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White Grape Pomace Effect on Laying Hens' Productivity, Egg Quality Traits, and Antioxidant Capacity Under Normal, Heat, and Cold Thermal Conditions

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Abstract: This study investigated the effect of white grape pomace (WGP) via a 6% level dietary supplementation on laying hens exposed to varying thermal stress conditions. The experiment was designed as a 2 × 3 factorial study, incorporating two dietary treatments (C and E) and three different thermal conditions: normal (NT: 22 °C), high stress (HST: 35 °C), and low stress (LST: 10 °C). Feed and water were provided ad libitum throughout the experiment. Results showed that the inclusion of 6% WGP in laying hens' diet did not demonstrate beneficial effects under HST conditions, but under LST conditions, the WGP showed higher final body weight (1849.38 g) compared to both groups from the HST conditions (C 1599.40 g and WGP 1592.59 g), and the AEW (average egg weight) was highly significantly higher ($p = 0.0001$) compared to the C or NT groups (both groups, 2nd week), and the HST conditions (both groups, 2nd, 4th, and 6th weeks). HDEP (hen-day egg production) registered highly significant values ($p = 0.0001$) for the WGP group under HST conditions compared to HST conditions (both groups, 4th and 6th weeks). The whole egg weight was highly significant ($p = 0.0001$) for the WGP group under LTS conditions compared to the C group and to HST conditions (both groups, 2nd week and 6th week). The yolk weight parameter registered highly significant ($p = 0.0001$) values for the WGP group under LTS conditions compared to the C group and HST conditions (both groups, 2nd week; C group, 4th week; both groups 6th week) and the NT conditions (C group, 2nd week). Our study indicates that dietary supplementation with 6% white grape pomace (WGP) has potential benefits in LST conditions but limited efficacy under HST conditions. Further research is needed to explore the mechanisms and optimal inclusion levels of WGP in diets for laying hens exposed to different temperatures, especially in HST conditions.

Keywords: antioxidant capacity; eggs; grape pomace; heat stress; low stress

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1. Introduction

The worldwide, continuously increasing temperatures and changing climate patterns have raised significant concerns regarding the welfare and productivity of laying hens, particularly during peak summer months when high thermal stress can severely impact their health and egg production, threatening the sustainability of poultry production systems [1].

Heat stress in laying hens leads to reduced feed intake, lower egg production, poor egg quality, compromised immune function, increased oxidative stress, and higher mortality rates [2,3].

Negative effects were also observed in laying hens exposed to low temperatures.

Cold stress in poultry increases dietary energy demand, feed intake, and feed conversion rate [3]. Kim et al. [4] reported changes in antioxidant status and lipid peroxidation under low thermal stress without stress-related responses. Other studies observed elevated antioxidant vitamins, reactive oxygen species, malondialdehyde, and corticosterone, alongside decreased insulin levels in laying hens' serum [5–7].

According to OECD/FAO [8] global food consumption is expected to rise by 1.3% annually, necessitating adaptive strategies and investments to ensure a resilient, sustainable food system while confronting climate change and population growth challenges.

Using agricultural byproducts in animal feed reduces land and water competition, saves resources, and supports a sustainable food system [9].

In the context of a circular economy, grape pomace serves as a sustainable antioxidant resource for farm animal feed, effectively mitigating heat or cold stress when combined with natural dietary additives and advancements in housing and climate control [10].

Grape pomace, a low-cost and available Romanian byproduct of the winemaking process, is a rich source of bioactive compounds (polyphenols, flavonoids, and dietary fibers). These bioactive molecules are known for their antioxidant, anti-inflammatory, and antimicrobial properties, which can offer various health benefits when included in farm animal feed formulations, also positively influencing the egg/meat quality [11].

The polyphenolic compounds present in grape pomace were highlighted for their ability to enhance the antioxidant status of poultry, with tested beneficial effects on gut microbial activity modulation histomorphology and functionality of the gut [12]. Herranz et al. [13] achieved enriched yolks with gallic acid and albumen Haugh units and yolk color score enhancement; although, they registered a reduction in shell thickness when including 50 g/kg of grape pomace in laying hens' diet.

Incorporating waste materials into poultry diets innovatively enhances animal health and productivity while supporting the circular bioeconomy by transforming agricultural byproducts into valuable feed, reducing waste, and promoting sustainable agriculture [14,15].

Furthermore, higher antioxidant enzyme activity and lower lipid peroxidation lead to overall improvements in the health, performance, and egg quality of the hens, particularly during storage, as evidenced by a study in which grape pomace flour was included in the feed of 74-week-old laying hens under heat stress [16].

We hypothesized that incorporating white grape pomace with recognized antioxidant potential into the diets of laying hens will mitigate the effects of different thermal stresses (high and low temperatures), improving productive performance, some quality traits, and the antioxidant capacity of eggs, efficiently enhancing the utilization of Romanian low-cost local natural available resources and reducing the need for synthetic additives.

2. Materials and Methods

2.1. Ethical Statement

The experiment was conducted according to the protocol (No. 601/05.02.2024) approved by the Commission of the National Research Development Institute of Animal Biology and Nutrition (IBNA-Balotesti, Romania) following the Romanian legislation (Law 206/2004, Ordinance 28/31.08.2011, Law 43/11.04.2014, Directive 2010/63/EU) for feeding, handling, and slaughtering procedures.

2.2. White Grape Pomace Purchasing and Their Proximal Chemical Analyses

The white grape pomace used in this study was sourced from a local vineyard farmer in Dâmbovița County, Romania. Traditionally, the waste generated after the grape harvest is considered to have no economic or productive value, becoming a significant disposal challenge for many Romanian farmers. Prior to incorporating the white grape pomace (WGP) into the laying hens' diet, the provided amount was dried in an oven (ECO CELL Blueline Comfort, Nuremberg, Germany) at a constant temperature of 65 °C for 48 h. Once dried, the WGP was weighed to assess its initial moisture content and then ground into powder using a laboratory hammer mill (Pulverisette 11, Fritsch, Industriestr 8, 55743 Idar-

Oberstaein, Germany) equipped with a 1 mm screen. To establish the proximate analyses, samples of white grape pomace (500 g) were analyzed for dry matter (DM), crude protein (CP), ether extract (EE), crude fiber (CF), ash, minerals, including copper (Cu), iron (Fe), manganese (Mn), zinc (Zn), and antioxidant activity, including polyphenols and antioxidant capacity using 2,2-Diphenyl-1-picrylhydrazyl (DPPH) radical-scavenging activity. The entire quantity of WGP was hammer milled and included in the laying hens' diet. The proximate composition, mineral content, and antioxidant activity of white grape pomace are presented in Table 1.

Table 1. The proximate composition, mineral and vitamin content, and antioxidant capacity of white grape pomace.

Parameters	White Grape Pomace
Proximate composition	
Dry matter (DM), %	92.17
Crude protein (CP), %	7.27
Ether extract (EE), %	5.21
Crude fiber (CF), %	14.44
Ash, %	3.33
Mineral content	
Cooper (Cu), ppm	9.27
Iron (Fe), ppm	147.02
Manganese (Mn), ppm	14.57
Zinc (Zn), ppm	10.11
Vitamin E content	
Alpha-tocopherol (mg/kg)	68.93
Delta tocopherol (mg/kg)	6.46
Gamma tocopherol (mg/kg)	19.61
Vitamin E total (mg/kg)	95
Antioxidant activity	
Total phenolic contents (mg GAE/100 g)	1254.78
Total flavonoid contents (mg catechin/100 g)	883.94
Antioxidant activity (DPPH, µmol TE/100 g)	11,020.40
Antioxidant activity (ABTS, µmol TE/100 g)	7923.94

Note: GAE (gallic acid equivalents), TE (Trolox equivalent), DPPH (2,2-diphenyl-1-picrylhydrazyl), ABTS (2,2′-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid).

The high DM content of the WGP sample suggests that white grape pomace has relatively low moisture, which is beneficial for storage and stability. The high CF content can be attributed to the presence of skins, seeds, stems, and other components. Previous studies have reported total dietary fiber levels for red grape pomace ranging from 51% to 56%, while for white grape pomace, values were found to range between 17% and 28% [17].

