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# Effects of dietary grape pomace powder supplementation on the performance, egg quality, hatchability, and blood parameters of laying quails (*Coturnix coturnix japonica*)

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## Abstract

This study investigated the effects of dietary supplementation with grape pomace powder (GP) on the performance, egg quality and hatchability, and blood biochemistry of quails (*Coturnix coturnix japonica*). A total of 200 quails (323.90 ± 1.991 g body weight) were randomly divided into four treatment groups, with five replicates of ten birds each. The treatments involved dietary supplementation with GP at 0% (0GP), 1% (1GP), 2% (2GP), and 4% (4GP) of the basal diet for eight weeks. The results indicated that GP significantly affected feed intake, egg production, and egg weight. The 1GP and 2GP treatments had higher egg production and a better feed conversion ratio (FCR) than the other treatments. The lowest egg production and poorest FCR in the study were in the 4GP group. Feed intakes and egg weights were lower in the supplemented groups than in the 0GP group. The 1GP, 2GP, and 4GP groups had higher eggshell breaking strength, Haugh unit, and egg albumen index values than the 0GP group. Plasma total cholesterol and high-density lipoprotein cholesterol concentrations in all GP-supplemented groups were lower than in the 0GP quails. The effects of GP supplementation on chick live weight and early embryonic mortality were significant, with GP supplementation considerably reducing early embryonic deaths compared to the 0GP group. In conclusion, this study showed that adding up to 2% GP to quails' diets had no negative effects on live performance, improved some egg quality traits, decreased early embryonic deaths, and may have helped reduce total lipid and cholesterol levels.

**Keywords:** blood profile, egg quality characteristics, grape pomace, hatching characteristics, Japanese quails

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## Introduction

To reduce feeding costs and preserve its global competitiveness, the poultry industry needs to find substitutes for conventional feed ingredients used in bird rations (Asghar *et al.*, 2021; Tufarelli *et al.*, 2022). Agro-industrial by-products are considered a suitable alternative, as they are produced in large amounts annually, mostly by the juice and winery industries, and present a significant issue for waste management and disposal (Erinle *et al.*, 2022). Türkiye is the sixth-largest grape-producing nation in the world (*Vitis vinifera sativa*) (Ayaz, 2024). Grape production is also particularly relevant because

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of the excellent nutritional and pharmaceutical properties of grape berries (both raw and dried forms) and their derivatives, such as peels, pomace, and seed extracts (Hafeez *et al.*, 2023; Almanza-Oliveros *et al.*, 2024). Grape pomace (GP) is an excellent source of basic bioactive substances such as phenolic acids, flavonoids, anthocyanin, catechins, and stilbenes, which have been shown to enhance growth, antibacterial and antioxidant responses, and poultry meat quality properties (Selim *et al.*, 2023). It has been reported that GP contains elevated levels of polyphenolic compounds, along with 25%–35% crude cellulose, 4%–10% hemicellulose, 5%–6% pectin, 8%–14% crude protein, 4%–10% crude fat, and 30%–45% non-nitrogen component. The total phenolic compound contents of GP from Emir and Kalecik Karası varieties were reported as 68.77 and 96.25 mg GAE/g, respectively (Özkan *et al.*, 2004). Karakaya *et al.* (2001) further reported that phenolic compounds were present at 3.99 mg/g in dried grapes and 2.21 mg/g in fresh red grapes. Karaman *et al.* (2021) reported that the concentrations of total phenolic compounds in Boğazkere grape seeds (GS), skin, and stem were 60250, 37875, and 62550 mg GAE/kg dry weight, respectively. Grape phenolic compounds, mainly catechins and proanthocyanidins, are scavengers of free radicals and counteract oxidative processes (Romero *et al.*, 2022). The dietary inclusion of grape by-products reduced lipid peroxidation in broilers (Aditya *et al.*, 2018; Nardoia *et a*l., 2020; Chowdhary *et al.*, 2021), and feeding grape products to laying hens increased the quality of their eggs but reduced feed consumption and egg weight. Compared to grape extract, GP supplementation has resulted in higher antioxidant activity in egg yolks (Romero *et al.*, 2022; Madkour *et al.*, 2024).

This study investigated the usability of bioactive substances in GP in animal nutrition, evaluating the effects of the dietary inclusion of GP on performance, egg quality, blood biochemistry, and hatchability parameters in quails.

## Materials and Methods

This experiment was conducted in accordance with the guidelines for the Care and Use of Animals and was approved by the Animal Research Ethics Committee of Niğde Ömer Halisdemir University (date: 03/11/2023, approval number: 2023/14).

