of Grape Pomace Powder

Ingredients	Percentage %
protein	16.2
lipid	6.4
Ash	9.4
Moisture	20.6
CHO	47.4

Table 2: Estimation of fatty acids of grape seed oil

Name	Con%
palmitic	7.08
Oleic	20.98
Lenolic	60.08
Lenolenic	0.5
Stearic	2.36

Table 3: Shows the phenolic compounds in grape pomace powder

Name	Con (ppm)
Resveratrol	192.5
Ferulic acid	87.4
Callic acid	85.4
Kaempferol	80.9
Quercetine	117.9
Rutin	124.9

The chicks were fed a starter diet (23.04% protein and 3021.45 kcal/kg feed) from day one until the third week of age. Afterward, a grower diet (20.06% protein and 3194.92 kcal/kg feed) was used until the end of the fifth week. Feed and water were provided freely.

Studied traits

Physiological Characteristics

Cellular Blood Indices

Blood samples were collected using anticoagulant-treated tubes from six birds per treatment at 35 days of age via the wing vein. The tests were performed in the Physiology Laboratory (Department of Animal Production - College of Agriculture / Al-Qasim Green University). The following characteristics were calculated:

Red blood cell counts according to the method described by Natt and Herrik (1952); white blood cell counts according to the method described by Al-Daraji *et al.* (2008); percentage packed cell volume (PCV) according to the method described by Archer (1965); hemoglobin (Hb) according to the method described by Al-Daraji *et al.* (2008); heterophile and lymphocyte counts, and the heterophil/lymphocyte ratio, according to the staining method described by Shen and Patterson (1983). Counting according to Campbell's method (1988).

Biochemical tests of blood

At 35 days of age, blood samples were taken from the birds immediately after slaughter. The samples were collected in tubes without an anticoagulant. The serum was then separated from the blood using a centrifuge at 3000 rpm for 15 minutes. The serum was then stored in a freezer at -20°C until chemical analysis. The following biomarkers were estimated in the blood serum using ready-made test kits from various companies:

-Glucose concentration (mg/100 ml blood). It was measured using a complete assay kit from Takeda, Japan, based on the method of Henry *et al.* (1982).

Total cholesterol (mg/100 ml blood) was estimated using a complete assay kit from Biolabo, France, according to the method of Franey and Elias (1968) [22].

Triglycerides (mg/100 ml blood) were also measured. It was estimated using a ready-made assay kit from the French company Biolabo, based on Franey and Elias (1968) [22].

Low-density lipoprotein (LDL) and very-low-density lipoprotein (VLDL) levels were calculated according to the equation Friedewal *et al.* (1972) [23]: $VLDL = TG/5$.

High-density lipoprotein (HDL) levels were estimated using a ready-made assay kit from the French company Biolabo, based on Burstein *et al.* (1970) [13].

Total protein and albumin concentration (g/100 ml of blood) were measured using a ready-made assay kit from the Japanese company Takeda, based on the Biuret Method (Wotton, 1964) [40]. Globulin concentration in serum was measured according to the method described by Bishop *et al.* (2000) [11] and the equation: $\text{Globulin concentration g/100 ml} = \text{Total Protein Concentration} - \text{Albumin Concentration}$

Blood Oxidation Indices

Measurements in this study included the activity of several enzymes and biomarkers in blood serum, as follows:

-Aminotransferases (ALT and AST): These enzymes were measured using a ready-made kit from the French company Bio Mérieux, according to the method developed by Reitman and Frankel (1957) [34].

Glutathione Peroxidase (GSH-PX): Its activity was measured using a ready-made commercial ISA kit from the American company Sigma-Aldrich, based on the method described by Sedlak and Lindsay (1968) [35].

-Superoxide Dismutase (SOD): Its activity was measured using a ready-made assay kit, according to the method developed by Flohe and Gunzler. (1984) [21].

Malondaldehyde (MDA): Serum malondialdehyde concentration was measured using Roche commercial assay kits, according to the method described by Buege and Aust (1978) [12].