2.3. Birds Performances, Housing, and Experimental Diets

The 6-week experiment involved 240 Lohmann Brown laying hens, aged 58 weeks, housed in three experimental rooms. Each room was assigned a Control (C) and an Experimental (E) group, resulting in a total of 6 Control and 6 Experimental groups. This 2 × 3 factorial design combined two dietary treatments (C and E) with three thermal conditions: normal, high stress, and low stress, ensuring that both diets were tested under each temperature condition, under permanent microclimate conditions controlled by a Big Dutchmann computer, with temperature and humidity parameters recorded

twice a day at 8:00 a.m. and 3:00 p.m. The registered daily average temperature and humidity were 22.44 °C ± 1.85 and 45% ± 1.02 for the normal temperature, NT, condition; 35 °C ± 1.50 and 63.88% ± 6.02 for the high-thermal-stress temperature, HST, condition, and 10 °C ± 1.50 and 40.00% ± 2.00 for the low-thermal-stress temperature, LST, condition.

Each thermal temperature experimental hall housed 80 birds, 40 birds/group, with 10 cages per group with 4 birds per cage (dimensions: front 610 mm; back: 745 mm; height: front 560 mm/back 450 mm; between levels: 688 mm; inclinations: 8°/14%). The lighting regime across all halls was maintained at 16 h of light per 24 h period. This setup was designed to analyze the effects of normal, high, and low thermal stress on the birds' overall productive performance and internal and external egg quality. Each cage was considered an experimental unit, and performance parameters were evaluated per pen. Feed and water were offered ad libitum during the entire experimental period. In the experimental room under high temperatures, the water was changed when it overheated, at 8 a.m. and again at 3 p.m. Each of the three groups from all experimental halls had a similar basal diet formulations shown in Table 2. Compared to the C diet, the E group included 6% white grape pomace. The diet structure was formulated using a specifically designed compound feed formulation software (Brill® Formulation, AGRIFOOD, Spain), in agreement with the feeding requirements of laying hens [18]. The diets were isoenergetic and isonitrogenous containing 18% CP and 2850.00 kcal/kg metabolizable energy (ME) per kg diet (Table 1). Average body weight (BW, g/hen) was measured at the beginning and the end of the experimental period. Daily production parameters were monitored and calculated: average daily feed intake (ADFI; g/hen/day); feed conversion rate (FCR; kg feed/kg egg); hen-day egg production (HDEP; %); average egg weight (AEW; g); and viability (%). No medical treatment was necessary or requested during the six-week experimental period. The difference between the amount of compound feed administered and the amount of leftover feed was used to calculate the ADFI parameter. The production parameter for FCR was given as kg of feed eaten for every kilogram of eggs produced. The HDEP parameter was calculated dividing the number of laid eggs divided by the number of hens and multiplied by 100.

Table 2. Diet formulation and proximal analysis results.

Specifications	C	E
Corn, %	39.06	49.66
Wheat, %	20.00	-
White grape pomace, %	-	6.00
Soybean meal, 46 CP %	26.33	28.25
Methionine, %	0.23	0.26
L-Threonine, %	0.01	0.01
Calcium carbonate, 38%	9.20	9.16
Monocalcium phosphate, %	0.56	0.66
Salt, %	0.37	0.37
Vegetal oil, %	3.19	4.58
Choline 60%	0.05	0.05
Premix *, 1%	1.00	1.00
Total ingredients, %	100.00	100.00
Calculated composition		
Metabolizable energy, kcal/kg	2850.00	2850.00
Dry matter, %	89.29	90.10
Crude protein, %	18.00	18.00
Crude digestible protein, %	15.00	14.50
Ether extract, %	5.53	7.14
Crude ash, %	2.26	2.58
Crude fiber, %	4.71	5.65
Calcium, %	4.19	4.19
Available phosphorus, %	0.38	0.38

Table 2. *Cont.*

Specifications	C	E
Calcium/Phosphorus	11.03	11.03
Sodium, %	0.18	0.18
Chloride, %	0.27	0.25
Lysine, %	0.89	0.90
Digestible lysine, %	0.80	0.81
Methionine, %	0.50	0.52
Digestible methionine, %	0.48	0.50
Methyionine + cysteine, %	0.85	0.80
Methyionine + cystine, %	0.72	0.72
Threonine, %	0.64	0.66
Tryptophan, %	0.20	0.19
Arginine, %	1.05	1.04
Linoleic acid (C18:2)	2.86	3.69
Metabolizable energy/Crude protein	158.33	158.33
Antioxidant analyzed composition		
Total vitamin E (mg/kg)	83.23	96.40
DPPH (mM echiv Trolox)	1.19	2.41
Total polyphenols, mg/g GAE	2.34	3.71

Note: * 1 kg Premix contains 1,100,000 IU/kg Vit. A; 200,000 IU/kg Vit. D3; 2700 IU/kg vit. E; 300 mg/kg Vit. K; 200 mg/kg Vit. B1; 400 mg/kg Vit. B2; 1485 mg/kg pantothenic acid; 2700 mg/kg nicotinic acid; 300 mg/kg Vit. B6; 4 mg/kg Vit. B7; 100 mg/kg Vit. B9; 1.8 mg/kg Vit. B12; 2000 mg/kg Vit. C; 8000 mg/kg manganese; 8000 mg/kg iron; 500 mg/kg copper; 6000 mg/kg zinc; 37 mg/kg cobalt; 152 mg/kg iodine; 18 mg/kg selenium.

2.4. Egg Collection and Their Internal and External Quality Assessment

During the entire experimental period (2, 4, and 6 weeks, respectively), a total number of 324 eggs were collected randomly (18 eggs/group/period collection) to assess the internal and external quality parameters of the eggs. The following measurements were realized: the egg weight and its components (albumen, yolk, and shell) were measured using a Kern EW6000-1M Electronic Balance, with a precision of 0.001 (Kern & Sohn GmbH, D-72336 Balingen, Germany), the egg freshness (albumen height and Haugh unit calculation), and eggshell breaking strength, measured using a Digital Egg Tester DET-6500 (NABEL Co., Ltd., Kyoto, Japan). The eggshell thickness was measured within a range of 0.10–0.60 mm, with an accuracy of $\pm$ 0.02 mm, with the concave part of the eggshell side down, using a digital micrometer with a range of 0–10 mm, with a measuring force of 1.5 N or less (Mitutoyo 547-360 ABSOLUTE Digimatic Thickness Gauge). The pH of the albumen and yolk was measured using a portable pH meter (Five Go F2-Food kit with LE 427IP67, Sensor Metler Tolledo, Greifensee, Switzerland). To measure the yolk color, the DET6500 uses natural light (artificial light varies greatly between locations depending on its light source), being estimated based on the DSM's YolkFan^{TM}. The CIE-Lab system (Commission Internationale de l'Eclaraige) with a customized aperture (8 mm/4 mm/1 $\times$ 3 mm), 2.6 s measuring time, high accuracy of 0.04, and an observer angle of 2°/10° was used to measure egg yolk color parameters (L*, a*, and b*) using a portable colorimeter 3nh YS3020 (Shenzhen Threenh Technology Co., Ltd., Beijing, China). The lightness (L*) scale, ranging from 0 (perfect black) to 50 (mid-gray) and up to 100 (perfect white), along with the saturation index a* (indicating green with negative values, red with positive values, and neutral at 0) and the saturation index b* (negative values representing blue, positive values representing yellow, and neutral at 0) were assessed using CIE—L* a* b* color reflectance coordinates.

2.5. Chemical Analyses of Samples

2.5.1. Proximate Analyses

The nutrient concentration of the samples (raw materials, compound feed, and eggs) was analyzed using standardized methods: the gravimetric method (BMT model ECOCELL Blueline Comfort, Nuremberg, Germany) was used to determine the DM concentration;

the Kjeldahl method (Kjeltek auto 1030—Tecator (FOSS Tecator AB, Höganäs, Sweden)) was used to determine CP concentration; extraction in organic solvents (FOSS Tecator AB, Höganäs, Sweden) was used to determine the EE concentration; a method with intermediary filtration (Fibertec 2010 System—Foss Tecator, Sweden) was used for the CF concentration; the procedures described in Regulation (CE) No. 152/2009 (Nabertherm Labotherm L15/11/P320 Comfort (Bremen, Germany)) were used for crude ash determination.