In total, 200 eight-week-old quails with an average body weight of 323.9 ± 1.99 g were randomly allocated to four treatment groups, with each group consisting of five replicates of ten birds each (eight female and two male quails per replicate). Each replicate group was housed in a 92 × 45 × 25 cm cage equipped with automatic waterers and feeders. The cages were kept in a completely enclosed, fan-ventilated building with a daily light schedule of 16 hours of light and eight hours of darkness. Quails were fed a commercial laying hen diet containing 18% crude protein and 2870 kcal metabolisable energy/kg (Table 1), supplemented with GP at 0%, 1%, 2%, or 4% for eight weeks.

The GP used in this study was from red grapes, and was produced by a private winery factory that processes grapes in Niğde Province, Türkiye. The grapes were cleaned and dried for 72 hours at 55 °C in a drying cabinet to produce the pomace. The pomace was then ground into powder using a 1 mm diameter grinder, and stored in air-tight containers in a cool environment until used. To produce the GP extract, 10 g of GP was dissolved in ethanol and water (50% ethanol), kept closed in a dark place for three hours, and then passed through a rotary evaporator at 50 °C, followed by filtration using a coarse filter paper (Shi *et al.*, 2003, Yilmaz & Toledo, 2006, Lafka *et al.*, 2007). The extracted powder was kept at -80 °C until the determination of its total antioxidant activity and total phenolic compound concentration.

The total phenolic compound concentration in the GP powder extract was determined using Folin-Ciocalteu reagent. For this purpose, 100 µL of GP powder extract with low density was combined with 900 µL of pure water, 5 mL of 0.2 N Folin-Ciocalteu reagent, and 4 mL of saturated sodium carbonate ($Na_2CO_3$) solution (7.5 g/L). The absorbance was then measured at 765 nm, after incubation for two hours at room temperature in the dark. The results are reported as milligram gallic acid equivalents per 100 g wet base (mg GAE/100 g wb) (Slinkard & Singleton,1977; Spanos & Wrolstad,1990).

The antioxidant activity of the GP was quantified using 2,2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS). A 2.45 mM potassium persulfate ($K_2S_2O_8$) solution containing 7 mM ABTS was made and kept in the dark at room temperature for 12–16 hours to produce a radical solution (ABTS + •). A well-plate of GP extract and standard Trolox concentrations was prepared, 1 mL ABTS + • was added to each 10 µL sample, and the plate was mixed in the spectrometer for six minutes. A slope

indicating the percentage of inhibition was obtained using the standard concentrations, and the ratio of the concentration to the slope was used to evaluate the antioxidant capacity of the GP, which was expressed as mM Trolox per 100 g of dry base pomace (Re *et al.*, 1999).

**Table 1** Ingredients and nutrient composition of the experimental diet

| **Feed ingredients (% inclusion)** | |
|---|---|
| **Maize** | 44.99 |
| **Soybean meal** | 29.05 |
| **Wheat** | 13.00 |
| **Vegetable oil** | 2.75 |
| **Limestone** | 8.3 |
| **Dicalcium phosphate** | 1.15 |
| **DL-methionine** | 0.16 |
| **NaCl** | 0.35 |
| **Vitamin – mineral premix*** | 0.25 |
| **Calculated analysis** | |
| **Crude protein (%)** | 18.00 |
| **Metabolisable energy (kcal/kg)** | 2870 |
| **Calcium (%)** | 3.50 |
| **Available phosphorus (%)** | 0.35 |
| **Methionine (%)** | 0.395 |
| **Lysine (%)** | 0.782 |
| **Linoleic acid (%)** | 1.03 |

*Supplied per kilogram of diet: 12 000 000 IU vitamin A, 2 400 000 IU vitamin $D_3$, 30 g vitamin E, 2.5 g vitamin $K_3$, 3 g vitamin $B_1$, 6 g vitamin $B_2$, 4 g vitamin $B_6$, 17 mg vitamin $B_{12}$, 25 g niacin, 8 g calcium D-pantothenate, 1 g folic acid, 50 g vitamin C, 545 mg D-biotin, 150 g choline chloride, 1.5 g canthaxanthin, 0.5 g apo-carotenoic acid ester, 80 g Mn, 60 g Zn, 60 g Fe, 5 g Cu, 1 g I, 0.5 g Co, 0.15 g Se

