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Effects of grapefruit juice supplementation on the performance, egg quality, and blood biochemistry of late-phase laying hens

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Abstract

This study investigated the effects of grapefruit juice supplementation on the performance, egg quality, and blood biochemistry of late-phase laying hens. A total of 144 Babcock White laying hens, aged 62 weeks and weighing 1603.05 ± 14.33 g, were divided into six groups. Each group was further divided into four replicates containing six hens each. Grapefruit juice was added to the hens' drinking water at concentrations of 0% (control), 0.25%, 0.50%, 1.0%, 2.5%, and 5.0% for four weeks. Grapefruit juice supplementation positively affected hen-day egg production and egg mass, but did not affect body weight, feed consumption, feed conversion ratio, or egg weight. The water consumption of the 5.0% group was higher than that of the other groups. Although grapefruit juice supplementation increased the Haugh unit, albumen index, and yolk index values, it decreased the eggshell thickness and egg yolk colour values. Serum glucose levels were lower in the 1.0% and 2.5% groups (134.83 mg/dL and 148.16 mg/dL, respectively) than in the control group (186 mg/dL), and serum immunoglobulin G levels were higher in the 1.0% and 2.5% groups than in the other groups. While the grapefruit juice supplementation increased the total antioxidant capacity of the hens, it reduced their gamma-glutamyl transferase values. No differences were observed between the groups in terms of high-density lipoprotein, low-density lipoprotein, cholesterol, alanine aminotransferase, alkaline phosphatase, or total oxidant status values. The results indicate that providing grapefruit juice to laying hens improves their performance, egg quality, and metabolism, depending on dose.

Keywords: citrus, egg yield, egg quality, Haugh unit, immune system

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Introduction

In modern laying-hen nutrition, the integration of natural and functional additives into animal diets is increasingly regarded as a sustainable approach to enhancing productivity, maintaining egg quality, and supporting animal health. The global ban on the use of antibiotic growth promoters is the main reason for the intensified interest in plant-based alternatives with bioactive properties (Aminullah *et al.*, 2025). However, the increasing concern of consumers about animal welfare, as well as the rearing

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conditions and management practices used in egg production, has also played a role (Sinclair *et al.*, 2022). It has been demonstrated that plant-based feed additives induce favourable changes in performance, gut structure, and the immune system, and that antibiotics can thus be replaced by plant-based feed additives with satisfactory outcomes (Afiouni *et al.*, 2023). Ayoob *et al.* (2023) found that dietary *Eucalyptus globulus* intake improved the growth performance and blood parameters of broilers and boosted their immune defence against diseases. Moreover, Abou-Elkhair *et al.* (2018) reported that the incorporation of natural, plant-based feed additives into the diet of layer hens improved their egg production and egg quality parameters by modulating the lipid content and antioxidative status of the eggs. The antimicrobial and anti-inflammatory traits of plant-based feed additives have also been investigated by several researchers (Mohammadi Gheisar & Kim, 2018).

Citrus fruits belong to the Rutaceae botanical family and are highly valued as cultivated fruits worldwide. These fruits are rich in essential nutrients such as vitamin C (ascorbic acid), vitamin A, vitamin E (tocopherols and tocotrienols), various B vitamins (including pantothenic acid), and dietary fibre, as well as in important minerals such as selenium, zinc, copper, iron, manganese, and potassium (Zou *et al.,* 2016; Lu *et al.,* 2023). Flavonoids contribute to the bitter taste of certain citrus fruits, such as grapefruits, lemons, and oranges (Okwu 2005), and possess a strong ability to influence the body's reactions to allergens, viruses, and carcinogens. They also exhibit various beneficial activities, including anti-allergic, anti-inflammatory, antimicrobial, and anti-cancer effects. Grapefruit (*Citrus paradisi*) fruits specifically contain several flavonoid glycosides, including narirutin, naringin, naringenin, quercetin, kaempferol, hesperidin, neohesperidin, didymin, poncirin, bergamottin, and nootkatone (Okwu & Emenike, 2006). Of these, naringin (a glycoside), naringenin (an aglycone), and hesperidin are the most abundant active ingredients found in the juice, seeds, and peels of grapefruit (Kiani & Imam, 2007).

Grapefruit's active ingredients have significant effects on metabolism. According to Gorinstein *et al.* (2006), grapefruit intake positively affects various types of serum lipids, particularly reducing serum triglyceride levels. The same authors also noted increased serum antioxidant activity in human models over a 30-day period. Naringin supplementation specifically has been found to have a number of health benefits, including antioxidant, antimicrobial, anti-inflammatory, anti-apoptotic, anti-mutagenic, and hypocholesterolaemic effects (Jeon *et al.*, 2004). In terms of the effects on poultry, Goliomytis *et al.* (2015) observed a significant improvement in the antioxidant activity of broiler meat from chickens supplemented with dietary naringin. Furthermore, Iskender *et al.* (2017) reported that dietary hesperidin supplemented to laying hens reduced egg yolk cholesterol, whereas naringin supplementation had no effect on yolk cholesterol levels. Hens supplemented with either hesperidin or naringin exhibited a stronger anti-inflammatory response compared to non-supplemented hens (Goliomytis *et al.*, 2019), and Gültepe *et al.* (2019) showed that dietary supplementation with lemon juice improved egg production and egg quality, without impairing metabolism.

While there are some studies regarding the effects of citrus fruits on poultry production and health in the literature, there are very limited studies on the effects of grapefruit on poultry production and health. The objective of this study was thus to explore the impacts of varying concentrations of grapefruit juice on the performance of laying hens. This study also aimed to assess how these different levels of grapefruit juice influenced egg quality and serological parameters in hens.

Materials and methods

The research protocol was approved by the Ethics Committee for Animal Experiments of Afyon Kocatepe University, with reference number: 49533702/62 (date: 03/05/2017).

