Research Article

Evaluation of the Productive and Physiological Effects of Grape Pomace, Its Nano-Form, and Its Seed Oil in Broiler Chickens Exposed to Cyclic Heat Stress

Forqan Q. Mohammed Ali, Fadhil Rasool Abbas Al-Khafaji*

Department of Animal production, Al-Qasim Green University, Iraq.

Abstract | The study aimed to evaluate the effects of dietary supplementation with grape pomace powder (GPP), grape seed oil (GSO), and nano-grape pomace powder on productive performance and selected blood cellular parameters of broiler chickens reared under cyclic heat stress conditions. A total of 420 Ross 308 broiler chicks were used and randomly distributed into seven treatments with three replicates each over a 35-day rearing period. The results demonstrated that live body weight and total weight gain were significantly ($P < 0.05$) improved in the supplemented groups, particularly in the treatment receiving grape pomace powder at the lower inclusion level, with similar improvements observed in the nano-grape pomace powder treatment. Some treatments showed a significant increase ($P < 0.05$) in feed intake and feed conversion ratio. Hematological parameters revealed a significant improvement ($P < 0.05$) in hemoglobin concentration and a reduction in the heterophil-to-lymphocyte (H/L) ratio, indicating enhanced health status and reduced heat stress effects in the supplemented groups. The study concludes that grape pomace powder, grape seed oil, and nano-grape pomace powder can serve as effective natural feed additives to improve productive performance and certain physiological traits in broiler chickens exposed to cyclic heat stress, particularly when used at appropriate inclusion levels, with nano-form supplementation showing promising potential.

Keywords | Grape pomace, Grape seed oil, Nanotechnology, Heat stress, Broiler, Chickens

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***Correspondence** | Fadhil Rasool Abbas Al-Khafaji, Department of Animal production, Al-Qasim Green University, Iraq; **Email:** dr-fadhilkafaji@uoqasim.edu.iq
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INTRODUCTION

Environmental factors are one of the most important factors affecting the health status and productivity of broilers, resulting in decreased productive capacity as well as changes in their blood cellular status. In this regard, from the other side, artificial antioxidants have been employed and because of their accumulation in body and consumer effect concerns there is a tendency to use natural ones to avoid these issues (Sen *et al*., 2010; Al-Saeedi *et al*., 2022, 2023).

One of these alternatives is grape pomace, which is made up of the waste material from grape juice (skin, pulp and seed) (Al-Jebory and Ibrahim, 2021). It is a source of phenolic compounds, natural antioxidants and can be served as feed additives for enhancing bird health and minimizing heat stress (Eder *et al*., 2025). A great number of studies specify that adding these by-products in the diet of chicken can be followed by the improvement in final live body weight, feed conversion ratio (FCR) and intestinal immune system as well as by increase in antioxidant activity. In this regard, Brenes *et al*. (2008) reported that grape pulp in poultry diets resulted in a lowered oxidative stress indicator and improvement of the intestinal structure, which led to improving nutrient absorption. Viveros *et al.* (2011) showed that phenolic compounds from grapes modulate intestinal microflora and elevate the level of beneficial bacteria such as *Lactobacillus* spp. which in turn helps for better absorption by intestines. Grape seeds are part of grape pomace and contain an average of 6–20% oil. Grape seed oil is used in various fields, whether for personal use in the daily diet or in the manufacture of cosmetics and pharmaceuticals, in addition to its use in animal diets as a nutritional additive (Gornas *et al.*, 2019). Grape seed oil is characterized by antioxidant and antibacterial and antifungal properties Because of its elevated levels of unsaturated fatty acids, notably linoleic acid (60%) and oleic acid (20%). These properties allow a reduction in stress.

The use of nano-compounds as feed additives increases bioavailability, improves absorption, and enhances the general health of birds (Kazemi, 2025). Nevertheless, they might have the potential toxicity risks associated based on material type, size, and dose employed; flounder of these factors' dependent can cause damage in vital organs like liver and kidney (Brohi *et al.*, 2017). A study by Mahmoud *et al.* (2025) reported that dietary supplementation of broiler chickens with zinc oxide nanoparticles under normal and heat stress conditions led to significant improvements in productive performance traits, such as weekly body weight gain, average feed intake, and feed conversion ratio, as well as enhanced hematological parameters. In heat-stressed broiler chickens, supplementation with nano-selenium reduced both cholesterol and glucose concentrations (Hammouda *et al.*, 2023).

This study aimed to investigate the effects of incorporating grape pomace powder, grape seed oil, and nano-grape pomace powder into broiler diets on productive performance and cellular blood parameters in broiler chickens exposed to heat stress during a 35-day rearing period, using modern scientific indicators to support the application of these additives as effective and natural alternatives in poultry nutrition.

MATERIALS AND METHODS

Study design and grouping

This trial was conducted at Al-Anwar National Poultry Company in Babil Governorate over a 35-day period, from December 12, 2024, to January 13, 2025. The study was carried out according to international ethical standards of animal handling. The study aimed to evaluate the effects of incorporating grape pomace powder, grape seed oil, and nano-grape pomace powder into broiler diets on productive performance. A total of 420 one-day-old unsexed Ross 308 broiler chicks were used and randomly allocated to seven treatments, each consisting of three replicates with 20 chicks per experimental unit. The treatments were as follows: T1, control without supplementation; T2, grape pomace powder at 0.5 kg/100 kg feed; T3, grape pomace powder at 1 kg/100 kg feed; T4, grape seed oil at 0.25 L/100 kg feed; T5, grape seed oil at 0.5 L/100 kg feed; T6, nano-grape pomace powder at 1 g/100 kg feed; and T7, nano-grape pomace powder at 3 g/100 kg feed. All chicks were exposed to cyclic high ambient temperatures ranging from 29°C to 36°C and then returning to 29°C, as detailed in the Table 1.