2.5.2. Minerals Assessment

The minerals' concentration assessment, for zinc (Zn), iron (Fe), copper (Cu), and manganese (Mn) in the raw materials, compound feed, and eggs, was conducted using flame atomic absorption spectrometry [Thermo Electron—SOLAAR M6 Dual Zeeman Comfort [19]. The results were expressed as μg/g (ppm) of the dried sample.

2.5.3. Vitamin E Assessment

For vitamin E, determination was accomplished using an RP HPLC analytical method described by Vărzaru et al. [20]. A high-performance liquid chromatograph (HPLC Finnigan Surveyor Plus, Thermo-Electron Corporation, Waltham, MA, USA) and a PDA-UV (292 nm) with a Hypersil BDS C18 column, with silica gel and dimensions of 250 × 4.6 mm and a particle size of 5 μm (Thermo-Electron Corporation, Waltham, MA, USA), were used. Chromatographic parameters were as follows: flow rate of 1.5 mL/min and a mobile phase of 4% water, using 96% methanol.

2.5.4. Determination of Total Polyphenols Content

The total polyphenol content of the plants was spectrometrically determined using the Folin–Ciocalteu method, as described by Untea et al. [21]. The reading of absorbance was performed at 732 nm, and gallic acid was used for the calibration curve, with the results being expressed as mg of gallic acid equivalent per gram of sample.

2.5.5. Determination of 2,2-Diphenyl-1-picrylhydrazyl for Measuring Antioxidant Capacity

The antioxidant activity of raw materials, feed, and egg samples was measured using 2,2-Diphenyl-1-picrylhydrazyl (DPPH) radical-scavenging activity as described by Untea et al. [22]. The absorbance was read at 517 nm using a spectrophotometer (Jasco V-530, Japan Servo Co., Ltd., Tokyo, Japan). The results were expressed as mM eq Trolox after a standard calibration curve was constructed by plotting percentage inhibition against different Trolox concentrations.

2.6. Statistical Analysis

All data were subjected to analysis of variance using the GLM procedure of the Minitab software (version 17, Minitab® Statistical Software). The data obtained were analyzed by two-way ANOVA (Analysis of Variance) following the following statistical model:

$$Yijk = \mu + \alpha i + \beta j + \alpha i\beta j + eijk$$

where Yijk = the variable measured for the kth observation of the ith treatment and jth temperature; μ is the sample mean, and αi is the effect of the ith treatment; βj is the effect of temperature; αiβj = the interaction of ith treatment and jth temperature, and εijk is the effect of error. The differences were highly significant when $p < 0.001$ and significant if $p < 0.05$.

3. Results

3.1. Productive Performance Parameters

Table 3 shows the body weight measured at the beginning and the end of the experiment, under different thermal stress conditions (NT, HST, and HST) and two different diets (C and WGP).

Table 3. Effect of dietary WGP supplementation on laying hens' body weight at the beginning and the end of the experiment.

Specifications	NT		HST		LST		SEM	*p*-Value
	C	WGP	C	WGP	C	WGP		
Initial body weight (g/layer)	1610.28	1678.33	1648.33	1658.15	1660	1653.71	15.14	0.9386
Final body weight (g/layer)	1787.22 ^{cd}	1822.78 ^{cd}	1599.40 ^{abef}	1592.59 ^{abef}	1734.17 ^{cdf}	1849.38 ^{cde}	15.789	<0.0001

Note: C—control diet; WGP—control diet supplemented with 6% white grape pomace; SEM, standard error of the mean; $^{a–f}$ Mean values within a row not sharing the same superscripts are significantly different at $p < 0.05$; NT (normal temperature); HST (high-stress temperature); LST (low-stress temperature).

The initial body weight of laying hens under NT, HST, and LST conditions registered no significant differences ($p = 0.9386$) between groups or thermal stress conditions. Significant differences ($p < 0.0001$) are observed in final body weights after 6 experimental weeks, indicating a statistically significant effect of the diets and temperature conditions. Under NT conditions, the laying hens from the C and WGP groups showed an increase in body weight (1787.22 g and 1822.78 g, respectively) compared to the initial weight (1610.28 g and 1678.33, respectively). In HST conditions, there was a significant decrease in final body weight for both the C (1599.40 g) and WGP (1592.59 g) groups, indicating that high stress negatively affected the weight gain of laying hens. Under LST conditions, the WGP group showed the highest final body weight (1849.38 g), suggesting that WGP might have a positive effect on weight gain under low-stress conditions compared to the C group.

The results obtained suggest that both temperature and group significantly influenced the final body weight of laying hens, especially HST which caused weight loss and WGP potentially enhancing the weight gain under LST conditions.

Table 4 presents the hens' production parameters data under different temperature conditions (NT, HST, and LST). At 2 weeks, under NT conditions, the ADFI parameter's values are highest ($p = 0.0001$) in the C group compared to the WGP group. Under HST or LST conditions, no significant differences ($p = 0.251$) can be observed between the two groups. The D×T interaction, evaluated at 2 weeks, registered highly significant differences ($p = 0.0001$) for the ADFI parameter.

Generally, a decrease in ADFI across all temperature conditions was observed, but hens under NT conditions with the WGP diet (121.23 g) still had a relatively high intake.

The ADFI evaluated at 4 and 6 weeks continues with the same trend, with hens in the NT condition with the C diet consistently exhibiting the highest ADFI, while hens under HST and LST conditions had significantly lower feed intakes. WGP supplementation slightly mitigated the reduction in feed intake under LST compared to HST conditions. The D×T interaction, evaluated at 4 weeks, registered significant differences ($p = 0.001$), while at 6 weeks a highly significant difference ($p = 0.0001$) was noticed, for ADFI parameter. It is important to note that, according to the Lohmann Tierzucht Management Guide [23], the average feed consumption for Lohmann Brown Classic hens ranges between 115 and 125 g/hen/day.

The hen-day egg production (HDEP) parameter at 2 weeks registered the highest value for the NT condition in the C group (93.25%) and was lower under HST, particularly in the C group. Under LST conditions, the WGP supplementation registered a higher percentage closer to the NT level. The D×T interaction, evaluated at 2 weeks, registered significant differences ($p = 0.010$) for the HDEP parameter.

At 4 weeks, the HDEP parameter was significantly higher ($p = 0.0001$) under NT and LST conditions compared to HST, with the highest values registered for the WGP groups compared to the C groups.

Table 4. Effect of dietary WGP supplementation on different thermal stress conditions on laying hens' productive performances.

Parameter	Period	Diet	NT	HST	LST	SEM	D	T	D×T
							p-Value		
ADFI (g/hen/day)	2 weeks	C	130.00 ^{a}	87.07 ^{d}	111.94 ^{c}	1.090	0.251	0.0001	0.0001
		WGP	121.23 ^{b}	93.64 ^{d}	119.49 ^{bc}				
	4 weeks	C	124.78 ^{a}	93.25 ^{d}	115.13 ^{c}	0.516	0.261	0.0001	0.001
		WGP	120.81 ^{b}	91.82 ^{d}	118.04 ^{bc}				
	6 weeks	C	122.70 ^{a}	94.73 ^{d}	109.41 ^{c}	0.608	0.052	0.0001	0.0001
		WGP	117.22 ^{b}	89.96 ^{e}	114.58 ^{b}				
HDEP (%)	2 weeks	C	93.25 ^{a}	88.48 ^{ab}	83.53 ^{b}	1.060	0.859	0.010	0.010
		WGP	90.48 ^{ab}	85.18 ^{b}	90.41 ^{ab}				
	4 weeks	C	95.63 ^{a}	86.27 ^{bc}	90.67 ^{ab}	0.877	0.170	0.0001	0.029
		WGP	96.43 ^{a}	84.39 ^{c}	96.91 ^{a}				
	6 weeks	C	94.84 ^{ab}	87.14 ^{c}	90.87 ^{bc}	0.832	0.083	0.0001	0.108
		WGP	97.22 ^{a}	85.98 ^{c}	95.85 ^{ab}				
AEW (g)	2 weeks	C	61.99 ^{b}	61.97 ^{b}	63.31 ^{b}	0.194	0.545	0.0001	0.0001
		WGP	62.28 ^{b}	59.97 ^{c}	64.93 ^{a}				
	4 weeks	C	64.27 ^{b}	59.75 ^{d}	62.94 ^{c}	0.139	0.0001	0.0001	0.0001
		WGP	64.89 ^{ab}	59.08 ^{d}	65.73 ^{a}				
	6 weeks	C	64.96 ^{a}	59.95 ^{c}	63.21 ^{b}	0.152	0.650	0.0001	0.0001
		WGP	64.89 ^{a}	58.12 ^{d}	65.40 ^{a}				
FCR (feed intake (kg)/egg production (kg))	2 weeks	C	2.36 ^{a}	1.64 ^{d}	2.14 ^{abc}	0.038	0.981	0.0001	0.011
		WGP	2.21 ^{ab}	1.87 ^{cd}	2.06 ^{bc}				
	4 weeks	C	2.15 ^{a}	1.89 ^{b}	2.08 ^{a}	0.024	0.0001	0.0001	0.054
		WGP	1.98 ^{ab}	1.87 ^{b}	1.87 ^{b}				
	6 weeks	C	2.15 ^{a}	1.85 ^{b}	1.95 ^{b}	0.022	0.0001	0.0001	0.057
		WGP	1.94 ^{b}	1.82 ^{b}	1.86 ^{b}				