Animals and experimental design

The study used 144 Babcock White late-phase laying hens, all of which were 62 weeks old at the start of the study. The mean initial body weight of the hens was 1603.05 ± 14.33 g. The hens were randomly divided into six groups of 24 hens each. Each group was further split into four replicates of six hens each. The study lasted for four weeks, and during this time, the laying hens were exposed to a light cycle of 16 hours of light followed by eight hours of darkness. The hens had unrestricted access to both feed and water. All groups were provided with a basal diet formulated according to the nutritional requirements of the birds, as outlined by the National Research Council (NRC, 1994). The composition of the basal diet is provided in Table 1. Drinking water was provided by a nipple drinking system attached to separate tanks for each group.

Table 1 Ingredients and nutritional composition of the basal diet provided to late-phase laying hens

Ingredients	(g/kg as-fed)
Maize	549
Sunflower meal (32% crude protein)	169.3
Full-fat soya	100
Limestone	78.7
Soybean meal (44% crude protein)	73.9
Dicalcium phosphate	17.3
Common salt	4.0
Vegetable oil	3.4
Vitamin-mineral premix[1]	2.5
L-lysine HCl	1.0
DL-methionine	1.0
Calculated nutritional values	**(g/kg, except where otherwise indicated)**
Metabolisable energy (kcal/kg)[2]	2778
Crude protein	170
Calcium[3]	37.1
Available phosphorus[3]	3.8
Sodium[3]	2.0
Methionine + cysteine[3]	7.1
Lysine[3]	8.3
Threonine[3]	6.1
Tryptophan[3]	2.0
Linoleic acid[3]	3.6
Analysed nutritional values	**(g/kg)**
Crude protein[4]	172
Ether extract[4]	49
Starch	369.6

[1] Provided per kg of diet: vitamin A: 12 000 000 IU, vitamin D_3: 3 000 000 IU, vitamin E: 35 000 IU, vitamin K_3: 3500 IU, vitamin B_1: 2750 IU, vitamin B_2: 5500 IU, nicotinamide: 30 000 IU, Ca-D-pantothenate: 10 000 IU, vitamin B_6: 4000 IU, vitamin B_{12}: 15 IU, folic acid: 1000 IU, D-biotin: 50 IU, choline chloride: 150 000 IU, manganese: 80 000 mg, iron: 60 000 mg, zinc: 60 000 mg, copper: 5000 mg, iodine: 2000 mg, cobalt: 500 mg, selenium: 150 mg, antioxidant (ethoxyquin): 15 000 mg. [2] Calculated using the equation described by Carpenter & Clegg (1956). [3] Calculated according to the methods of the NRC (1994). [4] Analysed as outlined by the Association of Official Analytical Chemists (1994).

The Star Ruby grapefruit cultivar used in this study was purchased from a local market in Afyonkarahisar, Türkiye. Juice was extracted using a commercial juicer to squeeze the grapefruits, followed by filtration to remove any residue. The extraction was carried out freshly every day throughout the study, and drinking water was prepared daily to ensure freshness. To prepare the drinking water, the water remaining from the previous day was drained, the tank was cleaned, and it was refilled with fresh water. The grapefruit juice was then added at the concentrations required for each treatment: 0% (control), 0.25%, 0.50%, 1.0%, 2.5%, and 5.0% of the volume of the drinking water tank. The components of the grapefruit juice were analysed using high-performance liquid chromatography, as described by Ribeiro & Ribeiro (2008).

Data collection

Water consumption was determined daily by subtracting the amount of remaining water in the tank from the amount of water initially provided. The live weights of the hens were measured both at the beginning and at the end of the study. Egg production was recorded daily, while feed consumption was

recorded weekly. The feed conversion ratio (FCR) was calculated using the feed consumption and egg mass data. Additionally, eggs were weighed weekly to determine their individual weights. Egg quality parameters, including the colour of the egg yolk, the albumen index, the yolk index, the eggshell thickness, and the Haugh unit, were determined at ends of the second and fourth weeks of the study. The Haugh unit was calculated based on the height of the albumen, which was measured using a digital calliper (CD-15CP, Mitutoyo Ltd., UK), as established by Haugh (1937). Yolk colour was assessed using the Roche Improved Yolk Colour Fan, which features 15 colour bands representing various shades of egg yolk colour. The yolk colour was compared to these bands on the colour fan to assign a specific colour value for each egg (YolkFan™, DSM Nutritional Products AG, Kaiseraugst, Switzerland). The albumen index and yolk index were determined using the methodology developed by Tılkı & Saatcı (2004).

At the end of the trial, three hens per replicate (12 hens per treatment) were randomly selected. Blood sampling and analysis were performed as outlined by Viana *et al.* (2021), with some modification of the anticoagulant used, which was sodium fluoride in the original study. Blood samples were collected directly from the hearts of the hens via cardiac puncture. The collected blood was placed in tubes containing ethylenediaminetetraacetic acid (EDTA). In the laboratory, the blood samples in the EDTA tubes were centrifuged at approximately 5000 rpm for 10 minutes. After centrifugation, the supernatant (serum) was carefully transferred from the tops of the tubes into Eppendorf tubes. The Eppendorf tubes containing the serum samples were then stored at −20 °C until further analysis. Serum biochemical parameters were analysed using an automated enzyme-linked immunosorbent assay analyser (Elisys Uno, HUMAN mbH, Wiesbaden, Germany). The analysed parameters included glucose, total cholesterol (CHO), high-density lipoprotein (HDL), low-density lipoprotein (LDL), aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), gamma-glutamyl transpeptidase (GGT), total antioxidant capacity (TAC), total oxidant status (TOS), and immunoglobulin G (IgG) concentrations.

Statistical analysis

The data were analysed using the generalised linear model procedure in the SAS statistical program (2008). The model included the grapefruit juice supplementation, the sampling week, and their interaction as fixed effects, while the replicates were included as random effects. The effect of grapefruit juice supplementation was considered throughout the supplementation period and was reported as the main effect of the grapefruit juice. Because blood samples were only collected at the end of the study, the effect of time was excluded from the model used to analyse the serum biochemical parameters. Data are presented as least square means ± the standard errors of the means (SEM), with statistical significance set at $P \leq 0.05$.

Results

The composition of the grapefruit juice used in this study is shown in Table 2, and the effects of the grapefruit juice supplementation on the hens' performance parameters are shown in Table 3.