Table 1: Average hourly temperature (°C) according to age.

Age (week)	6:00 AM	12:00 PM	6:00 PM	12:00 AM
1	34.01	34.65	35.22	34.20
2	30.92	35.30	35.50	30.32
3	29.80	36.60	36.80	29.20
4	29.42	36.60	36.20	29.30
5	29.60	36.80	36.40	29.10

Treatments

Grape pomace was obtained from local markets and dried at a temperature of 33–36 °C away from direct sunlight. The seeds were used to extract the oil by cold pressing, where the seeds were pressed in a mechanical press operating by pressure only, without using any heat. The nano-powder of grape pomace was synthesized in the lab of the Ministry of Science and Technology, where zinc nanoparticles were prepared using grape pomace extract according to the method of Elumalai with modifications, through the following steps:

Ten milliliters of grape pomace extract at a concentration of 20% were placed in a 1000 mL flask containing distilled water, then the flask was placed on a stirring device equipped with heating at different temperatures. After one minute from the start of stirring, 10 g of zinc sulfate ($ZnSO_4$) were added gradually with continuous stirring until obtaining a homogeneous mixture. Then, the solution was filtered to remove impurities, and sodium hydroxide was added to neutralize the acidity of the mixture. This was followed by transferring the mixture to a tightly sealed glass container and placing it in an oven at 200 °C for two hours to complete the reaction. To achieve separation of the filtered solution from the precipitates, the precipitates were collected and dried in an oven at 70 °C to remove residual moisture, yielding red-colored nanoparticle powder (Munir and Begum, 2019). A thin layer of the nanoparticles was placed onto a clean glass slide and allowed to dry at room temperature for 5 min prior to analysis. Surface morphology and particle size were examined using Atomic

Force Microscopy (AFM). Elemental composition and zinc confirmation were analyzed using energy-dispersive X-ray spectroscopy (EDX/EDXS) coupled with high-resolution electron microscopy in transmission mode. The nanoparticle characteristics were further determined by zeta potential analysis over a range of −160 to +160 mV. Finally, the obtained nanopowder was stored in a tightly sealed glass container in a dry, dark place until further use. The nutritional value, fatty acid profile and phenolic contents of grape pomace powder and grape seed oil were presented in Table 2.

Table 2: Nutritional value, fatty acids and phenolic contents of grape pomace powder and grape seed oil.

Item	Percentage
Nutritional value of grape pomace powder	
Protein	16.2
Lipid	6.4
Ash	9.4
Moisture	20.6
Carbohydrate	47.4
Fatty acid composition of grape seed oil	
Palmitic acid	7.08
Oleic acid	20.98
Linoleic acid	60.08
Linolenic acid	0.50
Stearic acid	2.36
Phenolic acids of grape pomace powder*	
Resveratrol	192.5
Ferulic acid	87.4
Gallic acid	85.4
Kaempferol	80.9
Quercetin	117.9
Rutin	124.9

* values are presented in ppm.

Dietary treatment

The chicks were fed a starter diet containing 23.04% crude protein and 3021.45 kcal/kg metabolizable energy from day 1 to 21 days of age. Thereafter, a grower diet containing 20.06% crude protein and 3194.92 kcal/kg metabolizable energy was provided until the end of the fifth week. Feed and water were supplied *ad libitum* throughout the experimental period. The composition of diet is described in Table 3.

Studied traits

Live body weight of birds and weekly body weight gain (g/bird)

The mean live body weight for each replicate was calculated at the end of each week for weeks 1–5 by carefully weighing all birds of the replicate using a precise electronic scale. The mean live body weight per bird was calculated according to the equation referred to by (Al-Fayyad, 1989).

$$\text{Mean live body weight (g/bird)} = \frac{\text{Total live weights of birds in the replicate at the end of the week (g)}}{\text{Number of birds in the replicate at the end of the week}}$$

As for the weekly body weight gain (g/bird), it was calculated as follows (Al-Fayyad, 1989):

Mean live body weight at the end of the week (g) – mean live body weight at the beginning of the week (g)

Table 3: Percentage of feed ingredients and chemical composition of the experimental diets.