Note: C—control diet; WGP—control diet supplemented with 6% white grape pomace; SEM, standard error of the mean; $^{a–e}$ Mean values within a row not sharing the same superscripts are significantly different at $p < 0.05$; NT (normal temperature); HST (high-stress temperature); LST (low-stress temperature); D (diet); T (temperature); ADFI (average daily feed intake); HDEP (hen-day egg production); AEW (average egg weight); FCR (feed conversion rate).

The D×T interaction, evaluated at 4 weeks, registered significant differences ($p = 0.029$) for the HDEP parameter. At 6 weeks, the situation was similar to 4 weeks; under the NT and LST conditions, the WGP group registered the highest values compared to the C and WPG groups under HST. However, WGP supplementation under LST conditions (95.85%) registered values reaching those of the C and WGP groups for NT conditions. At 6 weeks, no significant differences ($p = 0.108$) were detected for the D×T interaction in the HDEP parameter.

The AEW at 2 weeks under LST conditions with WGP had the highest value (64.93 g), while those under HST conditions, particularly with the control diet (59.97 g), had the lowest AEW values. At 4 weeks of evaluation, a similar pattern is observed, with the LST condition with WGP hens producing heavier eggs compared to their NT and HST counterparts.

At 6 weeks, in LST conditions the WGP hens still show the highest AEW, indicating that WGP supplementation can positively affect egg weight under low-stress conditions.

For the AEW parameter, highly significant differences ($p = 0.0001$) were observed in the D×T interaction at 2 weeks, 4 weeks, and 6 weeks.

The lowest FCR was registered at 2 weeks under HST conditions with the control diet (1.64), indicating more efficient feed conversion. However, under NT conditions, FCR was highest compared to the C diet (2.36). At 4 and 6 weeks, the FCR generally improves (decreases) under WGP supplementation across all temperature conditions, particularly under heat stress, suggesting that WGP may help hens utilize feed more efficiently in stressful environments. A significant statistical difference for FCR concerning the D×T interaction was observed only at 2 weeks ($p = 0.011$). At 4 weeks ($p = 0.054$) and 6 weeks ($p = 0.057$), only a tendency was noted.

Table 5 presents the egg percentage distribution of different size categories (small, middle, large, and extra large) during the 6 experimental weeks. The percentage of small eggs (<53 g) remained consistently low across all experimental weeks, with a peak production of 7.96% registered in the 6th week. The middle-sized eggs (53–63 g) registered the highest percentages, with a peak of 69.75% registered in the 2nd week for the WGP group. There was a noticeable decrease in the percentage of middle-sized eggs as the weeks progressed, especially on the 6th week (35.82% for the C group in the NT condition and 35.82% for the WGP group in the LST condition in week 6).

Table 5. The egg size percentage distribution (small, middle, large, and extra large) during the experimental period.

Egg Size Classification	Period	NT		HST		LST	
		C	WGP	C	WGP	C	WGP
Small (<53 g), %	2 weeks	0.88	1.33	1.27	4.46	0.72	0.45
	4 weeks	1.75	-	2.70	7.37	1.80	0.22
	6 weeks	-	-	4.31	7.96	0.84	0.43
Middle (53–63 g), %	2 weeks	61.23	62.67	57.46	69.75	46.17	35.23
	4 weeks	39.30	41.42	72.64	66.67	52.93	31.25
	6 weeks	35.17	41.11	71.38	69.32	51.46	35.82
Large (63–73 g), %	2 weeks	37.00	36.00	40.63	25.80	50.96	60.00
	4 weeks	52.40	54.81	24.32	25.32	42.79	62.07
	6 weeks	58.05	54.55	23.38	22.12	44.56	57.14
Extra large (>73 g), %	2 weeks	0.88	-	0.63	-	2.15	4.32
	4 weeks	6.55	3.77	0.34	0.64	2.48	6.47
	6 weeks	6.78	4.35	0.92	0.59	3.14	6.61

Note: C—control diet; WGP—control diet supplemented with 6% white grape pomace; NT (normal temperature); HST (high-stress temperature); LST (low-stress temperature); G (group); T (temperature).

The proportion of large eggs (63–73 g) increased throughout the experiment from the 2nd week, with the percentage ranging from 25.80% (WGP group—HST) to 62.07% (WGP group—LST), increasing by the 6th week, with values between 22.12% (WGP group—HST) and 58.05% (C group—NT). The extra-large eggs (>73 g) registered a low percentage but increased gradually over time, with the highest peak production registered on the 4th week for the C group in NT conditions and on the 6th week for the C group at 6.78%, and also a high percentage of extra-large eggs was noticed for the WGP group with LST conditions.

3.2. External and Internal Egg Quality Parameter

Table 6 presents the results for the external egg traits of the two groups and thermal stress conditions. The shape index values at 2 weeks registered no significant differences between groups or temperature ($p > 0.05$); at 4 weeks, no significant effects ($p > 0.05$) were noticed, and at 6 weeks, a significant interaction effect ($p = 0.013$) suggests that the combined effect of group and temperature significantly influences the shape index. Concerning the whole egg weight parameter at 2 weeks, significant differences were registered between groups ($p = 0.031$), temperatures ($p < 0.0001$), and their interaction

(p = 0.006), with significantly higher egg weight values for the C and WPG groups under NT and WGP with LST conditions compared to both groups for the HST condition and the C group with LST conditions.

Table 6. Effect of dietary WGP supplementation on different thermal stress conditions (NT, HST, and LST) on external egg quality parameters.

Parameter	Period	NT		HST		LST		SEM	*p*-Value		
		C	WGP	C	WGP	C	WGP		D	T	D×T
Shape index	2 weeks	88.98	88.42	88.38	88.78	87.68	87.53	0.321	0.823	0.104	0.689
	4 weeks	88.42	88.55	88.80	87.75	88.12	87.79	0.247	0.239	0.464	0.386
	6 weeks	86.58	87.69	87.80	86.32	87.72	87.85	0.251	0.824	0.193	0.013
Whole egg weight (g)	2 weeks	63.48 ^{ab}	62.42 ^{abc}	58.68 ^{d}	59.95 ^{cd}	61.07 ^{bcd}	64.60 ^{a}	0.398	0.031	0.0001	0.006
	4 weeks	62.41	62.46	62.04	61.30	62.73	63.41	0.489	0.996	0.262	0.704
	6 weeks	62.89 ^{ab}	63.58 ^{a}	60.88 ^{bc}	60.25 ^{c}	63.37 ^{ab}	64.13 ^{a}	0.357	0.586	0.0001	0.452
Eggshell weight (g)	2 weeks	8.47 ^{a}	8.54 ^{a}	7.15 ^{c}	7.35 ^{bc}	8.20 ^{ab}	8.63 ^{a}	0.120	0.173	0.0001	0.681
	4 weeks	7.73 ^{abc}	7.97 ^{ab}	7.26 ^{bc}	6.94 ^{c}	8.35 ^{a}	7.88 ^{ab}	0.116	0.270	0.0001	0.188
	6 weeks	7.99 ^{a}	8.30 ^{a}	7.16 ^{c}	7.36 ^{bc}	7.71 ^{abc}	7.84 ^{ab}	0.083	0.074	0.0001	0.824
Breaking strengths (kgF)	2 weeks	4.73	4.06	4.02	4.07	4.58	4.21	0.227	0.309	0.589	0.654
	4 weeks	4.06 ^{ab}	4.46 ^{ab}	4.50 ^{ab}	3.25 ^{b}	5.14 ^{a}	4.75 ^{a}	0.177	0.108	0.004	0.037
	6 weeks	4.25	4.39	4.20	3.90	4.61	4.63	0.152	0.825	0.102	0.694
Shell thickness (mm)	2 weeks	0.41	0.41	0.39	0.39	0.41	0.40	0.008	0.661	0.429	0.982
	4 weeks	0.39 ^{ab}	0.41 ^{a}	0.37 ^{b}	0.38 ^{ab}	0.39 ^{ab}	0.38 ^{ab}	0.006	0.176	0.069	0.147
	6 weeks	0.41	0.41	0.38	0.38	0.38	0.40	0.004	0.364	0.002	0.570