Catalase (CAT): The activity of this enzyme in serum was estimated by calculating the decrease in absorbance resulting from the consumption of the substrate (hydrogen peroxide), according to the method described by Aebi (1974) [1].

Results and Discussion

Hematological (Cellular) Characteristics

These are a set of parameters or measures used to assess a bird's general health and its physiological and immune response to the effects of diseases, stresses, or nutritional conditions. Cellular and biochemical blood profiles are a tangible indicator of the bird's adaptation to its surrounding environment (Ibrahim, 2013). Blood analyses also provide reliable information about the birds' health status. The values of the studied hematological parameters are an important variable in assessing a bird's ability to adapt and produce under any rearing conditions. Furthermore, they are a useful assessment alongside genetic, environmental, and management factors for the sustainability of traditional agriculture in arid and hot environments or environments experiencing extreme climatic variations between seasons. Therefore, they are effective indicators of internal balance and for evaluating nutritional, health, and adaptive aspects (Qusaibati, 2021). Consequently, the results in Table (5) showed inconsistent statistical significance for the cellular blood parameters. The addition of grape pomace, grape seed oil, and grape nanopowder had no significant effect on the red blood cell (RBC) and white blood cell (WBC) counts in the blood serum. The differences between the treatments were numerically insignificant, not reaching the level of statistical significance. This may indicate that the rearing conditions used in the research experiment were somewhat optimal, as they did not cause any adverse effects on the birds' health. Similarly, the feeding treatments used in the research experiment did not show any significant difference in the hemoglobin (Hb) index, the packed cell volume (PCV), or the lymphocyte count in the blood serum, as indicated in the same table. While the heterophil percentage index in blood serum varied with the experimental treatments, birds in treatment 2 achieved a significant reduction ($P \leq 0.05$) with the lowest mean of 28.33%, without significantly differing from treatments T4, T5, T6, and T7. Conversely, the control treatment recorded the highest mean of 35.33%, significantly outperforming all other treatments. Highly significant differences ($P \leq 0.01$) were also found among the experimental treatments in the heterophil-to-lymphocyte ratio

(H/L). The control treatment consistently outperformed all other treatments, achieving the highest mean of this index at 0.61%. The most significant reduction was observed in birds

in treatment 2, which recorded the lowest mean of 0.45%, without significantly differing from birds in treatment 4, which achieved a mean of 0.47%.

Table 5: Effect of grape pomace, grape seed oil and its nano powder on the physiological indicators of broiler chickens (mean \pm standard error)

Treatments	RBC	WBC	Hb	PCV	Lym	Hetro	H/L
T1	2.73 ± 0.51 a	21.40 ± 1.25 a	9.23 ± 0.15 a	27.70 ± 0.44 a	63.07 ± 0.33 a	35.33 ± 1.33 a	0.61 ± 0.02 a
T2	2.80 ± 0.21 a	20.93 ± 0.52 a	9.10 ± 0.55 a	27.30 ± 1.65 a	64.00 ± 2.08 a	28.33 ± 0.33 c	0.45 ± 0.02 d
T3	2.57 ± 0.12 a	20.90 ± 0.59 a	8.93 ± 0.09 a	26.80 ± 0.26 a	62.83 ± 0.33 a	30.50 ± 1.44 b	0.51 ± 0.02 bc
T4	2.67 ± 0.03 a	21.00 ± 1.15 a	9.10 ± 0.06 a	27.30 ± 0.17 a	63.57 ± 1.44 ab	30.17 ± 2.14 bc	0.47 ± 0.03 cd
T5	2.64 ± 0.21 a	21.33 ± 0.33 ab	9.17 ± 0.42 a	27.50 ± 1.25 a	63.33 ± 0.93 a	30.33 ± 1.45 bc	0.52 ± 0.02 bc
T6	2.55 ± 0.20 a	21.00 ± 1.15 a	9.37 ± 0.54 a	28.10 ± 1.61 a	63.82 ± 0.92 a	31.67 ± 1.76 bc	0.52 ± 0.02 bc
T7	2.55 ± 0.24 a	21.00 ± 0.58 a	8.97 ± 0.09 a	26.90 ± 0.26 a	63.05 ± 1.50 a	31.50 ± 1.32 bc	0.54 ± 0.02 b
Sig.	N.S.	N.S.	N.S.	N.S.	N.S.	*	**