Table 2 Composition of the grapefruit juice supplemented to late-phase laying hens

Ingredients	Quantity
β-carotene (mg/100 g)	0.470
α-tocopherol (mg/100 g)	0.102
Total phenolic matter (mg/100 g)	309.00
Iron (µg/L)	135.60
Zinc (µg/L)	433.40
Copper (µg/L)	19.64

Table 3 Effects of grapefruit juice (GJ) supplementation on the performance parameters of late-phase laying hens[1]

	Initial body weight (g)	Final body weight (g)	Feed consumption (g/hen/day)	Hen-day egg production (%)	FCR	Egg weight (g)	Egg mass (g/hen/day)	Water consumption (L/day)
Control	1568.88	1568.96	111.25	83.03[b]	0.05	65.16	54.05[b]	0.40[b]
0.25% GJ	1572.75	1621.08	108.12	88.24[a]	0.89	64.74	57.16[a]	0.33[d]
0.50% GJ	1596.13	1643.75	111.25	86.75[a]	0.96	65.22	56.52[a]	0.39[b]
1.00% GJ	1643.46	1685.75	112.50	84.82[ab]	0.03	65.15	55.26[ab]	0.36[c]
2.50% GJ	1610.08	1670.54	110.00	86.16[ab]	0.92	66.36	57.20[a]	0.40[b]
5.00% GJ	1624.33	1678.78	111.87	87.50[a]	0.95	64.91	57.36[a]	0.43[a]
***P*-value (GJ × time)**	-	-	0.802	0.753	0.700	0.111	0.103	0.747
***P*-value (GJ)**	0.635	0.199	0.714	0.050	0.101	0.347	0.033	<0.0001
***P*-value (time)**	-	-	0.010	0.017	0.227	0.949	0.017	0.114
SEM	35.28	36.28	2.05	1.27	0.04	0.53	0.83	0.00

[1] Data are represented as least square means. The values are averages of four replicate cages of six hens each per treatment (n = 24). Control: no GJ supplementation (plain water); 0.25% GJ: 0.25% GJ supplemented in drinking water; 0.5% GJ: 0.5% GJ supplemented in drinking water; 1% GJ: 1% GJ supplemented in drinking water, 2.5% GJ: 2.5% GJ supplemented in drinking water; 5% GJ: 5% GJ supplemented in drinking water.

[abc] Values with different superscript letters in the same column are significantly different ($P \leq 0.05$).

FCR: feed conversion ratio (feed consumption in g/egg mass in g), SEM: standard error of the mean.

While grapefruit juice supplementation increased the hen-day egg production and egg mass per hen values in all the treatment groups, the hen-day egg production only differed significantly between the 0.25%, 0.5%, and 5.0% treatment groups and the control group. The egg mass was also significantly higher for the 0.25%, 0.5%, 2.5%, and 5.0% groups than for the control group. Grapefruit juice supplementation did not have any effect on the feed consumption, FCR, or egg weight. The laying hens supplemented with 5.0% grapefruit juice in their drinking water consumed more water than those in the other groups (P <0.001). However, grapefruit juice supplementation did not have an effect on the final body weights of the birds at the end of the four-week period. There was an effect of time on the feed consumption, hen-day egg production, and egg mass (P <0.05), but there was no time effect on the FCR, egg weight, or water consumption. None of the measured performance parameters were affected by the time × treatment interaction (P >0.05, Table 3).

Statistically significant differences between the treatment groups were found for all examined egg quality parameters (Table 4).

Table 4 Effects of grapefruit juice (GJ) supplementation to late-phase laying hens on egg quality parameters[1]

	Haugh unit	Egg yolk colour	Eggshell thickness (mm)	Albumen index	Yolk index
Control	90.78^{b}	12.04^{ab}	0.40^{a}	11.63^{b}	43.02^{bc}
0.25% GJ	86.21^{c}	12.06^{a}	0.38^{b}	10.50^{c}	41.97^{c}
0.50% GJ	89.67^{b}	11.61^{c}	0.37^{b}	10.96^{bc}	44.36^{ab}
1.00% GJ	94.14^{a}	11.68^{c}	0.37^{b}	13.16^{a}	42.44^{c}
2.50% GJ	95.12^{a}	11.79^{bc}	0.38^{b}	12.94^{a}	45.24^{a}
5.00% GJ	95.73^{a}	11.77^{c}	0.37^{b}	13.05^{a}	45.57^{a}
SEM	1.15	0.09	0.00	0.40	0.68
***P*-value (GJ × time)**	<0.0001	<0.0001	0.715	<0.0001	0.402
***P*-value (GJ)**	<0.0001	0.001	0.000	<0.0001	<0.0001
***P*-value (time)**	<0.0001	0.090	0.185	<0.0001	<0.0001

[1] Data are represented as least square means. The values are averages of four replicate cages of six hens each per treatment (n = 24). Control: no GJ supplementation (plain water); 0.25% GJ: 0.25% GJ supplemented in drinking water; 0.5% GJ: 0.5% GJ supplemented in drinking water; 1% GJ: 1% GJ supplemented in drinking water, 2.5% GJ: 2.5% GJ supplemented in drinking water; 5% GJ: 5% GJ supplemented in drinking water.
[abc] Values with different superscript letters in the same column are significantly different (P ≤0.05).
SEM: standard error of the mean.

The addition of grapefruit juice to the drinking water of the hens resulted in a decrease in eggshell thickness across all experimental groups, in comparison to the control group (P <0.001). The eggs from the hens that consumed drinking water supplemented with 1.0%, 2.5%, and 5.0% grapefruit juice exhibited higher Haugh unit values than the eggs from the hens in the other groups (P <0.001). Grapefruit juice supplementation at 0.25% and 2.5% did not affect egg yolk colour; however, egg yolk colour values were lower in the 0.5%, 1.0%, and 5.0% groups (P <0.05). While the albumen index decreased in the 0.25% group, it increased in the 1.0%, 2.5%, and 5.0% groups. The yolk index was not affected by the lower levels of grapefruit juice supplementation (0.25%, 0.5%, and 1.0%), but was higher in the 2.5% and 5.0% groups (P <0.05). While there were time effects on the Haugh unit, albumen index, and yolk index values, egg yolk colour and eggshell thickness were not significantly influenced by time. There was also a time × treatment interaction effect for the Haugh unit, egg yolk colour, and albumen index values (P <0.05), whereas the eggshell thickness and yolk index values did not show a significant difference based on the time × treatment interaction.