Feed ingredients	Starter diet % (1-21 days)	Grower diet % (22-35 days)
Maize	30.0	40.0
Wheat	28.25	24.0
Soybean meal (48% protein)	31.75	24.8
Protein concentrate	5.0	5.0
Sunflower oil	2.9	4.4
Limestone	0.9	0.6
Dicalcium phosphate (DCP)	0.7	0.9
Vitamin and mineral premix	0.2	0.2
Table salt	0.3	0.1
Total	100	100
Chemical composition		
Crude protein (%)	23.04	20.06
Calculated metabolizable energy (kcal/kg diet)	3021.45	3194.92
Lysine (%)	1.27	1.07
Methionine (%)	0.41	0.38
Cystine (%)	0.35	0.30
Methionine + cystine (%)	0.82	0.78
Available phosphorus (%)	0.41	0.43
Energy:protein ratio (C/P)	131.14	159.26

Note: The chemical analysis of the diet was calculated according to (NRC, 1994) as follow: Protein concentrate Brocon-5 Special W: of Chinese origin; each kg contains (40% crude protein, 3.5% fat, 1% fiber, 6% calcium, 3% available phosphorus, 3.25% lysine, 3.90% methionine + cystine, 2.2% sodium, 2100 kcal/kg metabolizable energy, 20000 IU vitamin A, 40000 IU vitamin D3, 500 mg vitamin E, 30 mg vitamin K3, 15 mg vitamin B1 + B2, 150 mg B3, 20 mg B6, 300 mg B12, 10 mg folic acid, 100 µg biotin, 1 mg iron, 100 mg copper, 1.2 mg manganese, 800 mg zinc, 15 mg iodine, 2 mg selenium, 6 mg cobalt, 900 mg antioxidant (BHT).

Feed consumed (g/bird)

Feed intake was measured weekly on birds in each replicate for week one to five by weighing the provided feed at the beginning and subtracting remaining feed left at the end of each week. In case of mortality within the replicate, feed consumption was calculated according to the following equation (Al-Zubaidi, 1986).

$$\text{Daily mean feed intake } (g/bird) = \frac{\text{Amount of feed consumed during one week}}{(\text{Number of chicks at the end of the week} \times 7) + \text{number of days during which the dead birds consumed feed}}$$

Feed conversion ratio (g feed/g weight gain)

Feed conversion efficiency was calculated according to the equation referred to by (Al-Zubaidi, 1986).

$$\text{Daily mean feed intake } (g/bird) = \frac{\text{Amount of feed consumed during one week}}{(\text{Number of chicks at the end of the week} \times 7) + \text{number of days during which the dead birds consumed feed}}$$

Hematology

Blood samples were collected at 35 days of age from the brachial (wing) vein into anticoagulant-containing tubes, with six birds sampled per treatment group. Red blood cell (RBC) counts were determined according to the method of Natt and Herrick (1952), as described by Salman *et al.* (2025). White blood cell (WBC) counts were performed following the procedure of Al-Daraji *et al.* (2008). Packed cell volume (PCV) was measured according to Archer (1965), as cited by Alsudani *et al.* (2025), and hemoglobin (Hb) concentration was determined according to Al-Daraji *et al.* (2008). Differential leukocyte counts, including heterophils and lymphocytes, were assessed using the staining technique described by Shen and Patterson (1983), and the heterophil-to-lymphocyte (H/L) ratio was calculated according to Campbell (1988).

Statistical analysis

A completely randomized design (CRD) was used to evaluate the effects of different treatments on all measured traits (Duncan, 1955). Data were analyzed using one-way analysis of variance (ANOVA), and differences were considered statistically significant at $P < 0.05$.

RESULTS AND DISCUSSION

Live body weight (g)

The results of the statistical analysis shown in Table 4 indicate that treating the experimental birds with grape pomace, its seed oil, and its nano-powder from the first day of age did not significantly affect the mean live body weight, as numerical differences were found among treatments but did not reach the level of significance. However, this pattern changed at 7 days of age, where highly significant differences were observed in treatments T2 and T6 ($P \leq 0.01$) for this trait, with no significant difference between them. The lowest mean value was recorded in the control treatment, which did not differ significantly from treatments T3, T4, T5, and T7.

The same pattern continued in the second week of age, as highly significant differences ($P \leq 0.01$) were found for both treatments T2 and T6, with their superiority over all other experimental treatments, without significant differences between them. They recorded the highest mean for this trait compared with the control treatment T1, which recorded the lowest mean without differing significantly from treatments T3, T4, T5, and T7.

The highly significant superiority persisted into the third week of age, where treatment T2 recorded the highest mean value for this trait, exceeding all other experimental treatments except T6 and T7, which recorded mean values of 1000.67 and 1006.00 g, respectively, without significant differences among them. In contrast, the lowest mean value (965.00 g) was observed in the control treatment.

The highly significant superiority of treatment T2 was repeated by achieving the highest mean live body weight in the fourth week of age, with a mean of 1577.67 g, thus surpassing all other experimental treatments except treatments T6 and T7. Conversely, the control treatment

Table 4: Effect of grape pomace, grape seed oil, and its nano-powder on the weekly body weight of broiler chickens from 1 to 35 days of age under heat stress conditions.

Treatments	Age					
	One day	7 days	14 days	21 days	28 days	35 days
T1	41.67±0.33	197.00±5.19^{c}	505.33±5.33^{c}	965.00±3.21^{d}	1469.33±9.68^{c}	2003.33±3.33^{d}
T2	42.00±0.0	212.00±1.15^{a}	543.00±3.51^{a}	1011.67±1.2^{a}	1577.67±13.62^{a}	2266.67±17.63^{a}
T3	41.67±0.33	199.33±2.73^{bc}	518.67±7.22^{bc}	979.67±4.98^{c}	1499.67±16.04^{bc}	2224.0±15.14^{ab}
T4	42.00±0.0	202.00±1.15^{bc}	518.33±7.68^{bc}	990.33±2.03^{bc}	1512.00±21.00^{bc}	2260.67±27.28^{a}
T5	41.67±0.33	198.67±0.33^{bc}	518.33±5.78^{bc}	987.33±5.81^{bc}	1492.00±22.81^{bc}	2143.33±33.83^{c}
T6	41.67±0.33	206.33±2.03^{ab}	536.33±8.45^{ab}	1000.67±12.33^{ab}	1528.33±27.61^{abc}	2196.67±27.28^{b}
T7	42.00±0.0	200.33±1.86^{bc}	519.33±4.06^{bc}	1006.00±1.73^{ab}	1537.00±22.72^{ab}	2155.00±27.54^{c}
Significance level	N.S.	**	**	**	**	**