Blood Biochemical Index

This is a set of measures or standards that represent the chemical components of blood serum, reflecting the bird's vital functions and health status. It also identifies the presence of certain diseases, assesses their risk, and determines the bird's adaptation to its surrounding conditions. These measures include:

Albumin, Globulin, Total Protein, and Glucose

The results in Table (6) indicate highly significant differences ($P \leq 0.01$) caused by the feed additives at different levels of grape pomace, grape seed oil, and grape nanopowder, which together constituted the experimental treatments, in albumin concentration. Birds in treatment 2 achieved a significant advantage over all other experimental treatments except T4 and T6, recording the highest average for this parameter at 2.35 g/dl, while birds in treatment T1 recorded the lowest average for this index at 1.41 g/dl. Our results are consistent with those of Cao *et al.* (2020) [14], who observed a significant increase in serum albumin concentration in broiler chickens fed diets containing grape pomace extracts.

This significant improvement continued in the blood biochemical parameters, particularly in serum globulin concentration. Treatments 2 and 6 achieved the highest mean concentrations, both at 2.02 g/dL, surpassing only treatment T1, which lagged significantly ($P \leq 0.05$) with the lowest mean concentration of 1.71 g/dL. This finding is similar to those of Cao *et al.* (2020) [14], who also observed a significant increase in serum globulin concentration in broiler chickens fed diets containing grape pomace extracts. The significant response continued in the total serum protein concentration index, as recorded by birds in the second treatment group, which showed a highly significant advantage over the experimental treatments with the highest mean of 5.04 g/dL. This was not significantly different from the sixth treatment group, which recorded a mean of 4.44 g/dL, while the lowest

mean was 3.15 g/dL in the control group. Our findings are consistent with those of Cao *et al.* (2020) [14], who found a significant increase in total serum protein concentration in broiler chickens fed diets containing grape pomace extracts. Similarly, Erinle *et al.* (2022) [20] demonstrated a significant increase in total plasma protein concentration when broiler chickens were fed a diet containing 2.5% grape pomace.

The results of our study are consistent with those of Cao *et al.* (2020) [14], who found a significant increase in total plasma protein concentration when broiler chickens were fed a diet containing 2.5% grape pomace. Regarding serum glucose concentration, highly significant differences ($P \leq 0.01$) were found, resulting from the second treatment causing a decrease in serum glucose levels to 185.64 mg/dl. The control treatment recorded the highest average for this indicator at 235.72 mg/dl, without significant differences from any other treatments except for the birds in the second and sixth treatments. This result was consistent with that of Al-Mihna and Muhammad, who found significant differences in glucose concentration caused by adding grape pomace powder to broiler diets at levels of 2.5% and 5.0%. Serum glucose concentration decreased with regard to feeding treatments under normal growth conditions, specifically at the 5% level after 21 and 42 days of rearing. However, this finding was not consistent with the study by Erinle *et al.* (2022) [20], who found a significant increase in glucose concentration when grape pomace was added to broiler diets. The decrease in serum glucose levels caused by the addition of grape pomace to feed may be due to its protective effect on pancreatic beta cells from oxidative damage through the antioxidant activity of the proanthocyanidins it contains. In this case, the polyphenols inhibit glucose uptake by the kidneys by disrupting the action of the sodium-glucose cotransporter, a protein transporter found in the renal tubules (El-Alfy *et al.*, 2005) [19]. Meanwhile, Ding *et al.* (2013) [17] attributed the decrease in serum glucose levels resulting from

grape pomace feeding to the role of polyphenols in inhibiting or reducing carbohydrate digestion by inhibiting the enzymes α -glucosidase and α -amylase, and by inhibiting glucose transporters in the intestine. Alternatively, the significant

response may be attributed to the findings of El-Alfy *et al.* (2005) [19], who found that polyphenols modulate serum glucose concentration by stimulating peripheral glucose uptake.