The effects of grapefruit juice supplementation on the different serological indicators are shown in Table 5.

Table 5 Effects of grapefruit juice (GJ) supplementation to late-phase laying hens on serological indicators[1]

	Glucose (mg/dL)	CHO (mg/dL)	HDL (mg/dL)	LDL (mg/dL)	AST (U/L)	ALT (U/L)	LP (U/L)	TOS (µmol/dL)	TAC (mmol/L)	GGT (U/L)	IgG (mg/dL)
Control	186.75^{a}	120.58	17.16	38.58	199.16^{a}	1.66	363.2	123.98	1.07^{b}	34.30^{a}	115.33^{c}
0.25%GJ	180.25^{a}	10.9	19.63	42.45	216.80^{a}	10.6	377.22	124.82	1.19^{ab}	34.18^{a}	130.54^{bc}
0.50%GJ	181.09^{a}	112.36	18.27	38.9	211.18^{a}	13.18	320.1	104.32	1.35^{a}	35.54^{a}	141.18^{abc}
1.00%GJ	134.83^{c}	105.5	17.91	36.58	164.25^{b}	10.25	335.5	115.04	1.31^{a}	27.81^{b}	165.66^{a}
2.50%GJ	148.16^{bc}	102.58	17.41	38.33	161.50^{b}	11.91	386.9	116.16	1.30^{a}	26.83^{b}	159.75^{ab}
5.00%GJ	169.09^{ab}	114.41	17.58	46.58	187.25^{ab}	15.83	287.1	105.28	1.08^{b}	29.50^{b}	128.33^{c}
SEM	8.28	9.75	0.86	4.42	10.08	1.83	44.48	18.52	0.06	1.45	11.02
***P*-value**	<0.0001	0.823	0.432	0.643	<0.0001	0.316	0.599	0.947	0.02	<0.0001	0.014

[1] Data are represented as least square means. The values are averages of four replicate cages of six hens each per treatment (n = 24). Control: no GJ supplementation (plain water); 0.25% GJ: 0.25% GJ supplemented in drinking water; 0.5% GJ: 0.5% GJ supplemented in drinking water; 1% GJ: 1% GJ supplemented in drinking water, 2.5% GJ: 2.5% GJ supplemented in drinking water; 5% GJ: 5% GJ supplemented in drinking water.

[abc] Values with different superscript letters in the same column are significantly different ($P \leq 0.05$).

CHO: total cholesterol, HDL: high-density lipoprotein, LDL: low-density lipoprotein, AST: aspartate aminotransferase, ALT: alanine aminotransferase, ALP: alkaline phosphatase, TOS: total oxidant status, TAC: total antioxidant capacity, GGT: gamma-glutamyl transferase, IgG: immunoglobulin G, SEM: standard error of the mean.

Grapefruit juice supplementation in the drinking water had a significant impact on serum glucose levels. Compared to the control group, glucose levels were lower in all the grapefruit juice treatment groups. Specifically, the group supplemented with 1.0% grapefruit juice had the lowest glucose levels (P <0.001). Grapefruit juice supplementation also affected the TAC (P <0.05), AST (P <0.001), GGT (P <0.001), and IgG (P <0.05) levels. The supplementation of grapefruit juice resulted in an increase in IgG levels, with this increase being most pronounced in the group supplemented with 1.0% grapefruit juice. Serum AST levels were lower in the 1.0% and 2.5% groups than in the control group and the groups supplemented with lower doses of grapefruit juice, whereas the 5.0% group had AST levels similar to those of the other groups. The serum GGT levels in the 1.0%, 2.5%, and 5.0% groups were lower than those in the groups supplemented with lower doses of grapefruit juice and the control group. The levels of the other biochemical parameters, including the HDL, LDL, CHO, TOS, and ALT, were not significantly affected by grapefruit juice supplementation (Table 5).

Discussion

The iron, copper, and zinc contents of the grapefruit juice used in this study were lower than those reported by Gorinstein *et al.* (2001). Additionally, the total phenolic content was lower than that determined by Kelebek (2010). This inconsistency in the constituents of the various parts of the grapefruit, such as the juice, peel, or pomace, could be as a result of the concentrations of these components varying in response to the fruit's stage of growth (Singh *et al.*, 2021).

Supplementing late-phase laying hens' drinking water with grapefruit juice positively affected their hen-day egg production, water consumption, and egg mass per hen per day. Hen-day egg production was increased in all the experimental groups compared to the control group, indicating that grapefruit juice supplementation positively influenced egg production. However, grapefruit juice supplementation did not have statistically significant effects on other parameters, including feed consumption, FCR, egg weight, and body weight. This outcome emphasises the potential advantages of adding grapefruit juice to the diets of laying hens to improve their egg production parameters. The results of our study are consistent with the findings of Gültepe *et al.* (2019), who examined the effects of lemon juice supplementation. In both studies, incorporating the juice of a citrus fruit into the drinking water did not significantly impact egg weight, feed consumption, or FCR. The increases in egg yield and egg mass were believed to be linked to the flavonoid content of the citrus juice, and particularly the flavanones that are found in citrus fruits and are known to have beneficial effects on metabolism and various physiological processes (Yang *et al.*, 2023). It has been reported that dietary flavanone intake improves oestradiol, progesterone, follicle-stimulating hormone, and luteinising hormone concentrations, as well as the mRNA expression of genes related to egg yolk precursor synthesis in hens (Yang *et al.,* 2018; Amevor *et al.,* 2021). Moreover, grapefruit intake is known to interfere with oral oestrogen-replacement therapy, potentially because of its inhibitory effects on cytochrome P450 3A4 (CYP3A4), an enzyme involved in the metabolism of endogenous oestrogens (Spencer *et al.*, 2009). By reducing the activity of this enzyme, grapefruit consumption may lead to elevated circulating oestrogen levels. Oestradiol has been recognised as the key steroid responsible for the activation and stimulation of the production of the eggshell and internal egg components in hens (Hanlon *et al.*, 2022). The increased egg production rate in this study could thus have resulted from the increased secretion of reproductive hormones.

