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REGULAR ARTICLES

Effect of dietary pomegranate peel powder on productive traits, blood chemistry, economic efficiency and the expression of FSHR and LH-β genes in two strains of laying Japanese quail

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Abstract
The current experiment was designed to study the response of two strains of laying Japanese quail to diet containing different levels (0%, 3%, 6%, and 9%) of pomegranate peel (PP) powder for a period of 6 weeks, on egg production, blood chemistry, carcass traits, expression of FSHR and LH-β genes, and economic efficiency. A total of 576 6-week-old Japanese quails were made up of two strains: white quails (n=288) and brown quails (n=288). Four treatment groups were randomly distributed for each strain. Each group was subdivided into 6 replicates of 10 birds each. Results revealed that the white strain showed significant (P<0.05) higher values in final body weight (BW), egg quality parameters, weights of dressed carcass, and total return (TR) compared with the brown strain. Brown strain had higher hen day egg production % (HDEP%). A significant (P<0.05) interaction effects between genetic type and the dietary PP powder levels was recorded in growth traits, some egg production traits, and mRNA expression of FSHR and LH-β genes. Results concluded that all the dietary levels of PP powder up to 9% improved growth traits, egg production traits, fertility% (P=0.001), and hatchability% (P=0.007). Moreover, they have a safely biochemical effect on the level of urea (P=0.002) and the concentration of aspartate amino transferase (AST) (P<0.001). It also enhanced mRNA expression of FSHR and LH-β genes, but showed non-significant (P>0.05) influence on carcass traits. Economically, the net return (NR) was increased in quails fed on 6% and 9% PP powder diets.

Keywords Quail · Pomegranate peel · Egg production · Gene expression · Blood parameters · Economic evaluation

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Introduction

Recently, Japanese quail became an important experimental bird for scientific researches, due to rapid reproduction cycles and higher resistance to many diseases. Quails also give high production of eggs and meat (Jatoi et al. 2013). Additionally, quail is characterized by low cost of production and low initial investment. These reasons encourage farmers all over the world to raise Japanese quails on a commercial basis (Jeke et al. 2018). The cost of feeding alone constitutes 65 to 70% of the total cost of poultry production (Shambhvi et al. 2020), which leads many scientists to work on different cheap unconventional feed sources and try to improve their nutritional quality, to increase the productive and economic efficiency of poultry farms (Olorunfemi et al. 2006; Abeke et al. 2008).

More attention was tended to use natural feed additives particularly those of plant origin, due to their antioxidants, antibacterial, antifungal, and antimicrobial activities (Fawzia et al. 2020). In the last few years, the attempts to use phenolic compounds or extracts from different by-products of fruit processing industry to livestock feed has become a major area of interest to many researchers (Dai and Mumper 2010). The pomegranate (*Punica granatum*) belongs to the Punicaceae family, which is a nutrient food supplement that is rich in phyto chemical compounds (Ahmad et al. 2015). It is a widely spread fruit crop in the Mediterranean region, especially Egypt (Tayel and El-Tras 2010). Pomegranate fruit by-products are rich in most minerals and vitamins. They also possess biofunctional properties, such as antioxidant, antimicrobial activity, anti-cancerogenic, and anti-inflammatory properties (Zarfeshany et al. 2014). Pomegranate peel (PP) is the juice's residue left after extraction by pressing pomegranates in the juicer industry. Furthermore, PP powder is considered a valuable waste of the food industry as it is characterized by having bioactive compounds, especially, tannins, flavonoids, and phenolics with antioxidant properties and improvement digestion and metabolism (Nuamsetti et al. 2012). Polyphenolic compounds in PP might improve the immune status and animal health condition. The predominant polyphenol fractions in PP were caffeic acid, catechins, ellagic acid, p-coumaric acid, phenol gallic acid, and resocenol compounds (Rowayshed et al. 2013). Recent studies have proven the antioxidant potential effect of pomegranate in vivo and in vitro (Singh et al. 2002).

Quality of egg is considered the most important price contributing factor in both table, and hatching eggs (Khurshid et al. 2003). From the economic point of view, a laying flock success depends on the total number of quality eggs produced and the strain performance (Monira et al. 2003). Several investigators reported that using PP powder in poultry diets improved egg production (Saki et al. 2014). It was found that diet supplemented with PP powder led to improve egg production rate and enhanced the egg quality in laying Japanese quails (Hassan et al. 2019). Quail diet substitution with PP powder at a level 7.5% improved feed conversion ratio, egg production, egg numbers, egg weight, and egg mass (Rabia et al. 2017). The addition of PP powder (1%) significantly (P<0.01) decreased feed cost and the total cost of production (Kareem and Karwan 2019). Therefore, the current experiment was designed to elucidate the effect of diet containing different levels of PP powder on productive traits, blood chemistry, the expression of FSHR and LH-β genes, and economic efficiency in two strains of laying Japanese quail.

Materials and methods

The experiment was conducted at the department of Animal Wealth Development, the Experimental Animal Research Center, Faculty of Veterinary Medicine, Benha University, Egypt.

Birds, housing and management

A total of 576 6-week-old Japanese quails of two strains, white quails (n=288) and brown quails (n=288), were used in the experiment. Four treatment groups were randomly designed for each strain. Each group was subdivided into 6 replicates; each replicate consisted of 12 birds (8 females: 4 males mating ratio to ensure fertility aspects). Quails in each replicate were housed in battery cages. Measures of each cage were 60×50×37 cm (length×width×height). Cages were supplemented with sided feeders and nipples. All the housing, managerial, and hygienic conditions were as similar as possible for different experimental groups. The temperature was maintained around 28 °C throughout the experimental period. For lighting, 14 h of light were provided per day (12 h of natural daylight+2 h of artificial light), to maintain maximum fertility and egg production. Artificial supplementary fluorescent lamp lighting was utilized (2 fluorescent lamps, 36 W/2600 lx each).