Means within the same column with different superscript letters differ significantly. N.S. = Not significant. ** = $P \leq 0.01$

T1 gave the lowest mean live body weight without differing significantly from most of the other experimental treatments. This highly significant superiority was reflected positively in the fifth week of age in treatments T2 and T4, which continued their significant superiority over all experimental treatments. In contrast, the lowest mean was recorded in the control treatment.

The significant improvement achieved by the experimental chicks fed a diet containing ordinary and nano grape pomace powder and its dried seed oil may be due to the direct role of grape pomace powder in limiting or reducing the harmful effect of high temperatures by reducing energy consumption in metabolic processes, including panting, which contributes to preventing dehydration in birds, or the feeding strategy followed in this study under conditions of heat stress may have an important role in mitigating the negative effects of high temperatures (Ahmad *et al*., 2022). Alternatively, the significant increase could be attributed to the role of grape pomace powder in stimulating the bird's digestive system by increasing secretion of digestive enzyme or promoting utilization of digested product. This may be related to the enhancement of liver performance and its role in improving bird's immune status and suppressing growth of pathogenic microbes especially gut microbes that positively reflected by increasing productive performance traits. Moreover, the polyphenol content acts as an antioxidant and provides an inhibitory effect against heat stress conditions by directly lowering corticosterone secretion (Mode *et al*., 2009). It is also possible that the significant improvement specifically observed in the second treatment is due to grape pomace containing fat-soluble substances, essential fatty acids, and growth-promoting agents (Table 2) (Aljumaili *et al*., 2023).

The significant effect of the second treatment, it did not show significant differences with the six treatment for live body weight of broiler under heat stress up to 14 day old, and from the sixth and seventh treatments up to 28 days of age, and its significant similarity at the final weight (35 days) with the fourth treatment, can be attributed to the effect of the feeding diet that contained natural and nano grape pomace powder and its direct role in limiting or reducing the adverse impact of high temperatures, due to its ability to regulate body temperature, which is considered a closely related indicator of metabolic rate and is directly associated with acclimation. This indicates that the appropriate feeding conditions that were accompanied by the rearing conditions had the ability to deal with heat stress and reduce the levels of its effect, which was reflected in improved bird performance (Oni *et al*., 2025). The significant increase in birds fed diets containing grape pomace may also be due to compensatory growth of birds at the end of the experimental period, or due to the presence of phenols that act as antioxidants, which support birds exposed to an elevated ambient temperature (Al-Jiashi, 2018).

The closeness between the second and sixth treatments may be due to the effect of the nano grape pomace powder, which is characterized by being ultra-fine particles that greatly increase the reduction of the harmful effect of heat stress and reduce its severity first, and at the same time increase the specific effect of the active compounds contained in grape by-products through their action as antioxidants, thus improving metabolic processes, which is reflected positively in improving productive growth parameters (Mahmoud *et al*., 2025). In addition, the reason may be attributed to causing a high activation of vitamin E, which is one of the main antioxidants in the bird's body and is responsible for protecting phospholipids in cell membranes from oxidation by free radicals resulting from normal metabolic processes, and thus protects bird tissues from damage (Yausheva *et al*., 2018).

The weak performance shown by the birds of the control treatment can be explained by their being affected by the biological stress resulting from high temperatures during the period of conducting the experiment, which may cause a reduction in muscle development and building and immunity in general, due to its effect on the functions of the biological barrier of the digestive tract wall, which results in weak blood flow toward the intestine and other body tissues, leading to a clear decrease in oxygen concentration (Swain *et al*., 2021). Fathi *et al*. (2024) also clarified that adding dietary supplements in nano-powder form in the broiler diet may enhance physiological and productive performance and the health status of the bird, and increase the absorption of feed components.

Weekly body weight gain (g)

The results revealed significant ($P \leq 0.05$) differences in weekly body weight gain among the experimental treatments (Table 5). In the first week, birds in treatment T2 exhibited the highest mean gain (170.00 g), which did not differ significantly from T6. The lowest gain was observed in the control group (155.33 g), which was not significantly different from T3, T4, T5, and T7. In the second week, T2 maintained its significant superiority ($P \leq 0.05$) with the highest gain (331.00 g), surpassing all treatments except the control, which recorded the lowest gain (308.33 g). By the third week, birds in T7 showed the highest mean gain (486.67 g), significantly exceeding the control group, which had the lowest gain (459.67 g).

In the fourth week of age, the experimental treatments did not show a significant response, as the differences were numerical without reaching the level of significance. However, the situation differed in the fifth week of age, in which birds of treatment T2 recorded the highest mean of 689.00 g, thus surpassing all other experimental treatments. In contrast, birds of treatment T3 recorded the lowest mean of 524.33 g, without differing significantly from most experimental treatments.