Feed was offered to the quails daily and the daily feed intake was recorded. Eggs were collected daily, their weights were recorded, and the numbers of eggs per replicate were recorded. The feed conversion ratio (FCR) was calculated by dividing the amount of feed consumed by the weight of eggs produced by each replicate in each treatment group. Egg yield was calculated by dividing the number of eggs by the number of quails and multiplying by 100. Eggs were collected from each group every 14 days to determine egg quality characteristics. The collected eggs were weighed using a 0.01 g precision scale, and the lengths and widths of the eggs were measured using a digital calliper. Based on this data, the egg shape index (SI) was calculated using the following formula (Anderson *et al.*, 2004):

$$Egg\ SI\ (\%) = \frac{Width\ of\ egg\ (mm)}{Length\ of\ egg\ (mm)} \times 100$$

Eggshell breaking strength was measured using an egg force reader (Orka Food and Technology Ltd). The eggs were then broken onto a glass table, and the widths and lengths of the albumens and yolks, as well as the diameters of the egg yolks, were measured using a digital calliper (± 0.001 mm). The heights of the egg whites and yolks were measured using a tripod micrometre (Doyon *et al.*, 1986). Using the values obtained above, the albumen and yolk index and Haugh unit were calculated using the following formulae:

$$Egg\ albumen\ index\ (\%) = \frac{Height\ of\ egg\ white\ (mm)}{Average\ length\ and\ width\ of\ egg\ white\ (mm)} \times 100$$

$$Egg\ yolk\ index\ (\%) = \frac{Height\ of\ egg\ yolk\ (mm)}{Average\ length\ and\ width\ of\ egg\ yolk\ (mm)} \times 100$$

$$HU = 100\ log\ [H + 7.57 - 1.7\ W^{0.37}]$$

where HU: Haugh unit, H: albumen height (mm), and W: weight of the egg (g).

The colour of the egg yolk was measured using a colourimeter (CR-300, Minolta, Osaka, Japan), which uses reflectometry data in the L*, a*, and b* colour space to describe colour. Within this colour space, b* quantifies the colour position between blue and yellow and a* denotes the colour position between green and red, with b* < 0 for bluer colours and b* > 0 for yellower colours. L* indicates the clarity of the colour, with L* = 0 for black and L* = 100 for white (Mertens *et al.*, 2010). Egg yolk colour was also determined using the Roche yolk colour fan. The Roche yolk colour fan values ranged from 1 (light yellow) to 15 (orange).

After the eggs had been cleaned and dried, they were cracked open, separating the eggshell from its contents, and the shell weight was recorded using a precision balance (0.01 g). The thickness of the eggshells was measured at three sites on the cracked eggs (blunt, middle, and pointed part) using a digital gauge micrometre (0.01 mm), and the shell thickness was calculated by taking the arithmetic mean of these three readings (Chowdhury, 1987).

Blood biochemistry analysis was performed in the eighth week of the trial, with three quails randomly selected from each replicate of each treatment group and killed by cutting the ventral neck. Approximately 1 mL of blood from each quail was collected into a silicon and heparin-sanitised tube, and the tube was centrifuged for ten minutes at 3000 rpm. Using a commercial, automated biochemical analyser (DiaSys, Diagnostic Systems, Germany), the levels of glucose, triglycerides, total cholesterol, and high-density lipoprotein (HDL) components were measured.

Hatchability was investigated using eggs collected during the seventh week of the experiment, with a total of 400 eggs, 100 eggs per treatment group, being collected over seven days. These eggs were stored under appropriate humidity and temperature conditions, and cracked, thin-shelled, dirty, or abnormal eggs were excluded before incubation. The eggs were placed in an incubator, and for the 14-day development stage of incubation, the temperature was set at 37.7 °C and the relative humidity was 60%–65%; the eggs were also rotated 90° every two hours for these 14 days. After the 14-day development phase, the eggs were transferred to the outlet part of the incubator, and the temperature was adjusted to 37.3 °C and humidity to 75%. The chicks that hatched after the full 18-day incubation period were individually weighed and placed in a brooder cage. The stages of embryonic death were determined in the unhatched eggs according to Aygün *et al.* (2012). Using the data obtained, incubation parameters were calculated using the following formulae:

$$Fertility\ rate = \frac{No.of\ fertilised\ eggs}{No.of\ eggs\ set} \times 100$$

$$Early\ embryonic\ deaths = \frac{No.of\ embryos\ that\ died\ at\ 1-9\ d\ of\ incubation}{No.of\ fertilised\ eggs} \times 100$$