Birds, diet, and experimental design

Pomegranate peel (PP) was collected as residues from Qaha Company for Food Industry, Qalyubia Governorate, Egypt. PP was sun-dried for about 5 days. To avoid the growth of fungus, frequent flipping was considered until drying was complete. Then, PP was ground into powder. Proximate chemical composition of PP powder percentage (%) of dry matter basis was carried out according to Rabia et al. (2017), which was determined according to the procedures of the AOAC. (2000). Four iso-energetic and iso-nitrogenous diets were formulated and prepared for Japanese quails according to National Research Council (NRC 1994). The experimental diets mainly included yellow corn, soybean meal, corn gluten meal in addition to mineral and vitamin supplements. The diets were formulated to contain 0%, 3%, 6%, and 9% PP powder for control (PP_0), PP_3, PP_6, and PP_9, respectively (Table 1).

Data collection

Productive traits and egg quality parameters

Eggs were collected 2 times daily. At the end of the experiment, final body weight (BW) was individually recorded and egg weight was measured by using an electronic balance (PGL303, UK). Average daily feed intake (ADFI) and total feed intake (TFI) were measured throughout the experimental period (6 weeks). Hen day egg production (HDEP) was calculated as the number of eggs produced on each day divided by number of hens alive on each day.

Table 1 Ingredients composition and calculated chemical analysis of the experimental diets for laying Japanese quails (g/kg)

	Control	PP_3	PP_6	PP_9
Feed ingredients (% as fed)				
PP*	0.0	30.0	60.0	90.0
Yellow corn	575.0	563.00	533.0	498.0
Soybean meal (44%CP)	236.0	235.00	227.0	222.0
Corn gluten meal (60% CP)	74.0	80.0	88.0	95.0
Wheat bran	20.0	0.0	0.0	0.0
Soybean oil	21.0	17.0	17.0	19.0
L-lysine	2.0	3.0	3.0	4.0
DL-methionine	1.0	1.0	1.0	1.0
Vita. and min. mix†	3.0	3.0	3.0	3.0
Salt	4.0	4.0	4.0	4.0
Limestone	64.0	64.0	64.0	64.0
Total	1000.0	1000.0	1000.0	1000.0
Calculated chemical composition (%)‡				
CP	20.03	20.04	20.03	20.05
CF	3.48	3.61	3.87	4.12
Ca	2.52	2.52	2.52	2.52
Na	0.17	0.17	0.17	0.17
AvailableP	0.39	0.37	0.37	0.37
Lysine	1.03	1.12	1.1	1.2
Methionine	0.48	0.49	0.49	0.50
ME (kcal/kg)**	2900.9	2902.7	2900.6	2901.0

*PP, pomegranate peel powder (proximate chemical composition as % of dry matter basis according to Rabia et al. (2017), which was determined according to the procedures of the AOAC. (2000): organic matter (OM) 95.8%, crude protein (CP) 3.97%, crude fiber (CF) 12.1%, ether extract (EE) 2.34%, ash 4.19%, nitrogen-free extract (NFE) 77.4%, and metabolizable energy (ME) 2825 kcal/kg

**Metabolizable energy (ME kcal/g) was calculated according to the formula derived by Lodhi et al. (1976). ME (Kcal/g) = 1.549 + 0.0102 CP + 0.0275 EE + 0.0148 NFE – 0.0034 fiber

†Vitamin-trace mineral mixture: composition per 3 kg, vit. A 12,000,000 I.U.; vit. D_3 2,000,000 I.U.; vit. E 10,000 mg; vit. K_3 1000 mg; vit. B_1 1000 mg; vit. B_2 5000 mg; vit. B_6 1500 mg; vit. B_{12} 10 mg; niacin 30,000 mg; biotin 50 mg; folic acid 1000 mg; pantothenic acid 10,000 mg; choline chloride 500,000 mg; zinc 50,000 mg; manganese 60,000 mg; iron 30,000 mg; copper 10,000 mg; iodine 1000 mg; selenium 100 mg; cobalt 100 mg; calcium carbonate to 3 kg

‡Calculated according to (NRC for poultry 1994, nutrient requirements tables for Japanese quail page 45)

Feed conversion ratio (FCR)was measured at the end of the experiment on the basis of the amount of feed consumed in gram divided by egg mass in gram. For egg mass per bird, it was calculated as (average egg weight × egg number). For calculating fertility and hatchability percentages over the experimental period, a total of 320 eggs were collected from the eggs laid for 4 successive days (40 eggs/ each treatment) from each strain, then eggs were set on the hatchery. After hatching, percentages of fertility and hatchability were calculated as following:

Fertility (%)=(number of fertile eggs/total number of eggs set)×100; hatchability (%)=(number of hatched chicks/total number of set eggs) × 100.

External egg quality

During the last week (the 12th week of age) of the experiment, a total of 80 eggs (10 eggs from each experimental group of both white and brown strain) were collected to estimate the external and internal egg quality parameters. Egg width, egg length, and shell thickness (mm) were calibrated using traditional caliper (±0.01 mm). Egg shape index was computed as (egg width/egg length) × 100 according to Das et al. (2010).