For cumulative weight gain, birds of treatment T2 showed significant ($P \leq 0.05$) superiority over all experimental treatments with the highest mean of 2257.67 g, whereas the control treatment recorded the lowest mean of 1961.67 g, without differing significantly from birds of treatments T3, T4, and T5.

The significant increase in mean weight gain resulting from feeding grape pomace may be attributed to its content of secondary metabolites such as (resveratrol, rutin, quercetin, kaempferol, gallic acid, and ferulic acid), and its oil components (palmitic, linoleic, linolenic, and oleic) (Table 2). These are medically active compounds that directly influence body immunity because they act as antimicrobial agents against pathogens and lead to the sterilization of the digestive system. This, in turn, is reflected in an improved weight gain rate for broiler chickens. Furthermore, these compounds are effective in enhancing the absorption rate of major mineral ions in the diet, which contributes positively to weight gain (Ahmed *et al*., 2015). The benefits may also stem from the natural and nano components of grape pomace powder, particularly flavonoids and polyphenols, which are nutritionally and medically important for broilers. These compounds provide crucial support under non-normal stress conditions, such as high temperatures during the rearing period (Jahejo *et al*., 2019). Additionally, the observed advantages can be attributed to the feeding regimens' ability to mitigate heat stress, thereby improving both productivity and overall health outcomes in the birds.

The reason for the significant increase may also be explained by the role of grape plant extract and grape seed oil in increasing the utilization and better assimilation of the nutrients in the diet components used in the experiment, due to the seeds containing important active substances; therefore, these effects were reflected in the birds' growth rate and production parameters (Mohammed *et al*., 2013).

In general, heat stress causes accumulation of free radicals, leading to oxidative stress within the bird's body by causing gradual, unnoticed breakdown of body cells and tissues, which later results in deterioration of productive traits due to deterioration of its health. However, adding grape pomace as a powder and oil, with different compositions, resulted in a reduction in the stressful effect of high temperatures (Adekunle *et al*., 2017).

The significant response in body weight and weekly weight gain due to treatment with grape by-products may also be attributed to the occurrence of proliferation in muscle cell progenitors responsible for producing high levels of cells known as muscle stem cells, which are considered primary materials for skeletal muscle cells in the embryonic life of birds (Willemsen *et al*., 2010).

Weekly feed intake (g)

Table 6 shows that mean weekly feed intake was significantly ($P \leq 0.05$) influenced by the experimental treatments throughout the rearing period, except during the first and second weeks. In the third week, significant differences ($P \leq 0.05$) emerged, with birds in treatment T1 recording the highest mean feed intake of 651.67 g, although this did not differ significantly from treatment T2.

In the fourth week, significant differences were also observed. Birds in the control treatment T1 consumed the highest mean weekly feed intake of 881.00 g, not significantly different from T2, while birds in treatment T7 consumed the lowest amount, with a mean of 789.67 g.

Table 5: Effect of grape pomace, grape seed oil, and its nano-powder on the weekly weight gain of broiler chickens from 1 to 35 days of age under heat stress conditions (mean ± standard error).

Treatments	Age (Week)					Cumulative weight gain
	1	2	3	4	5	
T1	155.33 ± 4.91^{c}	308.33 ± 2.73^{b}	459.67 ± 8.09^{b}	504.33 ± 6.62	534.00 ± 8.71^{c}	1961.67 ± 3.18^{d}
T2	170.00 ± 1.15^{a}	331.00 ± 2.51^{a}	468.67 ± 2.72^{ab}	566.00 ± 12.5	689.00 ± 14.64^{a}	2257.67 ± 22.81^{a}
T3	157.67 ± 2.85^{bc}	319.33 ± 4.67^{ab}	461.00 ± 7.09^{b}	520.00 ± 12.17	524.33 ± 2.84^{c}	1982.33 ± 15.45^{d}
T4	160.00 ± 1.15^{bc}	316.33 ± 6.96^{ab}	472.00 ± 9.64^{ab}	521.67 ± 22.73	554.67 ± 8.51^{c}	2024.67 ± 27.28^{cd}
T5	157.00 ± 0.0^{bc}	319.67 ± 5.81^{ab}	469.00 ± 6.08^{ab}	505.33 ± 20.28	550.67 ± 17.9^{c}	2001.67 ± 33.72^{d}
T6	164.67 ± 2.02^{ab}	330.00 ± 6.43^{a}	464.33 ± 6.77^{ab}	527.67 ± 23.78	608.33 ± 17.67^{b}	$2095..00\pm27.18^{bc}$
T7	158.33 ± 1.85^{bc}	319.00 ± 2.52^{ab}	486.67 ± 5.24^{a}	531.00 ± 24.11	618.00 ± 5.29^{b}	2113.00 ± 27.54^{b}
Significance level	*	*	*	N.S.	*	*

Means within the same column with different superscript letters differ significantly. N.S. = Not significant. * = $P \leq 0.05$.

Table 6: Effect of grape pomace, grape seed oil, and its nanopowder on weekly and total feed consumption of broiler chickens from 1 to 35 days of age under heat stress conditions (mean ± standard error).