Table 6: Effect of grape pomace, grape seed oil, and grape seed nanopowder on broiler serum biochemical indicators (mean \pm standard error)

Treatments	Albumin g/dl(Globulin g/dl(Total protein g/dl(Glucose mg/dl(
T1	1.41 ± 0.06 d	1.71 ± 0.03 b	3.15 ± 0.15 e	235.72 ± 8.69 a
T2	2.35 ± 0.01 a	2.02 ± 0.03 a	5.04 ± 0.03 a	185.64 ± 2.97 b
T3	2.12 ± 0.08 b	1.91 ± 0.03 a	4.14 ± 0.06 b	210.50 ± 5.67 ab
T4	2.24 ± 0.03 ab	1.91 ± 0.03 a	4.04 ± 0.13 b	209.43 ± 3.15 ab
T5	2.08 ± 0.03 b	1.95 ± 0.02 a	3.77 ± 0.03 c	205.73 ± 3.11 ab
T6	2.27 ± 0.03 ab	2.02 ± 0.05 a	4.44 ± 0.03 ab	185.72 ± 3.15 b
T7	1.88 ± 0.03 c	1.90 ± 0.11 a	3.42 ± 0.005 d	203.00 ± 21.55 ab
Sig.	**	*	**	**

Triglycerides, VLDL, LDL, HDL, and Cholesterol

Table (7) data shows that the experimental treatments had a significant effect on the triglyceride index in the blood serum of broiler chickens. A significant decrease was observed in favor of the birds in treatment T2, which recorded the lowest average at 57.92 mg/dl. This result was significantly similar to all other experimental treatments except for the control treatment, which recorded the highest average at 68.97 mg/dl. This result is consistent with the findings of Al-Mihna and Muhammad, who observed significant differences in triglyceride concentration caused by adding grape pomace powder to the broiler diet at levels of 2.5% and 5.0%. Their findings showed a decrease in serum triglyceride concentration with respect to the feeding treatments under normal growth conditions, specifically at the 2.5% level after 21 and 42 days of rearing. The improvement in blood parameters may be attributed to the ability of grape pomace extract to enhance the activity of antioxidant enzymes and lipid peroxidases in the blood. This is due to its polyphenol content, which lowers triglyceride levels by inhibiting the action of cholesterol compounds that cross cell membranes (Kara *et al.*, 2016) [30]. Furthermore, Diego *et al.* (2003) [16] and Hanhineva *et al.* (2010) [26] confirmed that the polyphenols in grape pomace alter glucose, protein, amino acid, and lipid metabolism, thus affecting lipid metabolism in the bird's blood. This leads to a reduction in circulating free fatty acid levels, while simultaneously limiting fat absorption and accumulation in tissues.

The feed additives in the experimental treatments also had a significant effect on serum VLDL (very low-density lipoprotein) concentration. Significant differences ($P \leq 0.05$) were found in the second treatment, which significantly reduced the concentration of this protein, registering the lowest mean concentration of 11.69 mg/dl. Conversely, the control treatment showed the highest mean concentration of 14.41 mg/dl, without significantly differing from treatments T3, T4, and T7. The decrease in serum VLDL concentration caused by adding grape pomace to the diet can be attributed to the effect of the polyphenol content of grape pomace,

which reduces the size of VLDL particles while increasing lipoprotein lipase enzyme activity (Kehui *et al.*, 2016) [31]. Low-density lipoprotein (LDL) levels decreased significantly in birds in the second treatment group, achieving the lowest mean at 1.84 g/dl. The highest mean level was observed in the control group, reaching 2.94 g/dl, demonstrating a highly significant difference ($P \leq 0.01$) compared to all other treatments. This result is consistent with the findings of Al-Mihna and Muhammad, who found significant differences in LDL concentration caused by adding grape pomace powder to broiler diets at levels of 2.5% and 5.0%. Serum LDL concentration decreased with regard to feeding treatments under normal growth conditions, specifically at the 5% level after 21 and 42 days of rearing.