Internal egg quality

Measuring internal quality components of the eggs occurred by gently breaking the egg using a sharp scalpel and emptying the contents onto a clean petri dish then weighing it. The yolk was separated from the albumin to weigh the yolk. After that, albumin weight was calculated. Albumin height, yolk height, and yolk diameter in millimeter were estimated using traditional caliper. After that, yolk index was computed according to Romanoff and Romanoff (1949):

$$\text{Yolk index } (\%)(\text{yolk height}/\text{ yolk diameter }) \times 100$$

Haugh units (HU) was computed according to the formula of Haugh (1937).

$$HU = 100\log\left(Albumin\ height\ (mm) + 7.57 - 1.7\ egg\ weight\ (g)^{0.37}\right)$$

Biochemical parameters

Blood samples were collected from female quails, through puncturing the wing vein with a clean needle, then transferred to a clean centrifuge tubes and centrifuged at 3000 rpm for 10 min for separation of serum. Quantitative analysis was done for the following parameters: serum aspartate aminotransferase (AST) activity was calorimetrically determined according to the method described by (Murray 1984). Serum urea was determined according to Patton and Crouch (1977). Serum uric acid was determined according to (Young 2001). Serum creatinine was determined according to (Henry 1974). All biochemical parameters were determined using commercial diagnosing kits Centronic, Germany on Chem 7, USA.

Analysis of mRNA expression of follicle stimulating hormone receptor and luteinizing hormone genes

For gene expression analysis, pituitary and hypothalamus tissues were collected from control and treated female quail groups and then frozen at − 80 °C. The total RNA was isolated from the frozen tissues with TRIzol reagent according to the manufacturer's instructions (Invitrogen, Carlsbad, CA, USA). cDNA was produced from total RNA in 10 µl as a final volume according to the manufacturer's protocol of High Capacity cDNA Reverse Transcription Kits (Applied Bio systems) in T100 Thermal cycler BIO-RAD, English. Then, cDNA samples were stored at − 20 °C until use.

The expression of these genes was analyzed by real-time PCR using one pair of primer sequences for each gene in Table 2. PCR reactions occurred according to QuantiTect SYBR Green PCR Kit, Qiagenin an Applied Biosystem 7500 Fast Real-time PCR Detection system. Changes in the gene expression were computed from obtained cycle threshold (C_t) by the comparative $2^{-\Delta\Delta Ct}$ method (Livak and Schmittgen, 2001).

Carcass traits

At the end of the experiment (day 84 of age), 18 female quail from each group (3/replicate) were randomly selected, weighed, kept separately, and fastened for a period of 12 h before slaughtering, in order to measure the carcass traits. They were slaughtered as per the recommendation of the institutional committee, then they were processed by removing feather, viscera, head, neck, shanks, and feet. The dressed carcass was weighed and the dressing percentage was calculated according to the formula of (Brake et al. 1993):

$$\text{Dressing \%} = \frac{\text{dressed carcass weight}}{\text{live weight}} \text{ X } 100$$

Liver, intestine, gizzard, and heart were weighed individually, with the calculation of their percentages in relation to live BW.

Evaluation of economic efficiency

The economic evaluation of the experimental groups was measured via calculation of production costs, total returns (TR), and net return (NR). Production costs were differentiated into total fixed costs (TFC), total variable costs (TVC), and total costs (TC). TFC included the price of purchased quails, building rent values, equipment depreciation cost, veterinary management cost, labor, litter, water, electricity, and other miscellaneous costs. TVC included the TFI cost for each quail in each experimental group during the experimental period, by multiplying TFI per bird × cost of 1 kg diet. TR was calculated by the summation of manure selling return, the quail selling return, and egg selling return. The quail selling return was estimated by multiplying the final BW per gram × selling price per gram. The egg selling return was estimated by multiplying the total number of eggs produced per female in each group throughout the experimental period × selling price per egg. NR was calculated according to Kareem and Karwan (2019) by subtracting TC from TR.

Statistical analysis

All data were analyzed using the GLM procedures of the SPSS statistical software (SPSS/PC version 16). The model included the fixed effects of the genetic type of Japanese quail (two strains: white and brown), the pomegranate peel levels (four levels: control, PP_3, PP_6, and PP_9) and their interaction. Means were tested using Duncan multiple range test (Duncan 1955). Moreover, polynomial contrasts test was applied on productive traits and egg quality parameters, carcass traits, and the economic evaluation parameters. All values are displayed as a mean and a residual standard deviation. Figures were drawn by Microsoft Office Excel (2007) (Figs. 1 and 2).

Results

Productive traits and egg quality parameters

Effect of genetic type and dietary pomegranate peel levels on productive traits, fertility, and hatchability is presented in (Table 3). The obtained results revealed that the white strain showed significant (P<0.001) increase in final BW, and egg weight compared with the brown strain. Brown strain had significantly (P < 0.001) higher ADFI, TFI, and HDEP% over the white strain. While there were no significant differences between the two strains for FCR, egg mass, fertility%, and hatchability%.