$$Middle\ embryonic\ deaths = \frac{No.of\ embryos\ that\ died\ at\ 10-17\ d\ of\ incubation}{No.of\ fertilised\ eggs} \times 100$$

$$Late\ embryonic\ deaths = \frac{No.of\ embryos\ that\ died\ at\ 17-18\ d\ of\ incubation}{No.of\ fertilised\ eggs} \times 100$$

$$Hatchability\ of\ set\ eggs = \frac{No.of\ hatched\ chicks}{Total\ no.of\ set\ eggs} \times 100$$

$$Hatchability\ of\ fertile\ eggs = \frac{No.of\ hatched\ chicks}{Total\ no.of\ fertile\ eggs} \times 100$$

Statistical analyses were performed using general linear model techniques for randomised designs in IBM SPSS Statistics for Windows, version 23.0 (IBM Corp., Armonk, NY, USA). The significant differences between treatments were detected using Duncan's multiple range test. Significant differences were determined at $P$ <0.05.

## Results and Discussion

The GP powder used in this study had a total antioxidant capacity of 687.9 ± 7.60 µmol Trolox/g, and a phenolic compound concentration of 379.1 ± 21.6 mg GAE/g. Demirkol (2016) found that the total phenolic compound concentrations of extracts obtained from dried powdered pomace using four different drying temperatures and lyophilisers were between 19.858 and 34.959 mg GAE/g, with free radical scavenging values of 12.586 to 16.781 mg/L. Sağdıç *et al.* (2011) reported that the total concentration of phenolic compounds in the GP of four different grape varieties varied between 75.53 and 281.43 mg GAE/g. Kara *et al.* (2016) determined that the total phenolic compound concentration of GP was 733.37 mg GAE/100 g. Katalinic *et al.* (2010) determined the antioxidant activities of skin extracts from seven red and seven white grape varieties using DPPH, FRAP, $Fe^{2+}$-chelating ability, and β-carotene bleaching analyses. For the DPPH assay, they found that the antioxidant activities of the grape skin extracts of red varieties were between 58 and 139 mg GAE/L, and of white varieties were between 53 and 291 mg GAE/L. The higher total antioxidant capacity and phenolic compound content found for the GP used in this study may be because of differences in the analysis methods used.

The effects of increasing levels of dietary GP on the performance of the laying quails are presented in Table 2.

**Table 2** Laying performance of Japanese quails fed diets supplemented with different levels of grape pomace powder for eight weeks

| Parameters | | Treatment groups | | | | SEM | *P*-value |
|---|---|---|---|---|---|---|---|
| | | 0GP | 1GP | 2GP | 4GP | | |
| **Body weight (g)** | | | | | | | |
| **Initial** | **Female** | 330.66 | 333.00 | 332.39 | 330.82 | 2.055 | 0.972 |
| | **Male** | 301.10 | 299.54 | 302.25 | 298.94 | 0.256 | 0.958 |
| **Final** | **Female** | 358.43 | 357.22 | 356.96 | 350.07 | 2.554 | 0.663 |
| | **Male** | 313.83 | 321.98 | 329.77 | 308.23 | 0.445 | 0.338 |
| **Feed intake (g/d/bird)** | | 41.74$^{a}$ | 40.55$^{ab}$ | 41.55$^{a}$ | 39.19$^{b}$ | 0.281 | 0.004 |
| **FCR (kg feed/kg egg)** | | 3.38 | 3.35 | 3.36 | 3.54 | 0.039 | 0.259 |
| **Egg production (%)** | | 88.80$^{b}$ | 91.91$^{a}$ | 89.34$^{ab}$ | 82.82$^{c}$ | 0.517 | 0.000 |
| **Egg weight (g)** | | 14.04$^{a}$ | 13.62$^{d}$ | 13.91$^{b}$ | 13.77$^{c}$ | 0.019 | 0.000 |

$^{a, b, c, d}$ Means with different superscripts in each row differ significantly ($P$ <0.05).
0GP: 0% grape pomace, 1GP: 1% grape pomace, 2GP: 2% grape pomace, 4GP: 4% grape pomace, SEM: standard error of the mean, FCR: feed conversion ratio

There were no significant differences in live weights between the groups at the beginning or end of the study, and dietary supplementation of GP had no significant impact on the FCR. The feed intake of the group fed 4% GP was numerically lower than the intake of the quails fed 0%, 1%, or 2% GP ($P$ >0.05), and, although this was not statistically significant, a higher FCR was found for the 4% supplemented group. The lower feed intake and poorer FCR of the quails fed higher levels of GP (4%) could be attributed to the higher concentration of polyphenolic compounds in the GP used in our study.