Note: C—control diet; WGP—control diet supplemented with 6% white grape pomace; SEM, standard error of the mean; $^{a–d}$ Mean values within a row not sharing the same superscripts are significantly different at $p < 0.05$; NT (normal temperature); HST (high-stress temperature); LST (low-stress temperature); D (diet); T (temperature).

At 4 and 6 weeks, significant temperature effects ($p < 0.0001$) were noticed but with no significant interaction effects, suggesting temperature has a consistent effect on egg weight regardless of the group. For the eggshell weight parameter, it was observed that temperature resulted in significant effects at all periods ($p < 0.0001$), indicating a strong influence of temperature on eggshell weight, with no significant group or interaction effects. The breaking strength parameter at 4 weeks registered significant temperature and interaction effects ($p < 0.05$), suggesting that specific conditions impact eggshell strength. The shell thickness parameter at 6 weeks showed a significant temperature effect (p = 0.002), indicating that temperature influences shell thickness over time.

Table 7 presents the results for internal egg traits for the C and WGP groups under varying thermal stress conditions. For the albumen weight parameter at 2 weeks, a significant interaction effect of group and temperature (p = 0.033) was registered. At 4 and 6 weeks, a significant temperature effect at 6 weeks (p = 0.017) was registered with the highest values for the WPG group in NT and LTS conditions. The yolk weight registered significant differences across all weeks across groups (p = 0.0001 at 2 weeks; p = 0.035 at 4 weeks; p = 0.028 at 6 weeks) and temperatures ($p < 0.002$ for HST and $p < 0.0001$ for NT and LTS), indicating that both factors consistently affect yolk weight. The pH levels for the albumen show some significant temperature effects at 4 and 6 weeks ($p < 0.05$), suggesting that changes in albumen pH are temperature-dependent over time. The pH yolk showed significant temperature effects at 2 weeks and 6 weeks ($p \leq 0.025$), with an interaction effect at 2 weeks (p = 0.016), indicating that yolk pH is affected by both temperature and the combination of group and temperature. The albumen height registered no significant differences across groups, temperatures, or interactions, suggesting this parameter remains stable. At 2 and 4 weeks, the yolk fan color registered significant group differences ($p \leq 0.015$) and temperature differences ($p < 0.05$), indicating that color is influenced by both factors. At 6 weeks, a strong temperature effect (p = 0.010) and group effect ($p < 0.0001$) showed the continued influence of these factors over time. The Haugh unit parameter had no significant differences, indicating that the overall quality index remains unaffected by

the variables measured. The yolk height differences were noticed at various periods, with an interaction effect at 2 weeks ($p = 0.017$), indicating that a specific combination of factors affects yolk height. The yolk diameter registered significant differences observed mainly as temperature and group effects ($p < 0.05$), showing that these factors impact yolk size. The yolk index presented significant effects in the interaction at 2 weeks ($p = 0.015$) and at the group level at 6 weeks ($p = 0.0001$), suggesting that combined effects of group and temperature alter the yolk index. For albumen and yolk temperature, both parameters exhibited significant differences across all weeks (p-values < 0.05 for group, temperature, and interaction in some cases), indicating that the temperature of the egg components is highly influenced by the experimental conditions.

Table 7. Effect of dietary WGP supplementation on different thermal stress conditions (NT, HST, and LST) on internal egg quality parameters.

Parameter	Period	NT		HST		LST		SEM	*p*-Value		
		C	WGP	C	WGP	C	WGP		D	T	D×T
Albumen weight (g)	2 weeks	39.36	37.43	37.14	37.15	37.30	38.86	0.373	0.821	0.143	0.033
	4 weeks	39.37	38.08	40.03	39.22	38.27	38.88	0.469	0.457	0.383	0.482
	6 weeks	38.71	38.52	38.41	37.61	39.68	39.36	0.302	0.305	0.017	0.826
Yolk weight (g)	2 weeks	15.65 ^{bc}	16.45 ^{ab}	14.39 ^{c}	15.45 ^{bc}	15.57 ^{bc}	17.11 ^{a}	0.198	0.0001	0.0001	0.553
	4 weeks	15.31 ^{ab}	16.41 ^{a}	14.75 ^{b}	15.14 ^{ab}	16.11 ^{ab}	16.65 ^{a}	0.221	0.035	0.002	0.622
	6 weeks	16.19 ^{ab}	16.77 ^{a}	15.31 ^{b}	15.28 ^{b}	15.97 ^{ab}	16.93 ^{a}	0.160	0.028	0.0001	0.207
pH albumen	2 weeks	8.66	8.70	8.79	8.73	8.57	8.60	0.050	0.974	0.145	0.823
	4 weeks	8.81 ^{ab}	8.79 ^{ab}	8.81 ^{ab}	8.84 ^{a}	8.49 ^{b}	8.74 ^{ab}	0.047	0.211	0.025	0.220
	6 weeks	8.59 ^{b}	8.62 ^{ab}	8.64 ^{ab}	8.68 ^{ab}	8.14 ^{ab}	8.86 ^{a}	0.035	0.366	0.0001	0.987
pH yolk	2 weeks	6.48 ^{ab}	6.37 ^{ab}	6.53 ^{a}	6.50 ^{ab}	6.36 ^{b}	6.48 ^{ab}	0.022	0.873	0.025	0.016
	4 weeks	6.48	6.49	6.75	6.57	6.51	6.71	0.052	0.875	0.137	0.128
	6 weeks	6.42	6.40	6.67	6.57	6.53	6.44	0.043	0.244	0.019	0.826
Ht (mm)	2 weeks	7.13	6.50	6.73	6.06	6.78	6.76	1.270	0.453	0.326	0.295
	4 weeks	7.05	6.87	6.98	6.66	6.61	6.93	0.165	0.793	0.794	0.494
	6 weeks	7.01	6.54	6.26	6.47	6.83	6.68	0.145	0.511	0.202	0.417
Yolk fan color	2 weeks	4.90 ^{b}	6.00 ^{a}	5.00 ^{b}	5.60 ^{ab}	5.50 ^{ab}	6.10 ^{a}	0.102	0.0001	0.019	0.269
	4 weeks	5.00 ^{ab}	5.40 ^{ab}	4.50 ^{b}	5.10 ^{ab}	5.30 ^{ab}	5.70 ^{a}	0.131	0.015	0.013	0.880
	6 weeks	5.17 ^{b}	6.50 ^{a}	4.76 ^{b}	6.06 ^{a}	4.83 ^{b}	6.33 ^{a}	0.078	0.0001	0.010	0.721
Haugh unit	2 weeks	82.40	77.88	81.66	77.07	81.74	80.26	1.590	0.122	0.838	0.812
	4 weeks	83.01	82.10	82.59	80.82	79.62	81.86	1.090	0.924	0.630	0.530
	6 weeks	82.56	78.87	78.28	79.34	80.18	79.96	1.090	0.544	0.601	0.438
Yolk height (mm)	2 weeks	19.14 ^{ab}	18.93 ^{ab}	18.37 ^{ab}	17.99 ^{b}	17.93 ^{b}	20.18 ^{a}	0.287	0.178	1.142	0.017
	4 weeks	19.30 ^{a}	18.52 ^{ab}	18.70 ^{ab}	18.49 ^{ab}	18.04 ^{ab}	17.68 ^{b}	0.210	0.132	0.016	0.721
	6 weeks	18.06	18.36	17.70	18.30	18.85	18.96	0.221	0.288	0.053	0.812
Yolk diameter (mm)	2 weeks	39.51	39.28	37.17	39.68	39.82	39.58	0.433	0.273	0.216	0.117
	4 weeks	39.23 ^{ab}	41.81 ^{a}	38.60 ^{ab}	38.33 ^{b}	39.63 ^{ab}	40.46 ^{ab}	0.446	0.099	0.025	0.184
	6 weeks	39.72 ^{ab}	40.45 ^{ab}	42.89 ^{a}	34.71 ^{b}	38.81 ^{ab}	40.43 ^{ab}	1.02	0.181	0.763	0.012
Yolk index	2 weeks	0.48	0.48	0.49	0.45	0.46	0.51	0.009	0.811	0.764	0.015
	4 weeks	0.49	0.45	0.48	0.48	0.46	0.44	0.008	0.050	0.026	0.211
	6 weeks	0.45 ^{b}	0.46 ^{b}	0.44 ^{b}	0.55 ^{a}	0.50 ^{ab}	0.47 ^{b}	0.010	0.055	0.059	0.0001
Albumen temperature	2 weeks	11.86 ^{ab}	12.40 ^{a}	11.06 ^{ab}	10.58 ^{b}	12.15 ^{a}	7.77 ^{c}	0.195	0.0001	0.0001	0.0001
	4 weeks	9.88 ^{d}	10.96 ^{c}	9.29 ^{d}	9.70 ^{d}	16.90 ^{a}	14.19 ^{b}	0.148	0.057	0.0001	0.0001
	6 weeks	15.48 ^{a}	14.47 ^{ab}	13.30 ^{bc}	14.15 ^{ab}	10.98 ^{d}	11.86 ^{cd}	0.215	0.433	0.0001	0.017
Yolk temperature	2 weeks	11.11 ^{ab}	11.33 ^{ab}	10.09 ^{b}	9.82 ^{b}	12.55 ^{a}	7.32 ^{c}	0.256	0.0001	0.007	0.0001
	4 weeks	10.36 ^{c}	12.35 ^{b}	9.92 ^{c}	9.96 ^{c}	16.46 ^{a}	14.98 ^{a}	0.206	0.533	0.0001	0.0001
	6 weeks	14.21 ^{a}	14.38 ^{a}	12.13 ^{b}	12.44 ^{b}	10.17 ^{c}	11.72 ^{b}	0.200	0.019	0.0001	0.098