Table 2 Primer information for mRNA expression analysis

Gene	Primer sequences (5′—3′)	Reference	Cycle profile
FSHR	F: TAATGGAACCTGCCTGGATG R: GCACAGCAATGGCTAGGATAG	Cui et al., 2012	95 °C for 10 min, 40 cycles 95 °C for 15 s (denaturation), 60 °C for 1 min (annealing, extension)
LH-β	F: CCCAAAGTCATCCTACCCGT R: TATGGGGCAATCTATGGGGC	Han et al., 2017	
β-actin	F: ACCCCAAAGCCAACAGA R: CCAGAGTCCATCACAATACC	Gasparino et al., 2014	

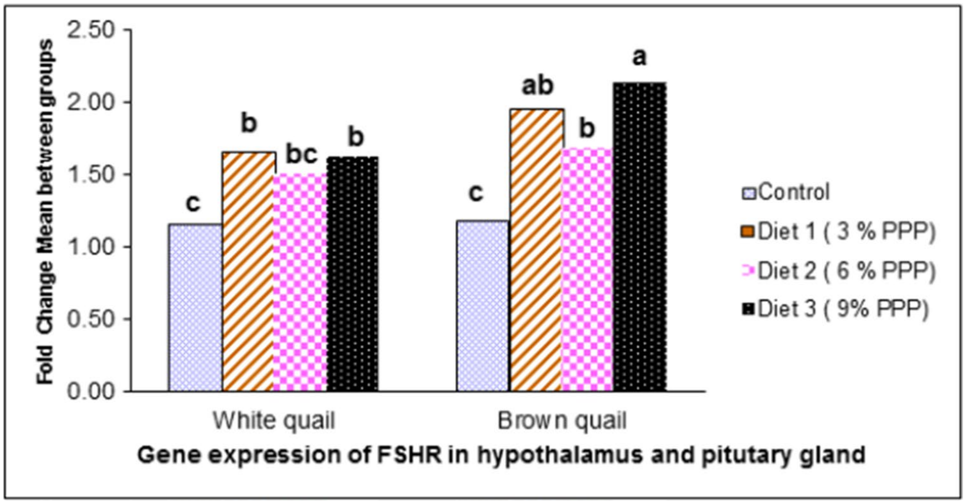

Fig. 1 Effect of genetic type and dietary pomegranate peel powder levels on follicular stimulating hormone receptor (FSHR) gene in two strains of Japanese quail. Values with the different letters on the bars are significantly different ($P<0.05$)

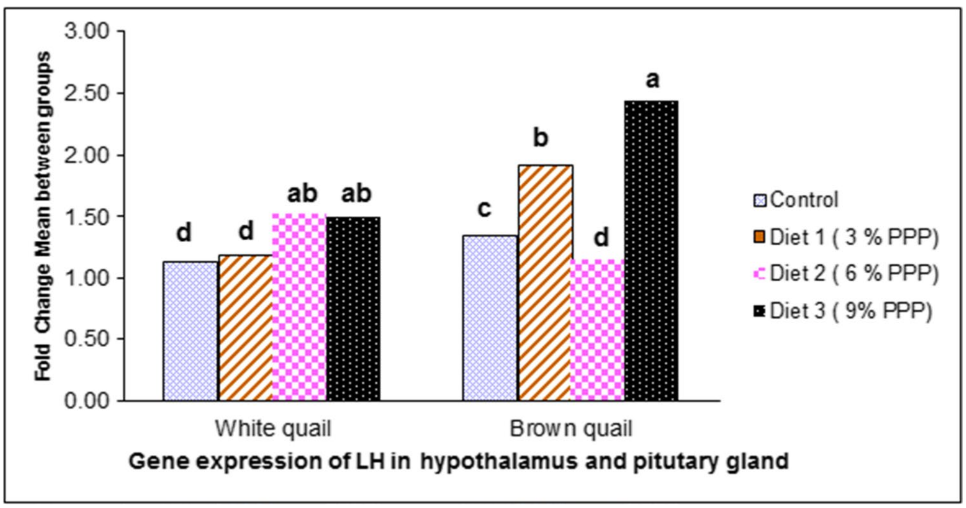

Fig. 2 Effect of genetic type and dietary pomegranate peel powder levels on Luteinizing Hormone (LH-β) gene in two strains of Japanese quail. Values with different letters on the bars are significantly different ($P<0.05$)

Regardless of the effect of strain, the dietary PP powder at different levels significantly ($P<0.001$) affects ADFI and TFI. PP powder 9% decreased ADFI (− 2.7 g/bird) and TFI (− 113.7 g/bird) compared with the control group.

Dietary PP powder at each level significantly ($P<0.05$) improved final BW, HDEP%, FCR, egg weight, egg mass, fertility%, and hatchability%. Quail group fed with 6% of PP powder recorded the highest ($P=0.021$) average final BW compared to the other experimental quail groups. Quail group fed with 9% PP powder diet reported the best ($P<0.001$) HDEP%, FCR, and egg mass. Quails that received 3% PP powder exhibited significantly ($P<0.001$) higher egg weight. Results also showed significant increase in fertility% ($P=0.001$) and hatchability% ($P=0.007$) for quail groups fed with 3%, 6%, and 9% PP powder diets compared to the control group.

Regarding interaction between genetic type and dietary PP powder levels, a significant difference was found in ADFI, TFI, HDEP%, FCR, egg weight, and egg mass, except for final BW, fertility%, and hatchability%.