It has been reported that by-products of grape juice or wine production are rich in polyphenolic compounds (Farahat *et al.*, 2017), and these polyphenols have been reported to reduce nutrient utilisation and suppress performance by forming tannin-protein complexes (Brenes *et al.*, 2016). Tannins are anti-nutritional substances found in grape skins and seeds that can form complexes with proteins and, to a lesser extent, carbohydrates and minerals (Addisu, 2016), and can thereby negatively affect nutrition utilisation. In this study, daily feed intake was lower in all the treatment groups supplemented with GP, compared to the control group ($P$ <0.05). Becker & Makkar (1999) reported that adding tannin-containing ingredients to feed can impart an unpleasant taste, which may reduce feed consumption. In contrast, Froes *et al.* (2018) reported that adding GP flour at different rates did not affect feed palatability and feed intake. Our study aligns with a previous study performed by Kumanda *et al.* (2019), who observed that introducing 7.5% dietary red GP decreased the total feed intake of chickens.

Egg weight was significantly higher in the control group than in the quails fed the GP-supplemented diets. However, egg production was significantly higher in the 1GP and 2GP groups than in the control and 4GP groups. The quails fed the diet containing 4% GP had the lowest egg production, compared to both the control and the other treatment groups ($P$ <0.05). The particularly low feed intake, poorer FCR, and consequently decreased egg production in the 4% GP-supplemented group could be attributed to the higher quantity of tannins received from the GP by this group.

Studies on grape by-products have mostly been conducted on laying hens. Kılınç & Karaoğlu (2020) showed that the dietary supplementation of GS oil and *Hypericum perforatum* L. extract to laying hens had no significant effect on their final live weight, feed intake, egg production, FCR, and egg weight. In the current study, differences were observed between groups in performance data, except for the final body weight and FCR. Mirghelenj *et al.* (2017) also examined the effects of different levels of GP supplementation (0.5%, 3%, and 4.5%) on the performance of laying hens, and concluded that feeding 4.5% GP did not affect the egg production rate, egg weight, egg mass or FCR, but decreased the feed intake. In contrast, El-Aaser *et al.* (2021) investigated the effects of dietary grape seed extract (GSE) supplementation (0, 600, 1200, and 1800 mg GSE/kg) on the productive performance of laying hens. They reported that hens fed a diet supplemented with 1200 mg GSE/kg diet had the highest egg production and egg mass and the best FCR. Contrary to our study, it was reported that the addition of 0%, 4%, and 6% GP to a laying hen ration did not affect egg production and feed intake (Kara *et al.*, 2016), and egg weight was higher in the 4% supplemented group than in the control group.

In our study, the highest egg weight was found for the control group. Kaya *et al.* (2014) investigated the effects of different doses of GS (0.5%, 1%, and 1.5%) and GSE (675, 1350, and 2025 mg/kg) on laying hens, and reported that feed intake and FCR did not significantly differ between treatment groups. However, adding GS and GSE to layer rations linearly increased egg production and quadratically affected egg weight compared to the control group. Hafez *et al.* (2023) reported no difference in feed intake, body weight, and FCR between the control diet and the experimental diets containing GSE in laying hens. In line with our results, Romero *et al.* (2022) reported that the dietary supplementation of both GP and grape extract decreased feed intake and FCR in laying hens. Unlike our study, though, these researchers stated that there were no differences between the groups receiving GP and grape extract in egg production or egg mass, although they found that the average egg weight was lower in the groups fed 30 g/kg and 60 g/kg GP, and in the group fed 0.5 g/kg grape extract, than in the control group.

Egg quality parameters were not affected by dietary GP supplementation, apart from the eggshell breaking strength, eggshell weight, Haugh unit, and albumen index (Table 3). The eggshell breaking strength was higher in the 1GP and 2GP groups than in the control and 4GP groups ($P$ <0.05). The Haugh unit and albumen index values were highest in the 1% supplemented group, at 94.67 and 14.45%, respectively, and lowest in the control group, at 92.64 and 12.93%, respectively ($P$ <0.05). High Haugh unit values in eggs are largely considered indicative of egg freshness and albumin quality (Zhu *et al.*, 2020), and the higher Haugh unit and albumin index values in the GP-supplemented groups can be attributed to the antioxidant activity of the GP. No significant differences were seen between the treatment groups in terms of egg yolk colour measures (L*, a*, and b*).