In this study, the body weight, FCR, and feed consumption of the hens did not differ significantly between the groups, whereas their water consumption levels differed. In particular, the group supplemented with 5.0% grapefruit juice had higher water consumption levels than all the other groups. Various components of grapefruit affect its sensory properties, such as colour, aroma, sweetness, and bitterness (Gous *et al.*, 2019), and the specific sensory properties of the grapefruit juice could be the reason for the increased water consumption in the 5.0% group. The improved palatability of the drinking water may have contributed to the increased water consumption observed in the 5.0% treatment group. It has been reported that the quality of drinking water is important, because of its impact on water intake and, consequently, on performance (Ebrahimi *et al.*, 2024). Based on the findings of the current study, it could be suggested that the improved productivity observed in the treatment groups might be attributed to their increased water intake. However, Raut *et al.* (2025) reported that poor water quality can negatively affect performance parameters even when water intake remains unchanged. Therefore,

future research should investigate how water quality affects intake and whether water consumption rate is linked to the performance of laying hens.

The Haugh unit, albumen index, and egg yolk index were all significantly higher in the groups supplemented with 1.0%, 2.5%, and 5.0% grapefruit juice than in the control group and the other treatment groups. In contrast, egg yolk colour and eggshell thickness were significantly lower in all the grapefruit juice-supplemented groups than in the control group. Gültepe *et al.* (2019) similarly reported that the addition of lemon juice to the diets of laying hens increased the Haugh unit but led to a lighter yolk colour. However, lemon juice supplementation had no significant effect on the eggshell thickness, albumen index, or yolk index. Boğa Kuru *et al.* (2019) stated that egg weight was negatively correlated with eggshell thickness. Considering the increased hen-day egg production and egg mass in our study, a possible reason for the reduced eggshell thickness could be the increased productive outcomes. The increase in egg production could also be a reason for the altered egg yolk colour.

Dietary polyphenol supplementation can enhance both the performance and egg quality of laying hens (Wang *et al.*, 2020). Zhu *et al.* (2020) observed that incorporating tea polyphenols into the diets of laying hens led to reduced feed consumption and improved FCR. However, high levels of tea polyphenols negatively affected eggshell and egg white quality. Zhou *et al.* (2021) found that administering tea polyphenols to laying hens during the late laying period resulted in increased egg production and improved egg white quality. Additionally, Chen *et al.* (2021) reported that adding magnolol to the diets of hens in the late laying stage enhanced both egg white quality and the Haugh unit. Li *et al.* (2022) similarly showed that dietary supplements containing 0.1%, 0.2%, and 0.4% naringin, administered over an eight-week period, increased both egg production and egg mass, which aligns with our findings. However, these authors also noted improvements in egg yolk colour and FCR, which were not observed in our study. These differences could be related to differences in the phenolic compound quantity naturally found in grapefruit and that used in previous studies.

Blood chemistry is an important tool for analysing the effects of various feed additives on the health of birds and for diagnostic purposes. It also serves as a key indicator for assessing the nutritional, pathological, and physiological status of the birds (Oleforuh-Okoleh *et al.*, 2015). In our study, glucose and GGT levels were significantly lower in the groups receiving grapefruit juice supplementation, while their TAC and IgG levels were higher. According to Ayoob *et al.* (2023), herbal products can significantly enhance immunity and resistance to diseases in broilers, and some studies have shown that dietary sweet orange supplementation enhanced serum levels of IgG and IgM in broilers (Pourhossein *et al.,* 2015; Pourhossein *et al.,* 2019). It has further been reported that flavonoids modulate the immune system by promoting T-cell responses, reducing reactive oxygen species production, and inhibiting inflammatory processes (Hoskin & Coombs, 2022). In this study, the unchanged TOS and increased TAC values suggested that grapefruit juice enhanced the antioxidant capacity of the laying hens, without reducing their oxidant status. For this reason, the effects of grapefruit intake on reactive oxygen species production should be investigated further.

In this study, there was a decrease in CHO levels in response to grapefruit juice supplementation, although this was statistically insignificant. The active ingredients in citrus fruits show inhibitory effects on cholesterol, LDL, and triglyceride synthesis by acting on the target genes and enzymes involved in lipid synthesis and beta-oxidation (Carvalho *et al.*, 2022). However, despite the numerical decrease in CHO levels, LDL, HDL, and CHO levels did not change significantly. The use of synthetic flavonoids can provide active ingredients at concentrations much higher than those naturally present in grapefruit. Therefore, the cholesterol-lowering effects of the fruit itself warrant further investigation. Additionally, our results indicated an increase in IgG levels, which is consistent with the results of Lien *et al.* (2008), who also reported elevated IgG levels.

Grapefruit juice supplementation also reduced the serum glucose levels in the 1.0% and 2.5% groups. In a previous study, naringenin administered at a dose of 50 mg/kg was found to decrease fasting blood sugar levels in the serum by lowering glycosylated haemoglobin levels and increasing insulin concentrations, thus demonstrating antihyperglycemic effects. This effect of naringenin was comparable to that of the standard anti-diabetic drug, gliclazide (Annadurai *et al.*, 2012). Additionally, Ortiz-Andrade *et al.* (2008) reported the hypoglycaemic effects of naringenin in a streptozotocin-nicotinamide rat model for diabetes at a dosage of 50 mg/kg. A possible underlying mechanism for the decreased serum glucose levels was that the naturally occurring active flavonoids in the citrus fruits increased insulin sensitivity and glucose uptake while lowering the activity of key enzymes involved in

gluconeogenesis (Lu & Yip, 2023). Therefore, it could be suggested that the reduced serum glucose levels found in our study were an effect of the naringenin that is naturally found in grapefruit juice.