As for High Density Lipoprotein (LDL) in the blood serum of broiler chickens, the birds in the second treatment continued to be distinguished by the highest average of 3.88 mg/dl without differing significantly ($P \leq 0.05$) with the birds in the sixth treatment, which came with an average of 3.87 mg/dl, and neither of them differed significantly with the third and fourth treatments. Meanwhile, the lowest average of this indicator was recorded in the birds in the control treatment, which reached 2.74 mg/dl, and which did not differ significantly with the birds in the fifth treatment, which recorded an average of 3.11 mg/dl. This result is consistent with that of Al-Mihna and Muhammad, who observed significant differences in HDL concentration caused by adding grape pomace powder to broiler diets at levels of 2.5% and 5.0%. Its concentration in blood serum increased with feeding parameters under normal growth conditions, specifically at the 5% level after 21 and 42 days of rearing. The increase in HDL (good cholesterol) and the decrease in LDL (bad cholesterol) resulting from the addition of grape pomace, which contains flavonoids that enhance the activity of the paraoxonase enzyme in HDL, leads to an increased cellular response through an increase in the number of receptors. The rise in HDL levels is associated with the formation of genistein, a polyphenol compound, which in turn activates mitogen kinase (Zagayko *et al.*, 2013) [42]. This

was confirmed by Chamorro *et al.* (2013) ^[15], who explained it by the high binding capacity of the phenols in grape pomace to cholesterol, thus increasing its excretion with the seeds. Highly significant differences ($P \leq 0.01$) in serum cholesterol concentration were found in the second treatment, which recorded a significant reduction with the lowest mean concentration of 118.50 mg/dl. In contrast, the control group showed the highest mean concentration of 177.55 mg/dl, outperforming all other treatments in the experiment. This result was consistent with the result obtained by Al-Mihna and Muhammad, who obtained significant differences in cholesterol concentration caused by adding grape pomace powder to the broiler diet at levels of 2.5 and 5.0%. The cholesterol concentration in the blood serum decreased in relation to feeding treatments under normal growth conditions, specifically at the 5% level after 21 and 42 days of rearing. This result was also consistent with the study by Erinle *et al.* (2022) ^[20], who obtained a highly significant reduction in cholesterol concentration in the blood serum

when 2.5% of grape pomace was added to the broiler diet. The decrease in serum cholesterol concentration, as measured by the experimental parameters, is likely due to the effect of grape pomace, specifically its polyphenol compounds, including catechin, gallic acid, and epicatechin. These compounds lower cholesterol concentration by inhibiting the pancreatic enzyme cholesterol esterase, which breaks down cholesterol in the feed, releasing free cholesterol. Furthermore, phenolic compounds bind to bile acids, reducing cholesterol solubility and thus decreasing its absorption under heat stress conditions (Hajati *et al.*, 2015) ^[25].

The variation in blood parameters observed in our study could be attributed to several factors, in addition to nutritional influences. These include variations in the amount of polyphenols, which is related to the genetic makeup of the grape variety, the type used, the extraction and processing techniques, the level of polyphenol addition to the feed, and its impact on other feed components.