Our findings on egg quality are in agreement with those reported by Kaya *et al.* (2014), who found that the supplementation of dietary GS and GSE at different levels to laying hens linearly increased the albumen index and Haugh unit value compared to the control group. These results are also supported by Romero *et al.* (2022), who reported an increase in the Haugh unit value of laying hens fed diets containing 60 g/kg GP, compared to the control treatment. Contrary to the results obtained in

the current study, some studies have reported that GP or GS had no significant effect on egg quality characteristics. For example, Silici *et al.* (2011) reported that the addition of GS to quail feed at levels of 0.5%, 1%, and 1.5% did not significantly affect the egg quality characteristics of Haugh unit, eggshell thickness, and egg yolk colour, compared to the control group. However, this was likely due to the low inclusion levels of GP used. Similarly, Kara *et al.* (2016) found that adding 4% and 6% GP to laying hen diets did not affect egg quality, and Herranz *et al.* (2024) reported no effect on the Haugh unit in a study evaluating the inclusion of GP (50 g/kg) in the diet of two genetic lines of chickens. On the other hand, Froes *et al.* (2018) stated that adding 0%, 2%, 4%, or 6% GP to quail diets linearly decreased egg weight, albumen weight, and egg specific gravity, whereas the Haugh unit and eggshell thickness were not affected by the treatments.

**Table 3** The egg quality characteristics of Japanese quails fed diets supplemented with different levels of grape pomace powder for eight weeks

| Parameters | Treatments | | | | SEM | *P*-value |
|---|---|---|---|---|---|---|
| | 0GP | 1GP | 2GP | 4GP | | |
| **Egg weight (g)** | 14.48 | 14.19 | 14.46 | 14.43 | 0.060 | 0.287 |
| **Eggshell breaking strength (kg/cm$^2$)** | 1.109$^b$ | 1.299$^a$ | 1.329$^a$ | 1.184$^b$ | 0.019 | 0.001 |
| **Eggshell weight (g)** | 1.67 | 1.59 | 1.65 | 1.55 | 0.018 | 0.058 |
| **Yolk weight (g)** | 4.67 | 4.58 | 4.52 | 4.61 | 0.032 | 0.381 |
| **Shape index (%)** | 78.84 | 79.31 | 78.59 | 79.42 | 0.172 | 0.279 |
| **Haugh unit** | 92.64$^b$ | 94.67$^a$ | 93.44$^{ab}$ | 93.79$^{ab}$ | 0.221 | 0.012 |
| **Yolk index (%)** | 48.61 | 48.22 | 49.08 | 47.71 | 0.344 | 0.551 |
| **Albumen index (%)** | 12.93$^b$ | 14.45$^a$ | 13.67$^{ab}$ | 13.75$^a$ | 0.136 | 0.001 |
| **Shell thickness (mm)** | 1.24 | 1.23 | 1.23 | 1.30 | 0.013 | 0.232 |
| **Yolk colour score** | 13.11 | 13.07 | 13.15 | 12.96 | 0.055 | 0.651 |
| **L* (lightness)** | 54.04 | 53.64 | 53.99 | 54.42 | 0.210 | 0.645 |
| **a* (redness)** | 16.39 | 15.48 | 16.19 | 15.55 | 0.210 | 0.325 |
| **b* (yellowness)** | 31.51 | 29.80 | 31.37 | 30.80 | 0.414 | 0.446 |

$^{a, b, c}$ Means with different superscripts in each row differ significantly ($P$ <0.05). SEM: standard error of the mean, 0GP: 0% grape pomace, 1GP: 1% grape pomace, 2GP: 2% grape pomace, 4GP: 4% grape pomace

In contrast with the egg yolk colour results obtained in this study, Froes *et al.* (2018) found that egg yolk pigmentation increased as the dietary GP level increased. This improvement in egg yolk colour was attributed to the anthocyanin content of the GP flour. Anthocyanin is the pigment that gives the purple colour to grapes (Abe *et al.*, 2007). Herranz *et al.* (2024) observed that the L* parameter was not affected by diet or chicken genetics; however, the a* parameter was significantly lower in the eggs of chickens consuming the GP-supplemented diet. Similarly, they found that the b* parameter tended to decrease when GP was included in the diets of the chickens.