The results of this study suggest that grapefruit juice may be a suitable additive for poultry diets. However, different cultivars and stages of vegetation are factors that may affect the ingredient composition of grapefruit juice. In future studies, this issue should be taken into consideration. In light of the results of this study, it could also be suggested that other products of grapefruit, such as the peel or pomace, could also be suitable additives, and these should be further investigated. However, these parts of the grapefruit can reduce the digestibility of a basal diet, and this should be taken into account. Although grapefruit juice seems to be more suitable from this point of view, its effects on nutrient digestibility should also be investigated. Limitations of this study include the lack of an analysis of the efficiency outcomes of the absorption of the components of grapefruit juice. Hence, the absorption efficiency of the grapefruit juice constituents should be investigated, as it is possible that the nutritive value of eggs could be enhanced by grapefruit supplementation. The effects of grapefruit supplementation on the chemical composition of the eggs should therefore be further investigated.

Conclusions

In conclusion, grapefruit juice is a possible alternative dietary additive for laying hens as it positively affects their productivity and the quality of the derived products. It also enhances metabolism and immunity and shows antioxidant properties. Further studies on grapefruit juice supplementation are recommended, particularly involving higher concentrations of grapefruit juice, extended trial durations, and other breeds of laying hens, so as to more comprehensively explore its effects on productivity and egg quality parameters in laying hens.

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Authors' contributions

ÜÖ: conceptualisation, data collection, sample analysis, writing (original draft, revision), and funding acquisition. MEO: validation and writing (original draft, revision). CU: data collection, sample analysis, data curation, and writing (original draft, revision). EEG: conceptualisation, methodology, data collection, sample analysis, and writing (original draft). İSÇ: conceptualisation, methodology, validation, and supervision. AI: data collection, sample analysis, data analysis, and writing (original draft, revision). İB: conceptualisation, methodology, data collection, data analysis, validation, writing (initial draft, revision), supervision, and funding acquisition.

Conflict of interest declaration

The authors declare that they have no conflicts of interest.

References

Abou-Elkhair, R., Selim, S., & Hussein, E., 2018. Effect of supplementing layer hen diet with phytogenic feed additives on laying performance, egg quality, egg lipid peroxidation and blood biochemical constituents. *Animal Nutrition*, 4:394–400. DOI: https://doi.org/10.1016/j.aninu.2018.05.009

Afiouni, A., Toghyani, M., & Landy, N., 2023. Performance, morphological responses of the small intestine, and humoral immunity of broilers fed oak acorn (*Quercus brantii*) as a substitution for antibiotic growth promoters. *Iranian Journal of Applied Animal Science,* 13:321–331.

Amevor, F.K., Cui, Z., Du, X., Ning, Z., Shu, G., Jin, N., Deng, X., Tian, Y., Zhang, Z., Kang, X., Xu, D., You, G., Zhang, Y., Li, D., Wang, Y., Zhu, Q., & Zhao, X., 2021. Combination of quercetin and vitamin e supplementation promotes yolk precursor synthesis and follicle development in aging breeder hens via liver–blood–ovary signal axis. *Animals*, 11:1915. DOI: https://doi.org/10.3390/ani11071915

Aminullah, N., Mostamand, A., Zahir, A., Mahaq, O., & Azizi, M.N., 2025. Phytogenic feed additives as alternatives to antibiotics in poultry production: A review. *Veterinary World*, 18:141–154. DOI: https://doi.org/10.14202/vetworld.2025.141-154

Annadurai, T., Muralidharan, A.R., Joseph, T., Hsu, M.J., Thomas, P.A., & Geraldine, P., 2012. Antihyperglycemic and antioxidant effects of a flavanone, naringenin, in streptozotocin-nicotinamide-induced experimental diabetic rats. *Journal of Physiology and Biochemistry*, 68:307–318. DOI: https://doi.org/10.1007/s13105-