Effects of genetic type and dietary pomegranate peel levels on egg quality traits are listed in Table 4. Based on strain effect, it could be reported that the white quails had a significantly ($P<0.001$) higher egg width, egg height, yolk height, yolk weight, albumin weight, shell thickness, and yolk index compared with the brown strain, except for haugh unit value which was higher ($P<0.01$) for the brown strain than the white one. Regardless of the effect of strain, the dietary PP powder at different levels did not affect egg width, egg height, albumin weight, yolk index, and egg shape index. However, there was a significant ($P<0.05$) difference between control and the dietary treated groups with PP powder for yolk height, yolk diameter, albumin height, yolk weight, shell thickness, and haugh unit. Quails fed with 3%, 6%, and 9% PP powder recorded significantly ($P=0.003$) higher value of

Table 3 Effect of genetic type and pomegranate peel powder level on productive traits, fertility and hatchability of laying Japanese quail

Parameter	Genetic type (G)		Dietary pomegranate peel powder level (PP)				RSD	*P*-values			
								G	PP		G×PP
	White	Brown	PP_0	PP_3	PP_6	PP_9			Linear	Quadratic	
Final BW (g/bird)	356.2	269.9	303.2^{c}	312.8^{ab}	320.8^{a}	315.4^{ab}	9.50	< 0.001	0.021	0.073	0.385
ADFI (g/bird)	35.4	36.6	37.6^{a}	35.7^{b}	35.8^{b}	34.9^{c}	0.10	< 0.001	< 0.001	< 0.001	< 0.001
TFI /bird (g/bird)	1488	1538	1579.9^{a}	1501.4^{b}	1503.1^{b}	1466.2^{c}	4.40	< 0.001	< 0.001	< 0.001	< 0.001
HDEP%	61.8	74.8	61.6^{c}	67.5^{b}	69.6^{b}	74.4^{a}	2.18	< 0.001	< 0.001	0.561	< 0.001
FCR	3.93	3.87	4.67^{a}	3.79^{b}	3.72^{b}	3.42^{c}	0.13	0.258	< 0.001	< 0.001	< 0.001
Egg weight (g)	15.1	12.7	13.6^{c}	14.1^{a}	13.9^{b}	13.9^{b}	0.003	< 0.001	< 0.001	< 0.001	< 0.001
Egg mass (g)	391.6	397.8	345.9^{c}	395.7^{b}	407.1^{b}	430.1^{a}	11.2	0.249	< 0.001	0.020	< 0.001
Fertility (%)	89.9	91.9	81.0^{b}	92.5^{a}	96.7^{a}	93.6^{a}	5.70	0.404	0.001	0.007	0.202
Hatchability (%)	81.2	87.5	72.9^{c}	86.0^{b}	91.8^{a}	86.5^{b}	8.19	0.078	0.007	0.014	0.158

PP_0, 0% pomegranate peel powder; *PP_3*, 3% pomegranate peel powder; *PP_6*, 6% pomegranate peel powder; *PP_9*, 9% pomegranate peel powder; *RSD*, residual standard deviation; *G×PP*, genetic type×gietary pomegranate peel powder level interaction. *ADFI*, average daily feed intake; *TFI*, total feed intake; *HDEP*, hen day egg production; *FCR*, feed conversion ratio

a, b, c means with different superscripts in each row are significantly different at $P<0.05$

Table 4 Effect of genetic type and pomegranate peel powder level on egg quality traits of laying Japanese quail

Parameter	Genetic type (G)		Dietary pomegranate peel powder level (PP)				RSD	*P*-values			
								G	PP		G×PP
	White	Brown	PP_0	PP_3	PP_6	PP_9			Linear	Quadratic	
Egg width (mm)	25.4	24.0	24.7	24.7	25.0	24.8	0.67	<0.001	0.631	0.829	0.639
Egg height (mm)	33.5	30.8	31.5	32.0	32.7	32.4	0.70	<0.001	0.281	0.593	0.01
Yolk height (mm)	10.9	9.80	9.00^{b}	10.7^{a}	10.5^{a}	11.2^{a}	0.81	0.003	0.003	0.243	0.26
Yolk diameter (mm)	21.8	21.7	20.7^{c}	21.8^{a}	22.5^{a}	21.7^{ab}	0.89	0.336	0.029	0.012	0.618
Albumin height (mm)	3.20	3.00	2.71^{b}	3.01^{ab}	3.20^{ab}	3.52^{a}	0.64	0.536	0.029	0.989	0.585
Yolk weight(g)	6.00	4.90	4.86^{b}	5.40^{b}	6.11^{a}	5.33^{b}	0.54	<0.001	0.040	0.010	0.03
Albumin weight (g)	7.71	5.70	6.03	6.81	7.10	7.03	0.37	<0.001	0.14	0.366	0.01
Shell thickness (mm)	0.27	0.24	0.25^{ab}	0.24^{b}	0.27^{a}	0.27^{a}	0.003	<0.001	0.005	0.515	0.771
Yolk index (%)	53.4	46.5	46.4	49.8	51.4	52.0	2.84	<0.001	0.085	0.541	0.002
Egg shape index (%)	77.7	79.3	78.2	79.3	78.5	78.0	2.43	0.111	0.774	0.477	0.138
Haugh unit	78.8	81.4	77.5^{b}	79.6^{ab}	81.2^{ab}	82.1^{a}	3.46	0.01	0.028	0.705	0.667

PP_0, 0% pomegranate peel powder; *PP_3*, 3% pomegranate peel powder; *PP_6*, 6% pomegranate peel powder; *PP_9*, 9% pomegranate peel powder; *RSD*, residual standard deviation; *G×PP*, Genetic type×dietary pomegranate peel powder level interaction

a, b, c means with different superscripts in each row are significantly different at $P<0.05$

yolk height and albumin height ($P=0.029$) compared to the control group. Quails groups fed with 6%, and 9% PP powder diet showed the highest ($P=0.005$) shell thickness. Haugh unit value was significantly ($P=0.028$) the highest for PP_9 dietary treated group.

Concerning interaction between genetic type and dietary PP powder levels, a significant ($P<0.05$) difference was found in egg height, yolk height, albumin weight, and yolk index.