Treatments	Age (Week)					Total feed consumption
	1	2	3	4	5	
T1	126.00±1.00	372.00±1.53	651.57±4.41[a]	881.00±4.93[a]	1001.67±6.01[d]	3.032.33±4.09[b]
T2	130.67 ±3.48	365.67±11.84	633.33±8.57[a]	861.67±6.01[a]	1176.67±14.53[a]	3168.00±30.92[a]
T3	126.67±2.03	370.67±11.05	627.00±5.77[b]	838.00±29.19[ab]	1032.67±25.22[c]	3014.00±44.09[b]
T4	136.67±2.03	370.67±11.05	622.00±5.77[b]	838.00±29.19[ab]	1031.67±25.22[c]	3004.00±44.09[b]
T5	129.00±2.08	357.33±7.62	617.00±9.54[b]	803.33±30.99[ab]	1041.67±14.81[bc]	2948.33±4.09[b]
T6	132.67±5.04	369.33±7.88	625.00±8.5[b]	830.67±22.26[ab]	1056.00±14.47[bc]	3013.67±30.05[b]
T7	128.00±4.36	360.67±3.38	622.33±1.33 [b]	789.67±34.74[b]	1085.00±10.41[b]	2985.67±25.75[b]
Significance level	N.S.	N.S.	*	*	**	**

Means within the same column with different letters differ significantly. N.S. = Not significant. * = $P \leq 0.05$. ** = $P \leq 0.01$.

During the fifth week, treatment T2 recorded the highest mean feed intake at 1176.67 g, showing a highly significant increase ($P \leq 0.01$) compared to all other treatments. Birds in treatment T1 consumed the lowest feed amount, 1001.67 g, without a significant difference from treatment T3, which had a mean feed intake of 1032.67 g.

Regarding total feed intake over the entire rearing period, treatment T2 achieved the highest mean total feed intake of 3168.00 g, showing highly significant superiority ($P \leq 0.01$) over other treatments. Birds in treatment T1 recorded the lowest total feed intake of 2948.33 g, with no significant differences from other treatments.

In general, heat stress reduces feed intake, which was observed in this study. However, treatments supplemented with natural and nano grape pomace by-products and grape seed oil showed a significant increase in feed intake compared with the unsupplemented control, particularly as the birds aged. This effect may be attributed to grape by-products, including their seeds, which are rich in essential compounds, beneficial acids, and aromatic oils. These components enhance the nutritional quality of the diet, improving palatability and stimulating appetite, thereby increasing feed intake (Ianni and Martino, 2020).

The improvement in mean feed intake in the different experimental treatments may be due to the role of grape pomace by-products, which significantly improved mean feed intake at a highly significant level despite rearing birds under high-heat environmental stress. This may be attributed to the role of feed additives in dissipating body heat and thus resisting heat stress, which led to the birds' appetite not being affected. The seed oil may also have an important role in palatability of the diet, which increased feed intake (Aljumaili *et al*., 2023).

Feed conversion ratio (g feed/g weight gain)

Data in Table 7 indicate that all treatments significantly ($P \leq 0.05$) affected feed conversion ratio (FCR) depending on the age of the broiler chickens during the study period. At early stages, the treatments did not significantly ($P > 0.05$) influence performance, and only numerical differences were observed.

A significant ($P \leq 0.05$) reduction in FCR appeared in the third week, with treatment T1 recording the highest mean of 1.42 g feed/g weight gain, not significantly different from most other treatments except T5 and T7, which had the lowest means of 1.31 and 1.28 g feed/g weight gain, respectively.

A similar pattern was observed in the fourth week, where birds in the control treatment (T1) showed a significant increase ($P \leq 0.05$) in FCR, reaching the highest mean of 1.74 g feed/g weight gain, while birds in treatment T7 recorded the lowest mean of 1.49 g feed/g weight gain.

In the fifth week, the experimental treatments again had a significant effect on FCR. Treatments T2, T6, and T7 recorded the lowest means of 1.71, 1.74, and 1.76 g feed/g weight gain, respectively. For total FCR, supplemented treatments were superior to the control, with T1 showing a highly significant ($P \leq 0.01$) highest mean of 1.54 g feed/g weight gain. The lowest total FCR was observed in birds of treatments T2 and T7 (1.44 g feed/g weight gain each), which did not differ significantly from treatment T6.

The inclusion of varying levels of natural and nano grape pomace powder, along with grape seed oil, had a significant impact on feed conversion efficiency. In the third week, this effect favored treatments T5 and T7, while in the fourth week, treatment T6 showed the greatest improvement. Subsequently, birds in treatments T2 and T6 exhibited a significant reduction in total FCR compared with the control. This improvement is likely due to the role of these natural plant extracts in enhancing weekly feed intake, accompanied by a significant increase

Table 7: Effect of grape pomace, grape seed oil, and its nanopowder on feed conversion ratio (g feed/g weight gain) of broiler chickens from 1 to 35 days of age under heat stress conditions (mean ± standard error).