011-0142-y
Association of Official Analytical Chemists, 1994. Official Methods of Analysis, Vol. I & II. Association of Official Analytical Chemists, Arlington, VA, USA.
Ayoob, A., Memon, A., Rajput, N., Arain, M., Lanjar, Z., Qureshi, M., & Muneir, P., 2023. Growth performance, blood components, immune response, and carcass traits in broiler chickens fed with *Eucalyptus globulus*. *Iranian Journal of Applied Animal Science*, 13:313–319.
Boğa Kuru, B., Kırmızıbayrak, T., Cengiz, M.M., & Adıgüzel Işık, S., 2023. The effect of egg weight on egg external quality characteristics and hatching performance in Pekin ducks. *Kafkas Universitesi Veteriner Fakültesi Dergisi*, 29:415–422. DOI: https://doi.org/10.9775/kvfd.2023.29657
Carpenter, K.J. & Clegg, K.M., 1956. The metabolizable energy of poultry feeding stuffs in relation to their chemical composition. *Journal of the Science of Food and Agriculture*, 7:45–51. DOI: https://doi.org/10.1002/jsfa.2740070109
Carvalho, B.M.R., Nascimento, L.C., Nascimento, J.C., Gonçalves, V., Ziegelmann, P.K., Tavares, D.S., & Guimarães, A.G., 2022. Citrus extract as a perspective for the control of dyslipidemia: A systematic review with meta-analysis from animal models to human studies. *Frontiers in Pharmacology*, 13:822678. DOI: https://doi.org/10.3389/fphar.2022.822678
Chen, F., Zhang, H., Du, E., Jin, F., Zheng, C., Fan, Q., Zhao, N., Guo, W., Zhang, W., Huang, S., & Wei, J., 2021. Effects of magnolol on egg production, egg quality, antioxidant capacity, and intestinal health of laying hens in the late phase of the laying cycle. *Poultry Science*, 100:835–843. DOI: https://doi.org/10.1016/j.psj.2020.10.047
Ebrahimi, N.A., Nobakht, A., İnci, H., Palangi, V., Suplata, M., & Lackner, M., 2024. Drinking water quality management for broiler performance and carcass characteristics. *World*, 5:952–961. DOI: https://doi.org/10.3390/world5040048
Goliomytis, M., Kartsonas, N., Charismiadou, M.A., Symeon, G.K., Simitzis, P.E., & Deligeorgis, S.G., 2015. The Influence of naringin or hesperidin dietary supplementation on broiler meat quality and oxidative stability. *PLoS One*, 10:e0141652. DOI: https://doi.org/10.1371/journal.pone.0141652
Goliomytis, M., Simitzis, P., Papalexi, A., Veneti, N., Hager-Theodorides, A.L., Charismiadou, M.A., & Deligeorgis, S.G., 2019. Influence of citrus flavonoids on laying hen performance, inflammatory immune response, egg quality and yolk oxidative stability. *British Poultry Science*, 60:272–278. DOI: https://doi.org/10.1080/00071668.2019.1587150
Gorinstein, S., Caspi, A., Libman, I., Lerner, H.T., Huang, D., Leontowicz, H., Leontowicz, M., Tashma, Z., Katrich, E., Feng, S., & Trakhtenberg, S., 2006. Red grapefruit positively influences serum triglyceride level in patients suffering from coronary atherosclerosis: studies in vitro and in humans. *Journal of Agricultural and Food Chemistry*, 54:1887–1892. DOI: https://doi.org/10.1021/jf058171g
Gorinstein, S., Martín-Belloso, O., Park, Y.S., Haruenkit, R., Lojek, A., Ĉíž, M., Caspi, A., Libman, I., & Trakhtenberg S., 2001. Comparison of some biochemical characteristics of different citrus fruits. *Food Chemistry*, 74:309–315. DOI: https://doi.org/10.1016/S0308-8146(01)00157-1
Gous, A.G.S., Almli, V.L., Coetzee, V., & de Kock, H.L., 2019. Effects of varying the colour, aroma, bitter, and sweet levels of a grapefruit-like model beverage on the sensory properties and liking of the consumer. *Nutrients*, 11:464. DOI: https://doi.org/10.3390/nu11020464
Gültepe, E.E., Iqbal, A., Çetingül, İ.S., Uyarlar, C., Özçınar, Ü., & Bayram, İ., 2019. Effects of lemon juice on performance, egg quality trait, and some blood parameters of laying hens in the late phase of production. *Acta Veterinaria Eurasia*, 45:56–62. DOI: https://doi.org/10.26650/actavet.2019.19005
Hanlon, C., Ziezold, C.J., & Bédécarrats, G.Y., 2022. The diverse roles of 17β-estradiol in non-gonadal tissues and its consequential impact on reproduction in laying and broiler breeder hens. *Frontiers in Physiology,* 13:942790. DOI: https://doi.org/10.3389/fphys.2022.942790
Haugh, R., 1937. The Haugh unit for measuring egg quality. *United States Egg Poultry Magazine*, 43:552–555.
Hoskin, D.W. & Coombs, M.R.P., 2022. Editorial: immune modulation by flavonoids. *Frontiers in Immunology,* 13:2022. DOI: https://doi.org/10.3389/fimmu.2022.899577
Iskender, H., Yenice, G., Dokumacioglu, E., Kaynar, O., Hayirli, A., & Kaya, A., 2017. Comparison of the effects of dietary supplementation of flavonoids on laying hen performance, egg quality and egg nutrient profile. *British Poultry Science*, 58:550–556. DOI: https://doi.org/10.1080/00071668.2017.1349297
Jeon, S.M., Park, Y.B., & Choi, M.S., 2004. Antihypercholesterolemic property of naringin alters plasma and tissue lipids, cholesterol-regulating enzymes, fecal sterol and tissue morphology in rabbits. *Clinical Nutrition*, 23:1025–1034. DOI: https://doi.org/10.1016/j.clnu.2004.01.006
Kelebek, H., 2010. Sugars, organic acids, phenolic compositions and antioxidant activity of grapefruit (*Citrus paradisi*) cultivars grown in Turkey. *Industrial Crops and Products*, 32:269–274. DOI: https://doi.org/10.1016/j.indcrop.2010.04.023
Kiani, J. & Imam, S.Z., 2007. Medicinal importance of grapefruit juice and its interaction with various drugs. *Nutrition Journal,* 6:33. DOI: https://doi.org/10.1186/1475-2891-6-33
Li, H., Hou, Y., Chen, J., Wu, H., Huang, L., Hu, J., Zhang, Z., Lu, Y., & Liu, X., 2022. Dietary naringin supplementation on laying performance and antioxidant capacity of Three-Yellow breeder hens during the