Biochemical indices

As described in Table 5, there were no significant differences between brown and white quails for all biochemical parameter. Regardless of the effect of strain, quails fed with diet PP_9 had the lowest level of urea ($P=0.002$). The lowest concentration of AST ($P<0.001$) was recorded for quails fed with 6%, and 9% PP powder diet, while, the highest concentrations of urea and AST were recorded in the control group.

Table 5 Effect of genetic type and pomegranate peel powder level on some biochemical indices of laying Japanese quail

Parameter	Genetic type (G)		Dietary pomegranate peel powder level (PP)				RSD	*P*-values		
	White	Brown	PP_0	PP_3	PP_6	PP_9		G	PP	G×PP
Urea (mg/dl)	4.09	4.93	5.18^{a}	4.52^{ab}	4.74^{ab}	3.62^{b}	0.65	0.210	0.002	0.147
Uric acid (mg/dl)	3.25	3.18	3.59	3.34	3.02	2.88	0.87	0.921	0.513	0.906
Creatinine (mg/dl)	0.318	0.358	0.377	0.302	0.348	0.327	0.07	0.213	0.39	0.441
AST (U/L)	70.6	65.4	119.7^{a}	74.1^{b}	32.3^{c}	45.8^{c}	19.7	0.528	<0.001	0.922

AST, aspartate amino transferase. PP_0, 0% pomegranate peel powder; PP_3, 3% pomegranate peel powder; PP_6, 6% pomegranate peel powder; PP_9, 9% pomegranate peel powder; *RSD*, residual standard deviation; *G×PP*, genetic type×dietary pomegranate peel powder level interaction

a, b, c means with different superscripts in each row are significantly different at $P<0.05$

Carcass traits

Referring to the effect of genetic type, weights of live and dressed carcass of white quails were significantly ($P<0.001$) higher than that of brown quails, but the percentages of intestine and gizzard in brown quails were significantly ($P<0.001$) higher than that of white (Table 6). Carcass traits were not significantly ($P>0.05$) affected with the dietary PP powder. The effects of interactions between genetic type and dietary PP powder levels were non-significant ($P>0.05$) for all carcass traits except for liver% ($P=0.039$).

Gene expression

The obtained results reported a significant up regulation of brown strain more than that of white strain for FSHR and LH-β genes. Quails that received 9% PP powder showed the highest mRNA expression for the two genes compared with the control group, which agreed with the phenotype (the increase of HDEP% in brown strain than in white strain was also clear in quails that received 9% PP powder diet than other groups). A significant interaction effect between genetic type and dietary PP powder levels was recorded in mRNA expression of the two genes.

Economic evaluation

In Table 7, the effect of genetic type showed that TFC, TC, quail selling return, and TR of white quails were significantly ($P<0.001$) higher than that of brown strain. Egg selling return of brown quails were significantly ($P<0.001$) higher than that of white strain, while there were no significant differences ($P>0.05$) between both strains in TFI cost and NR values. Regarding the dietary PP powder at different levels, there was a significant ($P<0.001$) higher value of TFI cost of the control group compared to the dietary treated groups with PP powder, and the lowest TFI cost value was recorded for quails fed with 9% PP powder diet. Quails fed with 6% and 9% PP powder diet exhibited significantly ($P<0.001$) higher NR values (0.433 and 0.468 $/ quail, respectively) compared with the control group. The effects of interactions between the genetic type and dietary PP powder levels were significant ($P<0.001$) for all

Table 6 Effect of genetic type and pomegranate peel powder level on carcass and some internal organs of laying Japanese quail

Parameter	Genetic type (G)		Dietary pomegranate peel powder level (PP)				RSD	*P*-values			
								G	PP		G×PP
	White	Brown	PP_0	PP_3	PP_6	PP_9			Linear	Quadratic	
Live wt. (g)	355.0	258.4	327.5	305.6	298.8	295.0	26.0	<0.001	0.257	0.657	0.904
Dressed wt. (g)	241.3	176.9	224.1	208.5	199.3	204.4	22.1	<0.001	0.292	0.473	0.856
Dressing %	68.0	68.4	68.5	68.4	66.6	69.3	4.65	0.845	0.954	0.379	0.690
Liver %	3.14	3.12	3.16	3.20	3.06	3.09	0.39	0.849	0.617	0.979	0.039
Intestine %	3.72	4.83	4.70	4.26	4.10	4.05	0.52	<0.001	0.099	0.490	0.629
Gizzard %	2.93	3.47	3.14	3.44	2.84	3.38	0.45	<0.001	0.880	0.541	0.110
Heart %	0.85	0.95	0.93	0.93	0.85	0.91	0.20	0.167	0.682	0.671	0.347

PP_0, 0% pomegranate peel powder; PP_3, 3% pomegranate peel powder; PP_6, 6% pomegranate peel powder; PP_9, 9% pomegranate peel powder; *RSD*, residual standard deviation; *G×PP*, Genetic type×dietary pomegranate peel powder level interaction

a, b, c means with different superscripts in each row are significantly different at $P<0.05$

Table 7 Effect of genetic type and pomegranate peel powder level on economic evaluation parameters of laying Japanese quails