Treatments	Age (Week)					Overall FCR
	1	2	3	4	5	
T1	0.81±0.02	1.15±0.02	1.42±0.02[a]	1.74±0.02[a]	1.87±0.03[a]	1.54±0.00[a]
T2	0.76±0.02	1.11±0.04	1.35±0.03[ab]	1.52±0.04[bc]	1.71±0.06[c]	1.40±0.01[c]
T3	0.80±0.01	1.14±0.02	1.36±0.05[ab]	1.65±0.08[ab]	1.87±0.06[a]	1.49±0.01[b]
T4	0.84±0.01	1.17±0.01	1.33±0.03[ab]	1.61±0.03[abc]	1.86±0.08[a]	1.48±0.03[b]
T5	0.80±0.0	1.12±0.04	1.31±0.03[b]	1.59±0.04[bc]	1.89±0.05[a]	1.48±0.00[b]
T6	0.78±0.02	1.12±0.01	1.35±0.01[ab]	1.58±0.06[bc]	1.74±0.07[c]	1.44±0.02[bc]
T7	0.78±0.01	1.14±0.01	1.28±0.01[b]	1.49±0.01[c]	1.76±0.03[c]	1.41±0.00[c]
Significance level	N.S.	N.S.	*	*	**	**

Means within the same column with different superscript letters differ significantly. N.S. = Not significant. * = P ≤ 0.05. ** = P ≤ 0.01.

in mean weekly weight gain. These results highlight the effectiveness of grape by-product supplementation in improving both performance and the economic feasibility of broiler production, particularly under high-temperature stress conditions.

The results of the study showed that FCR in broilers gradually increased with age from 1 to 35 days under heat stress conditions, a pattern consistent with the effects of heat stress documented in the scientific literature, where higher temperature leads to a decrease in feed consumption and the efficiency of its use in bioproduction (Cornescu *et al.*, 2024).

The significant improvement may be explained by grape pomace containing phenolic compounds and organic acids, especially ellagic acid (Ellagic) and coumaric acid (Coumaric), which contribute to improving the bird's health and immunity and reducing pathogenic effects through their ability to prevent the proliferation of harmful bacterial cells and cause their death, due to their effect in destroying the cell walls of harmful microbes, leading to changes in cytoplasmic membrane permeability and cessation of the basic cellular processes such as electron transport, phosphorylation, and enzyme-dependent reactions. This results in loss of control in the affected bacterial cells and leads to their death, thereby improving the digestion and absorption processes of the consumed feed under heat stress conditions (Bender *et al.*, 2020).

Improvement in feed conversion ratio is associated with increased body weight at hatch and the amount of feed intake, which may be attributed to improved bird weight gain, or to the fact that natural antioxidants present in grape seeds work to protect the gastrointestinal mucosa against oxidative stress and pathogens They also limit helminth activity in digestive disorders and reduce intestinal motility, leading to better absorption of nutrients (Aditya *et al.*, 2018).

The reason may also be related to what the experimental treatments showed of a noticeable improvement in feed conversion ratio, which was consistent with the advancement of bird age up to the mean cumulative feed conversion ratio. This might be due to the decline of nutrient requirements in older birds with age, and their digestive system became more efficient than before. Medicinal plant extracts, including grape, act to enhance digestibility, create balance in intestinal bacteria, and stimulate the secretion of digestive enzymes, which leads to increased bird growth (Shokraneh *et al.*, 2016).

In addition, the improvement that occurred in the studied productive parameters in the second, sixth and seventh treatment, may be explained by the role of nanotechnology in reducing the particle size of grape pomace powder and thus its ability for its nanoparticles to pass easily into the small intestine and then into the bloodstream, reaching the organs of the body. This can be considered a direct reason for the improved productive performance of birds in this treatment (Swain *et al.*, 2021).

Hematological (cellular) characteristics

The data in Table 8 indicate that feed supplementation with grape pomace, grape seed oil, and its nano-powder had no significant effect on red blood cell (RBC) counts, as only numerical differences were observed. In contrast, significant differences (P ≤ 0.05) were found in white blood cell (WBC) counts. Birds in the control treatment exhibited the highest mean WBC value of 23.47 × 10^3/µL, followed by treatment T4, which did not differ statistically from the other treatments. The lowest WBC mean was observed in birds of treatment T4, reaching 19.43 × 10^3/µL.

Regarding hemoglobin concentration (Hb), the experimental treatments produced significant differences (P ≤ 0.05). Birds in treatment T2 showed significant superiority over treatments T1, T3, and T5, recording the highest mean of 10.98 g/dL, while not differing significantly from the remaining treatments. The lowest Hb concentration was observed in the control group, with a mean of 9.39 g/dL, which did not differ significantly from treatments T3, T4, and T5.

Regarding the packed cell volume percentage (PCV), significant differences resulted from the experimental treatments. Birds of treatment T2 had a significant effect in reducing this index compared with only the control treatment T1, without differing significantly from the other experimental treatments, by recording the lowest mean of 26.93%. The highest mean was recorded by the control treatment and reached 32.33%.

The influence of the various experimental treatments on the percentage of lymphocytes (Lym) in blood serum was also significant (P≤ 0.05). Birds of treatment T1 recorded the highest mean for this trait, 58.00%, thus differing significantly from birds of only treatments T5 and T7. The lowest mean appeared in birds of treatment T7, which recorded 51.20%, without differing significantly from treatment T5, which recorded a mean of 51.71%.

A significant response also appeared in the percentage of heterophils (Heterophil) in broiler serum, as shown in the same table (Table 8). Birds of treatment T1 recorded a mean that differed significantly from birds of treatments T2, T3, and T6 only, without differing significantly from the remaining treatments, by recording the highest mean of 35.23%. The lowest mean for this index was 28.97%, recorded by birds of treatment T2, which did not differ significantly from all treatments except the control treatment.