Note: C—control diet; WGP—control diet supplemented with 6% white grape pomace; SEM, standard error of the mean; $^{a–d}$ Mean values within a row not sharing the same superscripts are significantly different at $p < 0.05$; NT (normal temperature); HST (high-stress temperature); LST (low-stress temperature); D (diet); T (temperature).

3.3. Yolk Color

The effects of the apple and white grape pomaces dietary supplementation under different temperature stress are shown within Table 8. The L* values at 2 weeks significantly differ between groups ($p < 0.0001$) and temperatures ($p < 0.0001$). The NT-C and LST-C groups had the highest L* values, indicating a lighter color, while the HST-WGP group had the lowest, indicating a darker color. The interaction between diet and temperature D×T is not significant ($p = 0.281$). At 4 weeks, there are significant differences in L* values between groups ($p < 0.0001$) and temperatures ($p = 0.004$). The LST-C group shows the highest lightness value (L* = 46.18), meaning the lightest yolk color, while the HST-WGP group shows the lowest value (L* = 43.39), which indicates the darkest yolk color. There was no significant interaction effect ($p = 0.235$) of diet and temperature. At 6 weeks, a significant group effect ($p = 0.025$) shows differences in L* values between groups, with the NT-C group recording the highest lightness values compared to the WGP-HST group which recorded the darkest yolk color. No significant differences for temperature ($p = 0.064$) and the D×T interaction ($p = 0.139$) were registered, suggesting that neither temperature nor the D×T interaction significantly affects L* at 6 weeks.

Table 8. Effect of dietary WGP supplementation under different thermal stress conditions (H, HST, and LST) on yolk color.

Parameter	Period	NT		HST		LST		SEM	*p*-Value		
		C	WGP	C	WGP	C	WGP		D	T	D×T
L*	2 weeks	44.59 ^{a}	43.68 ^{ab}	43.42 ^{ab}	41.59 ^{c}	44.79 ^{a}	42.78 ^{bc}	0.212	0.0001	0.0001	0.281
	4 weeks	44.53 ^{bc}	43.77 ^{bc}	44.93 ^{ab}	43.39 ^{c}	46.18 ^{a}	44.21 ^{bc}	0.206	0.0001	0.004	0.235
	6 weeks	45.17 ^{a}	44.39 ^{ab}	44.57 ^{ab}	43.70 ^{b}	44.45 ^{ab}	44.58 ^{ab}	0.160	0.025	0.064	0.139
a*	2 weeks	0.69 ^{b}	1.41 ^{a}	0.58 ^{b}	1.27 ^{a}	0.87 ^{b}	1.38 ^{a}	0.046	0.0001	0.048	0.369
	4 weeks	0.47 ^{b}	0.62 ^{ab}	0.09 ^{c}	0.57 ^{ab}	0.64 ^{ab}	0.87 ^{a}	0.046	0.0001	0.0001	0.101
	6 weeks	0.35 ^{b}	1.59 ^{a}	0.27 ^{b}	1.53 ^{a}	0.21 ^{b}	1.50 ^{a}	0.036	0.0001	0.155	0.934
b*	2 weeks	14.84 ^{b}	17.96 ^{a}	14.47 ^{b}	15.29 ^{b}	15.11 ^{b}	17.63 ^{a}	0.210	0.0001	0.0001	0.005
	4 weeks	15.89 ^{a}	15.36 ^{a}	13.42 ^{b}	15.49 a	15.18 ^{a}	16.21 ^{a}	0.190	0.002	0.0001	0.0001
	6 weeks	14.41 ^{d}	17.09 ^{a}	14.48 ^{d}	16.44 ^{ab}	14.98 ^{cd}	15.63 ^{bc}	0.161	0.0001	0.274	0.001

Note: C—control diet; WGP—control diet supplemented with 6% white grape pomace; SEM, standard error of the mean; $^{a–d}$ Mean values within a row not sharing the same superscripts are significantly different at $p < 0.05$; NT (normal temperature); HST (high-stress temperature); LST (low-stress temperature); D (diet); T (temperature). L* lightness scale, ranges from 0 (perfect black) to 50 (mid-gray) and 100 (perfect white); the saturation index a* (indicates green for negative values, red for positive values, and neutral at 0); the saturation index b* (negative values represents blue, positive values represent yellow, and neutral at 0).

The a* parameter at 2 weeks registered significant differences between groups ($p < 0.0001$) and temperatures ($p = 0.048$). Groups like NT-WGP and HST-WGP had higher a* values (a redness intensification of yolk color), compared to the NT-C and HST-C groups which registered lower values. No significant interaction ($p = 0.369$) was registered, indicating that the D×T interaction does not significantly affect the a* parameter at the 2-week period. At 4 weeks, both group ($p < 0.0001$) and temperature ($p < 0.0001$) have significant effects on a* parameter values. The LST-WGP group has the highest redness (a* = 0.87), while the HST-C group has the lowest (a* = 0.09). There was no significant interaction effect ($p = 0.101$). At 6 weeks, for the a* parameter, a significant group effect ($p < 0.0001$) shows statistical differences among groups, with the NT-WGP, HST-WGP, and LST-WGP groups showing higher redness yolk intensification. Temperature ($p = 0.155$) and the D×T interaction ($p = 0.934$) registered no significant effects.

For the b* parameter, at 2 weeks, it is noticed that all effects are significant: group ($p < 0.0001$), temperature ($p < 0.0001$), and D×T interaction ($p = 0.005$). This indicates that yellowness is highly influenced by group, temperature, and the D×T combination. The NT-WGP and LST-WGP groups showed the highest yellowness values, while the NT-C and HST-C groups showed lower values. At 4 weeks, the group ($p = 0.002$), temperature

($p < 0.0001$), and D×T interaction ($p < 0.0001$) all exhibited significant effects on b* values. The HST-WGP group registered the highest yellowness value compared to group HST-C. At 6 weeks, the group ($p < 0.0001$) and D×T interaction ($p = 0.001$) registered high statistical differences in yellowness yolk color, with the NT-WGP group showing the highest b* value.