Parameter	Genetic type (G)		Dietary pomegranate peel powder level (PP)				RSD	*P-values*			
								G	PP		G×PP
	White	Brown	PP_0	PP_3	PP_6	PP_9			Linear	Quadratic	
TFI cost ($/bird)	0.537	0.551	0.601[a]	0.523[b]	0.519[bc]	0.505[c]	0.01	0.294	<0.001	<0.001	<0.001
TFC/bird ($/bird)	1.27	1.00	1.11	1.16	1.12	1.14	0.08	<0.001	0.892	0.778	<0.001
TC ($/bird)	1.79	1.54	1.71	1.68	1.64	1.64	0.07	<0.001	0.336	0.804	<0.001
Quail selling return ($/bird)	1.37	1.04	1.17	1.20	1.24	1.21	0.03	<0.001	0.676	0.717	0.513
Egg selling return ($/bird)	0.791	0.897	0.827	0.812	0.841	0.898	0.03	<0.001	0.082	0.234	0.001
TR ($/bird)	2.17	1.93	2.00	2.01	2.07	2.10	0.03	<0.001	0.141	0.888	0.001
NR ($/bird)	0.363	0.389	0.270[c]	0.335[b]	0.433[a]	0.468[a]	0.04	0.203	<0.001	0.544	0.02

PP_0, 0% pomegranate peel powder; *PP_3*, 3% pomegranate peel powder; *PP_6*, 6% pomegranate peel powder; *PP_9*, 9% pomegranate peel powder; *RSD*, residual standard deviation; *G×PP*, genetic type×dietary pomegranate peel powder level interaction

a, b, c means with different superscripts in each row are significantly different at $P<0.05$

the economic evaluation parameters except for egg selling return (P=0.513).

Discussion

The main objective of the current study was to make comparative evaluation of the response of two strains of quails to diet containing different levels of PP powder.

In the present study, white strain had higher final BW than brown strain, which could be attributed to the higher body gain of this genotype. These findings are in line with those of Jessy et al. (2016) who reported significant variation of final BW between the white and brown quail strains. These results are also in agreement with the finding that the body weights were significantly affected by different types of color mutants or strains of quails, and the difference in the genetic development of flocks reared in different areas (Rahman et al. 2010). Jatoi et al. (2013) mentioned that white quails had lower BW than brown strain (p<0.05), this result was not in agreement with the findings of the present study. Concerning results of egg weight, the white strain showed significant higher egg weight compared with the brown strain. Consistent with these findings Fadhil et al. (2018) found that white quail strain produces heavier egg weight, revealing a remarkable role for plumage color in egg weight characteristics. Conversely, Ashok and Reddy (2010) have found that brown quails produce higher egg weight. Furthermore, brown quail strain exhibited higher HDEP than the white strain did. This result is in the same line with Jessy et al. (2016), who reported that brown quails had more egg production than white, where brown birds are lighter than white. In this context, the greater HDEP in brown quail strain may be attributed to a greater number of mature ovarian follicles in lighter weighed quails (Jatoi et al. 2013; Jessy et al. 2016). On the other hand, FCR, egg mass, fertility%, and hatchability% were nearly the same in both strains. Based on strain effect, it could be observed that white quails had a significant higher egg width, egg height, yolk height, yolk weight, albumin weight, shell thickness, and yolk index compared with the brown strain. This is in agreement with the findings of Jessy et al. (2016) and Hassan et al. (2019), who stated that egg width, egg height, and shell thickness values of eggs of brown strain were lower than those of white strains. These results might be due to the higher BW of white birds and higher egg weight comparing to the brown strain.

Herein, the dietary PP powder decreased the ADFI and TFI, where the lowest values were reported in the 9% PP group. These results were in agreement with Kareem and Karwan. (2019) who mentioned that quails fed with PP powder had lower FI (p<0.05) in comparison with the control group. Differently from the present study (Rabia et al. 2017) recorded that quails fed 7.5% of PP powder consumed more feed than on other dietary treatments. Decreasing TFI of all quails fed PP powder diets, especially in birds fed 9% PP powder, might be due to the fact that PP contains tannin which affects its odor causing it to be unpleasant and bitter (Khurshid et al. 2003).

Quail group fed with 6% PP powder diet recorded the highest final BW compared to the other experimental quail groups. Also, the addition of PP powder to quail diet at a level of 9% improved the FCR. These results might be attributed to the PP being characterized by having a content of crude fiber, carbohydrates, and total polyphenols. The predominant polyphenol fractions were caffeic acid, catechins, ellagic acid, p-coumaric acid, phenol gallic acid, and resocenol compounds (Rowayshed et al. 2013). Moreover, the improvement in quail productive performance by inclusion of PP powder in diet might be due to the effect of the antioxidant properties which can protect the intestinal mucosa against oxidative damage and pathogens via stimulation of gastro-intestinal enzymatic activity with improvement digestion and metabolism (Hassan et al. 2019; Rabia et al. 2017). Furthermore, PP has antimicrobial, antibacterial, antiviral, antifungal, and antimutagenic properties against a wide range of microorganisms (Negi et al. 2003).

Quails that received 3% PP powder exhibited significantly higher egg weight. Dietary PP powder at each level significantly improved fertility% and hatchability% compared to the control group. These results were found consistent with those of Saki et al. (2014) and Hassan et al. (2019) who reported the improvement in egg production traits with the supplementation of PP in quails. Therefore, PP powder, being considered as a promising potential dietary treatment for laying quails, might be due to its biological activities such as antioxidant potency, anti-inflammatory, antimicrobial effects, as well as tannins, flavonoids, and other phenolic compounds (Li et al. 2006).