Regarding the heterophil to lymphocyte (H/L) ratio, significant differences (P ≤ 0.05) were found, represented by the control treatment showing significant superiority over only treatments T2, T3, and T6, without having significant differences with the fourth, fifth, and seventh treatments, by recording the highest mean of 0.72%. The lowest means were recorded by birds of treatment T2 (0.50%), followed by treatment T6 with a mean of 0.53%.

The fact that treatment levels of normal and nano grape pomace powder as well as grape seed oil contents added to the broiler diet under heat stress which clearly had a significant effect on studied blood indices, except for mean RBCs, could possibly be used in justifying the explanation why there was a significant increase among treatments. The concentration of these levels were adequate for bringing about appreciable changes in the blood parameters of the cells, and higher concentrations may produce a harmful effect on these parameters. The reason may also be attributed to the effect of grape pomace on cellular blood parameters through improving lymphocyte numbers, especially in the second treatment. It is known that heat stress reduces lymphocyte counts and total protein and markedly increases body temperature; however, grape pomace acts to reduce body temperature by inhibiting the formation of prostaglandin compounds that increase body temperature.

The higher packed cell volume in the control treatment may be due to weak efficiency of oxygen transport in blood cells that may have been affected by high temperature, or it may be due to the absence of a significant response in red blood cells (Al-Saeedi *et al*., 2021; Al-Jebory *et al*., 2024; Arkan *et al*., 2025), which did not give a significant indicator. Thus, their formation in blood plasma was not enhanced, and this may be related to conditions associated with heat stress or due to an increase in erythropoietin production under heat stress. In contrast, the groups of birds treated with grape pomace and its oil showed an increase in cellular blood indicators due to their ability to reduce the harmful effect, which increased oxygen transport among cells and thus increased hemoglobin concentration (Oni *et al*., 2025).

Table 8: Effect of grape pomace, grape seed oil, and its nanopowder on cellular blood indices of broiler chickens under heat stress conditions (mean ± standard error).

Treatments			RBC ($\times10^6/\mu L$)			H/L ratio
T1	2.66±0.29	23.47±0.29^{a}	9.39±0.35^{b}	32.33±2.52^{a}	58.00±2.08^{a}	35.23±2.89^{a}	0.72±0.09^{a}
T2	2.97±0.18	20.10±0.32^{ab}	10.98±0.49^{a}	26.93±1.48^{b}	56.00±1.15^{a}	28.97±0.61^{bc}	0.50±0.01^{c}
T3	2.63±0.12	20.57±0.47^{ab}	9.54±0.28^{b}	28.17±1.04^{b}	54.37±0.84^{ab}	30.53±0.69^{b}	0.56±0.02^{bc}
T4	2.33±0.15	19.43±0.78^{b}	10.07±30.20^{ab}	28.63±0.84^{b}	57.93±1.51^{a}	33.00±2.38^{ab}	0.63±0.07^{abc}
T5	2.74±0.26	20.43±1.29^{ab}	9.71±0.24^{b}	29.13±0.72^{ab}	51.71±0.58^{bc}	33.60±0.40^{ab}	0.64±0.03^{abc}
T6	2.72±0.31	21.21±1.45^{ab}	10.55±0.8^{a}	30.57±0.42^{ab}	54.97±0.66^{ab}	30.57±1.23^{b}	0.53±0.02^{c}
T7	2.42±0.13	21.80±1.33^{ab}	10.78±0.84^{a}	31.67±2.4^{ab}	51.20±1.60^{bc}	33.33±1.67^{ab}	0.69±0.05^{ab}
Significance level	N.S.	*	*	*	*	*	*

RBC: Red Blood Cells; WBC: White Blood Cells; Hb: Hemoglobin; PCV: Packed Cell Volume; Lym: Lymphocytes; Hetro: Heterophils; H/L: Heterophil to Lymphocyte Ratio. Means within the same column with different superscript letters differ significantly. N.S. = Not significant. * = P ≤ 0.05.

These results are consistent with the study of Mahmoud *et al*. (2025), who found that heat stress causes a noticeable and significant effect on cellular blood indicators of broiler chickens, and also concluded that nano plant dietary supplements improved these parameters in experimental birds reared under heat stress conditions.

CONCLUSIONS

The study demonstrated that feed additives (grape pomace, grape seed oil, and its nanopowder) significantly influenced both productive and physiological characteristics of broiler chickens. Supplementation with grape pomace powder at a rate of 0.5 kg/100 kg feed produced the most favorable outcomes in terms of live body weight, weight gain, and total feed intake, while also reducing the heterophil-to-lymphocyte (H/L) ratio, suggesting a mitigation of heat stress effects. Moreover, the results obtained with the nano-powder were comparable to those of conventional grape pomace at lower concentrations. These findings indicate that the feed additives tested in this study, at the doses applied, are effective in enhancing the productive performance and physiological resilience of broilers under heat stress conditions.

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NOVELTY STATEMENT

The first research using grape pomace and nanopowder on broiler

AUTHOR'S CONTRIBUTION

All authors contributed equally to the conception and design of the experiment. Data collection, laboratory analyses, and statistical analysis were carried out collaboratively. All authors participated in data interpretation, manuscript drafting, and critical revision, and approved the final version of the manuscript for publication.

Generative AI and AI assisted technology statement

The authors declare that no generative AI and AI assisted technology was used in the creation of this manuscript.

Conflict of interest

The authors have declared no conflict of interest.

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