3.4. Proximate Composition and Oxidative Stability of Yolk Under Different Thermal Stress Conditions

As presented in Table 9, no significant differences ($p > 0.05$) were found for DM%, with values ranging from 51.50% in the HST-WGP group to 52.15% in the LST-WGP group, regardless of diet or temperature effects. The highest CP content, which was statistically significant ($p = 0.0001$), was observed under LST conditions in both the C and WGP groups compared to the WGP group under HST conditions. For ash concentration, highly significant differences ($p = 0.0001$ for temperature; $p = 0.015$ for the D×T interaction) were observed in the LST-WGP group compared to the NT-C, NT-WGP, and HST-WGP groups.

Table 9. Effect of dietary WGP supplementation under different thermal stress conditions (NT, HST, and LST) on yolk proximate composition and its oxidative stability.

Parameter	NT		HST		LST		SEM	*p*-Value		
	C	WGP	C	WGP	C	WGP		D	T	D×T
DM%	51.88	51.80	51.51	51.50	51.57	52.15	0.106	0.297	0.103	0.161
CP%	17.09 ^{ab}	17.05 ^{ab}	16.85 ^{ab}	16.80 ^{b}	17.26 ^{a}	17.27 ^{a}	0.0566	0.745	0.0001	0.937
EE%	27.98	28.03	27.85	27.89	27.87	28.29	0.0885	0.178	0.408	0.376
Ash%	1.56 ^{bc}	1.54 ^{bc}	1.63 ^{ab}	1.52 ^{c}	1.64 ^{ab}	1.68 ^{a}	0.0135	0.132	0.0001	0.015

Note: C—control diet; WGP—control diet supplemented with 6% white grape pomace; SEM, standard error of the mean; $^{a–c}$ Mean values within a row not sharing the same superscripts are significantly different at $p < 0.05$; NT (normal temperature); HST (high-stress temperature); LST (low-stress temperature); D (diet); T (temperature).

Figure 1 presents the total polyphenol concentrations which registered highly significant differences ($p = 0.0001$), with the highest polyphenol content being recorded in the NT-C and WGP groups with values of 1.63 and 1.59, respectively, significantly higher values compared to HST-C group (1.36) and LST-WGP group (1.34). No significant effects of diet ($p = 0.833$) or the D×T interaction ($p = 0.244$) were observed.

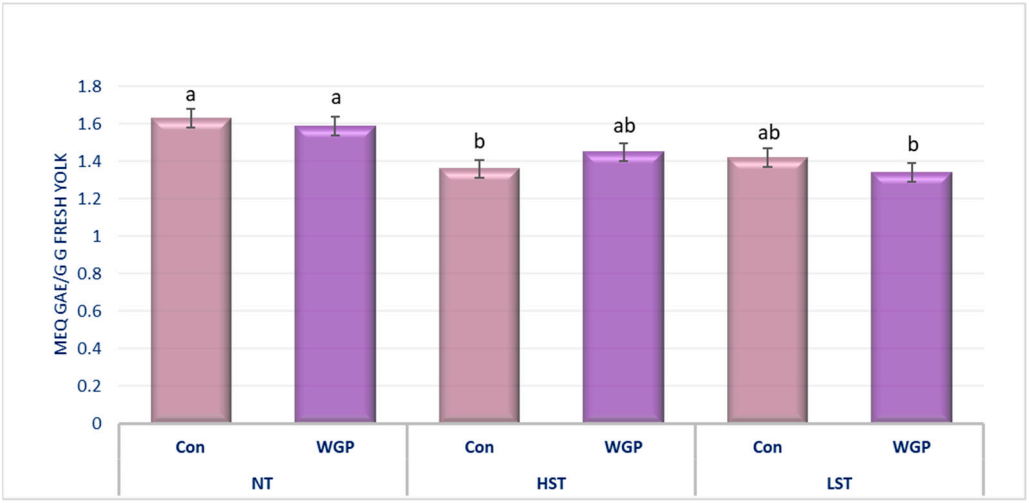


Figure 1. Total polyphenol concentration determined in yolk samples (mEq GAE/g g fresh yolk); ^{a,b} Mean values not sharing the same superscripts are significantly different at $p < 0.05$.

Figure 2 displays the DPPH results, with highly significant D×T interaction effects ($p = 0.0001$), where the highest antioxidant capacity was observed in the NT-WGP group

(3.47). The HST-WGP group recorded lower DPPH values (3.22) compared with the NT-WGP group, while the LST-C group recorded the lowest antioxidant capacity (2.02). Neither diet ($p = 0.059$) nor temperature ($p = 0.058$) independently influenced DPPH concentration.

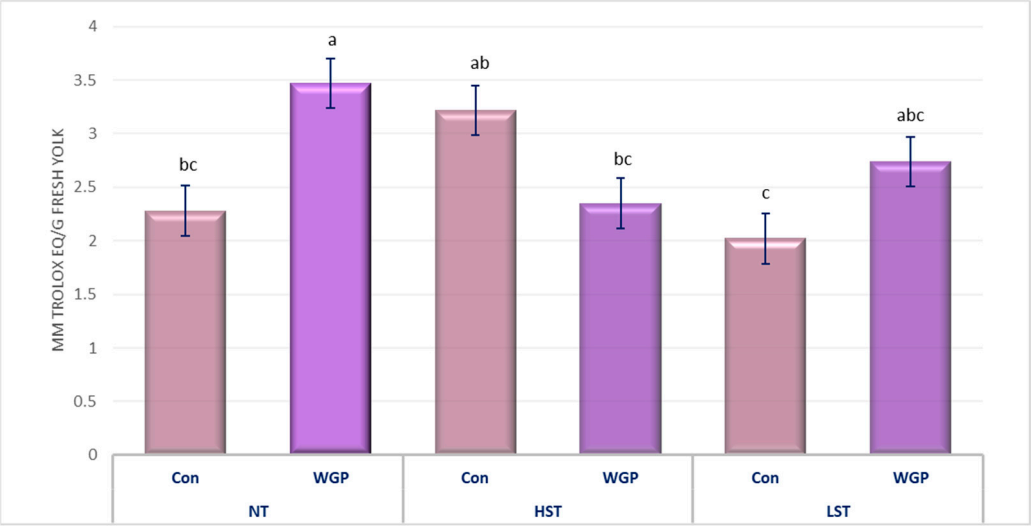


Figure 2. DPPH concentration determined in yolk samples (mM Trolox Eq/g fresh yolk), $^{a–c}$ Mean values not sharing the same superscripts are significantly different at $p < 0.05$.

4. Discussions

4.1. Productive Performance Parameters

Under HST conditions, both the C and WGP groups registered a significant decreasing in final body weight compared to all other groups under NT and LST conditions, highlighting the adverse impact of high temperatures on weight gain, likely due to reduced feed intake and increased energy expenditure for heat dissipation as an adaptive method [24]. Mashaly et al. [25] found similar findings in heat-stressed laying hens, reporting a significant reduction in both body weight (19%) and feed consumption between C and E groups. A recent study [26] noticed that high temperature–humidity index levels (>81) significantly reduced laying hens' body weight. Interestingly, in our study, under LST conditions, the WGP group outperformed the C group, suggesting a beneficial effect of WGP supplementation in colder environments. Based on these results, we assumed that WGP supplementation in laying hens' diet may positively influence metabolic efficiency, which contributed to the observed weight gain. Our hypothesis is supported by the antioxidants' and fibers' beneficial effects present in WGP, which could potentially help the body's adaptation to low temperatures. Zhou et al. [27] reported that a cold tolerance test (4 °C metabolism chamber) performed on mice indicated that dietary high concentrations of grape seed flour (54.04 mg/g) could be beneficial in maintaining body temperature suggesting that an increased energy expenditure positively correlated with more heat production. Also, Gollucke PB [28] stated that grape juice provides high energy and fructose intake that may lead to body weight gain.