There were significant differences between control and the dietary treated groups with PP powder for yolk height, yolk diameter, albumin height, yolk weight, shell thickness, and haugh unit. Quail groups fed with 6%, and 9% PP powder showed the highest shell thickness. Haugh unit value was significantly the highest for PP_9 dietary treated group. It is widely accepted that an increase in yolk quality by PP may be attributed to the antioxidant potency, which can protect yolk membrane against oxidation and damages, and increase standing up quality of yolk. The traits of the egg have a very important role in the development of chick embryo and hatchability, especially egg weight, egg shape index, and shell thickness which give us an indication about the content of the egg for incubation process. Based on strain effect, it could be reported that the white quails had a significant higher egg width, egg height, yolk height, yolk weight, albumin weight, shell thickness, and yolk index% compared with the brown strain. Consistent with these findings, Jessy et al. (2016) and Fadhil et al. (2018) reported that the proportion of yolk in the white quail eggs is larger ($P<0.01$) than those in brown quail eggs, which may be correlated with their higher values of the egg weight. Meanwhile, Khawaja et al. (2013) noticed that brown strain had a better egg quality than white strain, which is not in agreement of the present study finding.

It was found that birds fed with 6% and 9% PP powder had safely reduced values of urea, and AST, compared to the control group. These results agreed with the finding of Rahmani et al. (2016) and (Shahira et al. (2020) who suggested that diet supplemented with PP powder showed a potential hepatoprotective effect depending on the presence of phenolic and antioxidant compounds in PP powder. Sadeghi (2015) also recorded that pomegranate extract has nephro-protective role.

The current results reflect the effect of strain type on carcass traits, as the weights of live and dressed carcass of white quails were higher than that of brown strain. This result might be attributed to the higher growth performance of white quails over the brown. These results came in accordance with Fadhil et al. (2018) who reported an obvious higher productive parameter including carcass weight of the white strain compared with the brown strain. In contrast to the present results, Inci et al. (2016) reported higher values of carcass dressing% of the brown strain.

Herein, the reduction in dressing percentages associated with the dietary inclusion of PP may be compensated by increasing the percentage of reproductive organs to be more effective in egg production. Actually, this hypothesis needs to be more confirmed in a further study. These results are in agreement with Svihus (2014) who indicated that diet containing PP improved the percentages of intestine and gizzard. The current results are also in accordance with Shahira et al., (2020) who reported that the dietary antioxidant supplementation did not have any significant effect on the liver relative weight of broilers. Also, Fawzia et al. (2020) stated that dietary supplementation of PP extract at 100, 150, and 200 mg did not affect all carcass traits.

The current results reflect that increasing egg production was associated with increasing mRNA expression of FSHR and LH-β genes in quails fed 9% PP powder. Unfortunately, we could not find any previous study that refers or explains the analysis of mRNA expression of (FSHR) and (LH-β) genes for laying quails fed with diet containing PP powder. Reham et al. (2020) stated that diet supplemented with 0.3% *Moringa oleifera* seed powder upregulated the expression of mRNA of FSHR in laying quail, which is associated with higher egg production. Another study showed that breeding geese fed on diet supplemented with 80 mg/kg vitamin E gives the highest egg number which is associated with the increase in mRNA expression of FSHR and LHR genes (Yin et al. 2019). The current study agrees with Elizabith et al. (2000) who stated that FSH exert its role by its specific receptor (FSHR) in the development and maturation of the follicles and cooperates with LH in the ovulation process.

In the current study, PP powder could also be utilized in quail diet to improve the economics of production and minimize the expenses of production. TFC, TC, quail selling return, and TR of white quails showed significant higher values compared to the brown strain. These results might be attributed to the heavier weights of the white quails than the brown one. These findings agree with those of Rahman et al. (2010), who stated that the body weights were significantly affected by different types of color mutants or varieties of quails. Egg selling return values of brown quails were significantly higher than those of white strain, the reason might be due to the higher HDEP% for the brown strain over the white strain (Jessy et al. 2016).

The present results revealed that the addition of PP powder to quail diets reduced the feed cost, saving about $ 32.01, 35.21, and 35.85/ton for PP_3, PP_6, and PP_9, respectively. Meanwhile, the highest feed cost/ton was found for the control diet. Feed is the major input item of the production costs in poultry farms, and thus has an important role in the economics of quail farm. Furthermore, it is insistent to give more attention to the effective utilization of feed without negatively affecting the growth performance traits (Shambhvi et al. 2020). Quails fed with 6% and 9% PP powder diets showed significantly higher NR compared to the control group. These results were in the same line with Kareem and Karwan (2019) and Raeesi et al. (2010) who recorded that quails fed PP diets had higher ($P<0.01$) TR compared with control group.

Conclusion

In summary, the white strain showed higher final BW, egg weight, egg width, egg height, yolk height, yolk weight, albumin weight, shell thickness, and yolk index compared with the brown strain. Meanwhile, brown strains had higher ADFI, TFI, and HDEP% value over the white strain. The white strain of quail showed higher carcass weight than the brown strain. All the dietary levels of PP powder up to 9% improved growth traits, egg production rate, and the mRNA expression of FSHR and LH-β genes. In both quail strains, 9% PP powder dietary treatment decreased the levels of serum urea and AST in quails fed with 6% and 9% PP powder. Economically, the net return (NR) was increased in laying quails fed on 6% and 9% PP powder diets.

Author contributions All authors contributed to the study conception and design. Material preparation and data collection were performed by all authors. Data analysis was performed by Eman Ramadan Kamel, Basant Mohamed Shafik, and Maha Mamdouh. The original draft of the manuscript was written, reviewed, and edited by Eman Ramadan Kamel and Fathy Attia Ismaiel Abdelfattah. All authors read and approved the final manuscript.

Data availability Data will be made available on reasonable request.

Declarations

Ethics approval The ethical approval was obtained from the Institutional Animal Care and Use Committee of Animal Care and Welfare, Benha University, Faculty of Veterinary Medicine, Egypt (Approval No. BUFVM 03–09-2020).

Conflict of interest The authors declare that they have no conflict of interest.

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