Table 7: Effect of grape pomace, grape seed oil and its nano powder on the biochemical indicators of broiler blood serum (mean \pm standard error)

Treatments	Triglyceride mg/dl(VLDL mg/dl(LDL g/dl(HDL mg/dl(Cholesterol)mg/dl(
T1	68.97 ± 0.81 a	14.41 ± 0.26 a	2.94 ± 0.06 a	2.74 ± 0.35 c	177.55 ± 3.15 a
T2	57.92 ± 1.1 b	11.69 ± 0.03 c	1.84 ± 0.12 c	3.88 ± 0.03 a	118.50 ± 3.16 c
T3	65.77 ± 3.52 ab	13.17 ± 0.61 abc	2.36 ± 0.08 b	3.53 ± 0.03 ab	140.65 ± 1.16 b
T4	58.35 ± 1.5 a	12.39 ± 0.32 bc	2.24 ± 0.03 b	3.55 ± 0.03 ab	142.24 ± 3.18 b
T5	62.46 ± 2.8 ab	12.27 ± 0.6 bc	2.38 ± 0.03 b	3.11 ± 0.03 bc	147.45 ± 1.8 b
T6	65.62 ± 2.82 ab	12.45 ± 0.55 b	2.30 ± 0.03 b	3.87 ± 0.32 a	140.35 ± 7.16 b
T7	65.49 ± 3.12 ab	13.42 ± 0.55 ab	2.37 ± 0.26 b	3.39 ± 0.26 b	148.27 ± 8.54 b
Sig.	*	*	**	*	**

Oxidation Index

The results in Table (8) showed highly significant differences ($P \leq 0.01$) between the increase and decrease in all studied oxidation indices. These differences resulted from the feed additives of grape pomace, grape seed oil, and grape nanopowder. The second treatment significantly reduced the concentration of Malondialdehyde (MDA) in the blood serum of its birds, with the lowest mean of 71.28 (ng/ml). This was followed by the third and fourth treatments, which recorded a mean of (76.18 and 78.63) (ng/ml), without any significant differences between them according to the study conditions. The control treatment had the highest concentration of this index, with a mean of 110.68 (ng/ml), significantly outperforming all the experimental treatments. This result is consistent with the findings of Bander (2017) ^[10]. It also aligns with Cao *et al.* (2020) ^[14].

The birds in the second treatment group continued to exhibit significant advantages, significantly reducing ($P \leq 0.01$) superoxide dismutase (SOD) levels, recording the lowest mean at 353.44 pg/ml. In contrast, the highest mean was found in the control group, achieving 451.44 pg/ml, thus significantly outperforming all other experimental treatments. This result is consistent with Cao *et al.* (2020) ^[14]. The experimental treatments also had a significant effect on

aspartate aminotransferase (AST) concentration. The highest mean AST concentration was found in the control group (no feed additives), reaching 62.84 U/I, surpassing all other experimental treatments. In contrast, the birds in the second treatment significantly reduced the detrimental effect of this enzyme ($P \leq 0.01$) compared to the other experimental treatments, registering the lowest mean AST concentration at 26.24 U/I. This finding is consistent with Cao *et al.*'s (2020) ^[14] and Erinle *et al.*'s (2022) ^[20] studies.

This was followed by findings regarding the alanine aminotransferase (ALT) enzyme, whose activity decreased with the feed supplementation in the second treatment, which achieved the lowest average level of 16.34 U/I. In contrast, the significant effect of feeding conditions was evident in the control group, which recorded the highest average level of 33.63 U/I, demonstrating a highly significant advantage ($P \leq 0.01$) over all other experimental treatments. This result is consistent with Cao *et al.* (2020) ^[14] and Erinle *et al.* (2022) ^[20].

The same pattern was observed with serum catalase (CAT) concentration, which decreased significantly in the birds of the second treatment group. This indicates that this group was superior to the other treatments in reducing harmful indicators that broiler chickens may encounter during their

rearing stages due to the feed additive. This is demonstrated by the results in the same table, which showed the lowest average concentration of CAT at 21.78 pg/ml. In contrast, the birds in the control group, which had the highest average concentration of this indicator at 40.89 pg/ml, showed a highly significant advantage ($P \leq 0.01$) over the other treatments. This result is consistent with Cao *et al.* (2020) [14]. The same pattern was observed for serum glutathione (GSH) concentration. A significant increase was found in the control group, whose birds exhibited the highest mean GSH level at 2.63 ng/ml, demonstrating their superior response to the experimental conditions compared to all other treatments. In contrast, the second treatment group showed a highly significant improvement ($P \leq 0.01$) in this indicator, with the lowest mean level at 1.83 ng/ml, indicating their significant ability to induce a substantial reduction in GSH concentration. Our findings are consistent with Cao *et al.* (2020) [14].