Regardless of the thermal stress conditions, Goni et al. [29] reported that incorporating grape pomace into laying hen diets improved antioxidant status but had minimal effects on body weight. Brenes et al. [30] found that grape seed extract did not significantly affect body weight in broilers but improved antioxidant capacity. Previous studies [31] suggested that antioxidant supplementation can alleviate some negative effects of heat stress on poultry performance. Reis et al. [32] declared that a 1–3% addition of grape pomace flour in the diet of laying hens can be beneficial for their health and performance, particularly in enhancing egg production and quality, while also providing antioxidant benefits. Dietary inclusion of 4% and 6% grape pomace in laying hen diets had no significant effects on weight, feed intake, or egg production [33]. Similar findings were indicated by Dae Kim et al. [34],

who observed that low temperatures in laying hens significantly affect final body weight, feed intake, and FCR. Hafeez et al. [35] reported that incorporating grape seed extract at levels of 250–750 g/kg into laying hens' diets under normal temperature conditions did not significantly influence feed intake, body weight gain, or FCR. In contrast, Sayago-Ayerdi et al. [36] found that including grape seed byproducts above 6% in laying hens' diets under similar conditions negatively impacted feed intake. In our study, under NT conditions, the WGP group exhibited significantly lower ADFI, likely due to the increased dietary CF content. In contrast, under HST and LST conditions, ADFI did not differ significantly between the C and WGP groups across all periods, except at the 6th week under LST, where the WGP group showed a significantly higher ADFI. Overall, temperature and the D×T interaction had a highly significant effect on ADFI across all periods (2nd, 4th, and 6th weeks). Our findings align with previous research demonstrating that heat stress adversely affects feed intake in poultry. De Souza et al. [37] observed that broilers exposed to continuous or cyclical heat stress at 32 °C experienced a reduction in feed intake ranging from 25% to 45%. Similarly, Mitchell and Carlisle [38] reported a 29% decrease in feed intake and a 20% reduction in body weight in domestic fowl subjected to 35 °C for 14 days. These studies corroborate our results, highlighting the significant impact of elevated temperatures on poultry feed consumption and growth performance. The HDEP parameter was highly influenced by HST conditions on both C and WGP groups (especially at the 4th and 6th weeks) compared to NT and LST conditions. While no significant differences were observed between the C and WGP groups under LST conditions, the WGP group exhibited a slight increase in HDEP across all three periods suggesting a potential positive influence of WGP supplementation under LST conditions. This improvement may be attributed to enhanced nutrient absorption, as reported by Hosseini Vashan et al. [39], who found that including grape pomace at levels of 20, 40, and 60 g/kg in the diets of heat-stressed broilers (exposed to 37 °C for 6 h daily from days 25 to 42) improved nutrient absorption.

Similarly, Selim et al. [40] reported that incorporating grape pomace at 3%, 6%, and 9% into laying hens' diets under normal temperature conditions led to increased egg production rates and egg weights. The FCR value under NT conditions was higher, indicating less efficient feed utilization compared to HST or LST conditions. On the other hand, a lower FCR value for HST conditions is most probably due to reduced feed intake and egg production in both groups.

4.2. External and Internal Egg Quality Parameters

The external and internal egg quality parameters data showed that some egg quality traits, including weight, pH, strength, and temperature, were significantly influenced by both group and temperature. The interaction between these factors also plays a role, especially for parameters like yolk weight, pH, and shell thickness, indicating complex interactions affecting egg quality.

Our study's results indicate that HST conditions adversely affected whole egg weight, eggshell weight, and shell thickness compared to NT and LHS conditions. Similar results were reported in heat stress conditions by [25] which registered a significant decline in egg quality, including reductions in egg weight, shell weight, shell thickness, and specific gravity.

Kim et al. [4], under low temperature conditions of 12 ± 4.5 °C, found no significant ($p > 0.05$) differences concerning the egg weight, mass, and production.

Sahin et al. [41] found that dietary antioxidants improved egg production and quality under heat stress. Kara et al. [33] observed that incorporating 4% grape pomace into the diets of laying hens under normal temperature conditions led to a significant egg weight increase, while other egg quality parameters remained unchanged. According to Herranz et al. [13], a supplementation of 50 g/kg of grape pomace in laying hens' diet significantly enriched the egg yolks with gallic acid, improved albumen Haugh units and yolk color scores, and reduced the eggshell thickness. Kim et al. [26] observed a reduced eggshell thickness and strength and Haugh units under severe heat stress (33 °C). Additionally,

Attallah et al. [42] stated that the antioxidant properties of grape pomace (0.5%, 1%) may help mitigate oxidative stress, which can be exacerbated by heat, potentially leading to improved yolk quality.

Our results showed that WGP supplementation can enhance some productive performance metrics, especially under LST conditions.

4.3. Yolk Color

The data obtained in our study showed that all color parameters (L*, a*, and b*) were influenced by diet and temperature effects to varying extents, with significant group effects during all periods for each parameter. The D×T interaction effects are less significant, compared to both group and temperature. The L* parameter generally shows significant changes due to group and temperature, and the a* parameter is more influenced within groups, with temperature also exerting an influence at certain periods, whereas the b* parameter is significantly influenced by group, temperature, and their interaction, particularly noticeable in earlier weeks. The addition of WGP to the diet of laying hens has been found to improve the yolk color score, a benefit that is especially significant under heat-stress conditions. Research indicates that diets supplemented with grape pomace significantly increased yolk color scores, with one study reporting a 9.36% increase when 50 g/kg of grape pomace was included in the diet [13]. While specific studies on heat stress were not detailed, the overall benefits of grape pomace in enhancing yolk color and reducing lipid peroxidation suggest a positive impact on egg quality during stressful conditions [32,33]. Dae-Kim [34] reported an intensification of eggshell color under low thermal stress compared to normal temperatures, whereas Kim et al. [26] found that heat stress (33 °C) significantly diminished yolk color. Ronceros et al. [43] found that a 5% grape pomace inclusion produced darker, redder yolks, while higher inclusions (7% and above) resulted in lighter yolks with diminished yellow and red tones.

4.4. Antioxidant Capacity of Eggs Obtained Under Different Thermal Stress Conditions

Numerous studies have shown that polyphenols from grape pomace possess strong antioxidant properties that help alleviate the oxidative stress caused by extreme thermal stress conditions in poultry, reducing lipid peroxidation and enhancing overall antioxidant capacity [44]. In our study, the highest polyphenol concentrations were observed under normal temperature (NT) conditions. Both the HST and LST conditions registered lower polyphenol concentrations. Neither the type of WGP supplementation diet nor the D×T interaction significantly influenced the polyphenol content. The highest DPPH value recorded in the NT-WGP group suggested that NT conditions combined with the WGP diet (rich in polyphenols) promoted a higher antioxidant capacity in the yolk. The DPPH value observed for LST conditions was higher in the WPC group compared to the C group but without significant statistical differences. Reis et al. [32] found that a 3% dietary inclusion of grape pomace prevented the reduction in monounsaturated fatty acids in the yolk of stored eggs compared to the control group. Additionally, laying hens fed grape pomace (at 1%, 2%, or 3% inclusion levels) exhibited higher glutathione peroxidase and superoxide dismutase activities, along with greater total antioxidant capacity against peroxyl radicals in their serum, while showing the lowest levels of lipid peroxidation compared to the C group. Also, red grape pomace has been linked to increased antioxidant levels in eggs, which may mitigate oxidative stress associated with high temperatures. Studies indicate that dietary GP can increase the deposition of phenolic compounds in eggs, suggesting a transfer of these antioxidants from the diet to the egg [13]. Additionally, the antioxidant properties of grape pomace may help maintain the integrity of egg lipids during storage, thereby enhancing the nutritional value of eggs produced under heat stress [40]. Affordable and country-specific feed ingredients with unique properties, bioactive properties, and adequate inclusion levels can help alleviate thermal stress in laying hens [45,46].

5. Conclusions

This study aimed to explore the potential of locally available white grape pomace, a waste byproduct rich in antioxidants and polyphenols, as a dietary inclusion for laying hens under normal, heat, and cold thermal conditions. While the inclusion of 6% white grape pomace did not significantly impact productive performance or egg quality parameters overall, it demonstrated promising benefits under low thermal stress conditions for the following: average egg weight, hen-day egg production, eggshell weight, breaking strength, yolk weight, a* color parameter, and DPPH value. These findings highlight the potential of white grape pomace as a functional feed ingredient, particularly in mitigating the challenges of low thermal stress in poultry production.

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