The effects of dietary GP supplementation on blood biochemical parameters are reported in Table 4. The glucose and triglyceride concentrations were not affected by GP supplementation ($P$ >0.05). However, although it was not statistically significant, the triglyceride level was reduced by 13.85%, 18.61%, and 15.73% in the 1%, 2%, and 4% GP groups, respectively, compared to the control group. The inclusion of graded levels of GP in the quails' diets significantly decreased the concentrations of plasma total cholesterol and HDL cholesterol compared to the control diet. The lowest total cholesterol and HDL cholesterol levels were in the 2% GP-supplemented group.

The improved blood profile observed in the GP-supplemented quails could be attributed to the polyphenol content of GP. Polyphenols have been shown to lower the risk of diabetes, cardiovascular disease, and other chronic diseases by promoting the excretion of fat, cholesterol, and bile acids in the urine, as well as reducing chronic inflammation (Arvik *et al.*, 2018). Total cholesterol levels in the 1%,

2%, and 4% GP groups were lower than in the control group. Grape-derived polyphenols have been found to substantially improve serum indicators related to cardiovascular disease (Annunziata *et al.*, 2021), and polyphenols have antioxidant and anti-inflammatory properties, and can lower blood pressure and improve HDL levels (Gopi *et al.*, 2020). The benefits of polyphenols have been identified as being related to their interactions with the gut microbiome (Reda *et al.*, 2021; Abd El-Hack *et al.*, 2023). Zhang *et al.* (2022) reported that polyphenols affected the structure and function of the intestinal microbiota, enabling beneficial bacteria to produce metabolites such as short-chain fatty acids, which play an important role in promoting hormone secretion.

**Table 4** The blood biochemical parameters of Japanese quails fed diets supplemented with different levels of grape pomace powder for eight weeks

| Parameters (mg/dL) | Treatment groups | | | | SEM | *P*-value |
|---|---|---|---|---|---|---|
| | 0GP | 1GP | 2GP | 4GP | | |
| **Glucose** | 261.85 | 254.28 | 255.00 | 252.14 | 2.408 | 0.539 |
| **Total cholesterol** | 217.28[a] | 184.42[ab] | 153.00[a] | 157.71[a] | 0.081 | 0.010 |
| **HDL cholesterol** | 118.55[a] | 99.62[ab] | 77.94[b] | 81.20[b] | 5.955 | 0.048 |
| **Triglyceride** | 148.45 | 127.93 | 120.81 | 125.09 | 8.431 | 0.686 |

[a, b, c] Means with different superscripts in each row differ significantly ($P$ <0.05). 0GP: 0% grape pomace, 1GP: 1% grape pomace, 2GP: 2% grape pomace, 4GP: 4% grape pomace, SEM: standard error of the mean, HDL: high-density lipoprotein

Similar results were reported by Kaya *et al.* (2014), who found that dietary supplementation of GS and GSE decreased serum cholesterol and glucose concentrations in laying hens. This decrease in total cholesterol concentration was attributed to the polyphenolic compounds and insoluble fibre structures in GS, which increase the excretion of lipids and accordingly reduce the cholesterol level (Kaya *et al.*, 2014). In contrast, Kara *et al.* (2016) reported that laying hens supplemented with 4% and 6% GP had no significant differences in total cholesterol and triglycerides, compared to the control group. However, they reported a significant decrease in the glucose levels in hens supplemented with GP (Kara *et al.* 2016). Kılınç & Karaoğlu (2020) observed that GS oil added to laying hen diets did not affect total cholesterol and triglyceride concentrations, whereas significant variations among groups were reported for the levels of serum HDL. Ebrahimzadeh *et al.* (2018), in their study on broiler chickens fed 5%, 7.5%, and 10% GP, reported that there were no effects on glucose and total cholesterol concentrations, but triglyceride and HDL cholesterol levels differed significantly between the treatment groups. Farahat *et al.* (2017) found that HDL cholesterol and total cholesterol levels did not significantly differ between treatments in broilers fed GS extract. These conflicting results for the effects on lipid and glucose metabolism could be attributed to the varying levels of GP extract supplemented and varying levels of bioactive compounds and/or anti-nutritional factors in the GP used, in addition to other factors such as the duration of the experiment, the poultry breed used, and the environmental conditions.