These results were consistent with several studies that obtained significant improvements in serum oxidation markers when broiler chicken diets were treated with grape pomace powder. The significant improvement was directly proportional to the increase in the addition level from 1% to

2%, including studies by Gadde *et al.* (2017), Mehdi *et al.* (2018), and Shang *et al.* (2020) [24, 32, 37].

The decrease in the studied oxidation markers due to the addition of grape pomace may be attributed to its phenolic content, which possesses a high capacity to inhibit lipid oxidation by suppressing free radical activity and enhancing the efficiency of the first stage of oxidation. This is reflected in the slower formation of hydroxyloxides, including peroxides, thus reducing oxidation markers, most notably MDA (Wang *et al.*, 2008; Kanbur *et al.*, 2011) [38, 29]. It is well-established that plant-derived antioxidants can significantly reduce damage caused by oxidative compounds by neutralizing free radicals before they can attack body cells, thus preventing damage to proteins, enzymes, and lipids. Grape pomace dietary byproducts may increase blood levels of phenolic compounds by raising the concentration of alpha and beta tocopherols (vitamin E) in blood plasma, thereby improving the antioxidant status of broiler chickens (Irene *et al.*, 2019) [28]. The improvement in oxidative stress indicators resulting from dietary treatment with grape pomace and its oil may be due to its potent antioxidant properties, as described by Wang *et al.* (2010) [39]. They indicated that the phenols present in grape pomace have an antioxidant activity twenty times greater than that of vitamin E and fifty times greater than that of vitamin C, making it a highly significant antioxidant (Al-Jumaili *et al.*, 2025; Al-Saeedi *et al.*, 2023; Al-Saeedi *et al.*, 2025) [5, 7, 8].

Table 8. Effect of grape pomace, grape seed oil, and grape pomace nanopowder on the oxidative stress indicators of broiler chickens (mean \pm standard error)

Treatments	MDA (ng/ml)	SOD (pg/ml)	AST (U/l)	ALT (U/l)	CAT (pg/ml)	GSH-px (ng/ml)
T1	110.68 ± 4.61 a	451.44 ± 5.71 a	62.84 ± 0.29 a	33.63 ± 1.73 a	40.89 ± 1.73 a	2.63 ± 0.03 a
T2	71.28 ± 0.57 d	353.44 ± 3.17 c	26.24 ± 0.29 f	16.34 ± 0.29 d	21.78 ± 0.57 e	1.83 ± 0.03 d
T3	76.18 ± 2.32 d	405.89 ± 2.86 b	31.19 ± 0.29 e	21.29 ± 0.29 c	26.24 ± 0.29 cd	2.33 ± 0.03 b
T4	78.63 ± 0.87 d	401.47 ± 2.86 b	41.09 ± 0.29 c	29.21 ± 0.29 b	29.21 ± 2.6 bc	2.03 ± 0.03 c
T5	90.98 ± 0.57 c	401.94 ± 2.86 b	33.17 ± 0.29 d	23.27 ± 0.29 c	31.14 ± 0.29 bc	2.23 ± 0.03 b
T6	93.98 ± 2.03 c	401.94 ± 2.86 b	45.44 ± 0.002 b	23.06 ± 0.29 c	34.59 ± 3.17 b	2.21 ± 0.03 b
T7	103.78 ± 5.48 b	402.44 ± 2.86 b	46.04 ± 1.45 b	23.49 ± 1.16 c	35.99 ± 0.29 b	2.29 ± 0.06 b
Sig.	**	**	**	**	**	**

Conclusion

The current study concludes that grape pomace powder, its oil, and its nano-powder improved the physiological performance and oxidative parameters of broiler chickens under heat stress.

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