late laying period. *Poultry Science*, 101:102023. DOI: https://doi.org/v10.1016/j.psj.2022.102023
Lien, T.F., Yeh, H.S., & Su, W.T., 2008. Effect of adding extracted hesperetin, naringenin and pectin on egg cholesterol, serum traits and antioxidant activity in laying hens. *Archives of Animal Nutrition*, 62:33–43. DOI: https://doi.org/10.1080/17450390701780318
Lu, K. & Yip, Y.M., 2023. Therapeutic potential of bioactive flavonoids from citrus fruit peels toward obesity and diabetes mellitus. *Future Pharmacology*, 3:14–37. DOI: https://doi.org/10.3390/futurepharmacol3010002
Lu, X., Zhao, C., Shi, H., Liao, Y., Xu, F., Du, H., Xiao, H., & Zheng, J., 2023. Nutrients and bioactives in citrus fruits: Different citrus varieties, fruit parts, and growth stages. *Critical Reviews in Food Science and Nutrition*, 63:2018–2041. DOI: https://doi.org/10.1080/10408398.2021.1969891
Mohammadi Gheisar, M. & Kim, I.H., 2018. Phytobiotics in poultry and swine nutrition – a review. *Italian Journal of Animal Science*, 17:92–99. DOI: 10.1080/1828051X.2017.1350120
National Research Council, 1994. Nutrient Requirements of Poultry: 9th revised edition. The National Academies Press, Washington, DC, USA. DOI: https://doi.org/10.17226/2114
Okwu, D. & Emenike, I., 2006. Evaluation of the phytonutrients and vitamin contents of citrus fruits. *International Journal of Molecular Medicine and Advance Sciences*, 2:1–6.
Okwu, D., 2005. Phytochemicals, vitamins and mineral contents of two Nigerian medicinal plants. *International Journal of Molecular Medicine and Advance Sciences*, 1:375–381.
Oleforuh-Okoleh, V., Ogunnupebi, J., & Iroka, J., 2015. Assessment of growth performance and certain blood constituents of broiler chicks given banana leaf as a phytoadditive. *Asian Journal of Poultry Science*, 9:242–249. DOI: https://doi.org/10.3923/ajpsaj.2015
Ortiz-Andrade, R.R., Sánchez-Salgado, J.C., Navarrete-Vázquez, G., Webster, S.P., Binnie, M., García-Jiménez, S., León-Rivera, I., Cigarroa-Vázquez, P., Villalobos-Molina, R., & Estrada-Soto, S., 2008. Antidiabetic and toxicological evaluations of naringenin in normoglycaemic and NIDDM rat models and its implications on extra-pancreatic glucose regulation. *Diabetes, Obesity and Metabolism*, 10:1097–1104. DOI: https://doi.org/10.1111/j.1463-1326.2008.00869.x
Pourhossein, Z., Qotbi, A.A., Seidavi, A., Laudadio, V., Centoducati, G., & Tufarelli, V., 2015. Effect of different levels of dietary sweet orange (*Citrus sinensis*) peel extract on humoral immune system responses in broiler chickens. *Animal Science Journal*, 86:105–110. DOI: https://doi.org/10.1111/asj.12250
Pourhossein, Z., Qotbi, A.A.A., Seidavi, A., Laudadio, V., Mazzei, D., & Tufarelli, V., 2019. Feeding of dried sweet orange (*Citrus sinensis*) peel on humoral immune response of broiler chickens. *International Journal of Recycling of Organic Waste in Agriculture*, 8:361–367. DOI: https://doi.org/10.1007/s40093-019-0272-8
Raut, R., Biswas, B.K., Taylor-Bowden, T., Kilonzo-Nthenge, A., Pokharel, B., Tabler, T., & Maharjan, P., 2025. Effects of chronic exposure of specific water quality parameters in poultry drinking water on dietary amino acid digestibility and early broiler performance. *Poultry Science*, 104:104835. DOI: https://doi.org/10.1016/j.psj.2025.104835
Ribeiro, I.A. & Ribeiro, M.H., 2008. Naringin and naringenin determination and control in grapefruit juice by a validated HPLC method. *Food Control*, 19:432–438. DOI: https://doi.org/10.1016/j.foodcont.2007.05.007
Sinclair, M., Lee, N.Y.P., Hötzel, M.J., de Luna, M.C.T., Sharma, A., Idris, M., Islam, M.A., Iyasere, O.S., Navarro, G., Ahmed, A.A., Curry, M., Burns, G.L., & Marchant, J.N., 2022. Consumer attitudes towards egg production systems and hen welfare across the world. *Frontiers in Animal Science*, 3:995430. DOI: https://doi.org/10.3389/fanim.2022.995430
Singh, V., Chahal, T.S., Grewal, S.K., & Gill, P.S., 2021. Effect of fruit development stages on antioxidant properties and bioactive compounds in peel, pulp and juice of grapefruit varieties. *Journal of Food Measurement and Characterization*, 15:2531–2539. DOI: https://doi.org/10.1007/s11694-021-00841-w
Spencer, E.A., Key, T.J., Appleby, P.N., Van Gils, C.H., Olsen, A., Tjønneland, A., Clavel-Chapelon, F., Boutron-Ruault, M.-C., Touillaud, M., & Sánchez, M.-J., 2009. Prospective study of the association between grapefruit intake and risk of breast cancer in the European Prospective Investigation into Cancer and Nutrition (EPIC). *Cancer Causes & Control*, 20:803–809. DOI: https://doi.org/10.1007/s10552-009-9310-6
Tılkı, M. & Saatcı, M., 2004. Effects of storage time on external and internal characteristics in partridge (*Alectoris graeca*) eggs. *Revue de Medecine Veterinaire*, 155:561–564.
Viana, E.F., Carvalho Mello, H.H., Carvalho, F.B., Café, M.B., Leandro, N.S.M., Arnhold, E., & Stringhini, J.H., 2022. Blood biochemical parameters and organ development of brown layers fed reduced dietary protein levels in two rearing systems. *Animal Bioscience*, 35:444–452. DOI: https://doi.org/10.5713/ab.21.0145
Wang, W.-w., Wang, J., Zhang, H.-j., Wu, S.-g., & Qi, G.-h., 2020. Effects of *Clostridium butyricum* on production performance and intestinal absorption function of laying hens in the late phase of production. *Animal Feed Science and Technology*, 264:114476. DOI: https://doi.org/10.1016/j.anifeedsci.2020.114476
Yang, J.X., Chaudhry, M.T., Yao, J.Y., Wang, S.N., Zhou, B., Wang, M., Han, C.Y., You, Y., & Li, Y., 2018. Effects of phyto-oestrogen quercetin on productive performance, hormones, reproductive organs and apoptotic genes in laying hens. *Journal of Animal Physiology and Animal Nutrition*, 102:505–513. DOI: https://doi.org/1111/jpn.12778
Yang, S., Yang, R., Zhou, X., Yang, S., Liao, F., Yao, B., Zhu, B., & Pongchan, N.-L., 2023. Effects of dietary supplementation of flavonoids from Moringa leaves on growth and laying performance, immunological and

antioxidant activities in laying ducks. *Journal of Applied Poultry Research*, 32:100318. DOI: https://doi.org/10.1016/j.japr.2022.100318

Zhou, L., Ding, X., Wang, J., Bai, S., Zeng, Q., Su, Z., Xuan, Y., & Zhang, K., 2021. Tea polyphenols increase the antioxidant status of laying hens fed diets with different levels of ageing corn. *Animal Nutrition*, 7:650–660. DOI: https://doi.org/10.1016/j.aninu.2020.08.013

Zhu, Y.F., Wang, J.P., Ding, X.M., Bai, S.P., Qi, S.R.N., Zeng, Q.F., Xuan, Y., Su, Z.W., & Zhang, K.Y., 2020. Effect of different tea polyphenol products on egg production performance, egg quality and antioxidative status of laying hens. *Animal Feed Science and Technology*, 267:114544. DOI: https://doi.org/10.1016/j.anifeedsci.2020.114544

Zou, Z., Xi, W., Hu, Y., Nie, C., & Zhou, Z., 2016. Antioxidant activity of Citrus fruits. *Food Chemistry*, 196:885–896. DOI: https://doi.org/10.1016/j.foodchem.2015.09.072