Adding GP to Japanese quails' diets had a significant impact on the chicks' live weights and early embryonic mortality rates, as shown in Table 5. The control group's average chick live weight was 10.32 g, which was considerably higher than those of the GP-supplemented groups. There was no significant difference between the groups in terms of fertility rate. However, the fertility rate was numerically higher with increasing GP supplementation, compared to the control group. Similarly, the hatchability of set eggs and fertile eggs was also higher in the supplemented groups than in the control group, although this difference was non-significant. Supplementing GP also significantly reduced early embryonic mortality rates compared to the control group, highlighting the potential of GP as a protective agent during crucial early stages of development. These results may be attributed to the antioxidant effects of the GP, which may have prevented the formation of free radicals in the egg yolk. The results obtained in this study are consistent with those reported by Bocsan *et al.* (2022), who emphasised the antioxidant properties of GP and its beneficial effects on bird health. Chedea *et al.* (2021) showed that the polyphenols found in GP can effectively decrease oxidative stress. This could explain the reduced early embryonic mortality with GP supplementation.

As found in this study, Hafez *et al.* (2023) reported that the fertility rate in laying hens receiving 250, 500, and 750 g/kg GSE was higher than in the control group. Hafez *et al.* (2023) explained that GSE reduces blood malondialdehyde levels in chickens, which increases fertility by reducing lipid peroxidation. They further reported that there was no difference between the groups in the hatchability of set eggs and the hatchability of fertilised eggs, and stated that the hatchability of fertilised eggs was higher in the GSE-supplemented groups than in the control. The higher fertility rate, hatchability of set eggs, and hatchability of fertilised eggs in the GP-supplemented groups could be attributed to the antioxidant capacity of the GP. Şimşek *et al.* (2015) reported that the fertility rate in quails was increased by the dietary supplementation of cinnamon and rosemary oils ($P$ >0.05), but that the hatchability of set eggs and hatchability of fertile eggs were not affected by the additives ($P$ >0.05).

**Table 5** The hatchability parameters of eggs from Japanese quails fed diets supplemented with different levels of grape pomace powder for eight weeks

| Parameters | Treatment groups | | | | SEM | *P*-value |
|---|---|---|---|---|---|---|
| | 0GP | 1GP | 2GP | 4GP | | |
| **Chick live weight (g)** | 10.32[a] | 10.15 [ab] | 9.92[b] | 10.07[ab] | 0.043 | 0.013 |
| **Hatchability (%)** | | | | | | |
| **Fertile eggs** | 84.24 | 93.05 | 96.18 | 92.30 | 0.052 | 0.205 |
| **Set eggs** | 78.75 | 87.50 | 93.75 | 91.25 | 0.413 | 0.124 |
| **Fertility rate (%)** | 93.75 | 93.75 | 97.50 | 98.75 | 0.042 | 0.208 |
| **Embryonic mortality (% of fertile eggs)** | | | | | | |
| **Early (0–9 d)** | 14.51[a] | 1.39[b] | 2.50[b] | 3.81[b] | 0.730 | 0.008 |
| **Middle (9–17 d)** | 0.00 | 4.16 | 1.31 | 2.63 | 0.972 | 0.512 |
| **Late (17–18 d)** | 1.25 | 1.39 | 0.00 | 1.25 | 0.523 | 0.800 |

[a, b, c] Means with different superscripts in each row differ significantly ($P$ <0.05). 0GP: 0% grape pomace, 1GP: 1% grape pomace, 2GP: 2% grape pomace, 4GP: 4% grape pomace, SEM: standard error of the mean

## Conclusion

This study was conducted to determine the effects of GP supplementation to laying quail rations on performance, egg quality, serum parameters, and hatchability. The addition of GP had a significant effect on feed intake, egg production, and egg weight, with egg production being higher in the 1% and 2% GP-supplemented groups than in the control group, and feed intake being lower in the GP-supplemented quails than in the control quails. Egg quality criteria such as eggshell breaking strength, Haugh unit, and albumin index were improved by GP supplementation, highlighting the role of dietary polyphenols in improving egg quality parameters. The fact that the Haugh unit and albumen index values, which are accepted as indicators of egg freshness, were higher in the GP-supplemented than in the control group can be attributed to the antioxidant activity and phenolic compound content of GP. This study showed significant changes in blood cholesterol levels with increasing GP supplementation, with lower total cholesterol levels observed in the 1%, 2%, and 4% GP groups than in the control group. Additionally, GP supplementation reduced the early embryonic mortality rate, suggesting its potential protective role during crucial developmental stages. As a result, the inclusion of 1% and 2% GP in the diets of laying quails could increase egg production, and the antioxidant and phenolic compound content of GP could significantly enhance some egg quality characteristics and reduce early embryonic deaths.

**Conflicts of interest**

The authors declare that they have no conflicts of interest.

**Additional information**

This study is taken from the doctoral thesis of N.M.A. Zebari.

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