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REGULAR ARTICLES

Response of two strains of growing Japanese quail (*Coturnix Coturnix Japonica*) to diet containing pomegranate peel powder

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Abstract

Four hundred and eighty 1-day-old Japanese quail chicks from white and brown strains, 240 birds from each strain, were divided into 4 treatment groups with 6 replicates each (10 birds/replicate). Both strains of quail were fed diets containing 0%, 3%, 6%, and 9% pomegranate peel (PP) powder for a period of 42 days. Results revealed that the final body weight (BW), total feed intake (TFI), body weight gain (BWG), gizzard percentage, and eviscerated carcass weight of white growing quails were significantly ($P < 0.05$) higher than the brown strain. Quail group fed with 6% PP powder had the highest ($P < 0.001$) average final BW and BWG ($P < 0.001$). The dietary PP powder at different levels significantly ($P = 0.032$) decreased TFI, but had no significant effect on carcass traits except for the liver percentage. Quails fed diet 3% and 9% PP powder had significant ($P < 0.001$) increased count of immune cells, and improved antioxidant potency. White quails fed diet 3% and 6% PP powder had greater expression of hepatic GHR gene. The expression of hepatic IGF-1 gene was significantly ($P < 0.05$) higher for brown quails fed diet 6% and 9% PP powder. White quails recorded the highest value of total return (TR). Quails fed diet 6% and 9% PP powder significantly ($P < 0.001$) recorded higher net return (NR) values. In conclusion, dietary inclusion of PP powder at levels up to 9% for Japanese quail strains improved the growth performance, increased antioxidant properties, enhanced hepatic gene expression, and did not show any adverse influence on carcass quality and blood indices of Japanese quail. Moreover, it increased the NR.

Keywords Pomegranate peel · Growth performance · Quail · Gene expression · Carcass traits · Economics of production

Introduction

Poultry meat is considered one of the main sources of nutrients for consumers. In common, consumer preference, nutrient content, availability, and low expenses of production have made poultry meat a serious source of animal protein (Hassan et al. 2017). Production of meat and egg of quail has globally become a financially reasonable activity and has progressively developed. Economically, quail production is highly profitable due to its high growth rate, early onset of lay, rapid reproduction cycles, and low feed consumption (Kareem and Karwan 2019). Many plumage color variants of Japanese quails were reported by Traux and Johnson (1979), Somes (1976, 1979), and Roberts et al. (1978). The body weights of Japanese quail were significantly affected by different types of color mutants or strains of quails (Rahman et al. 2010). Additionally, strain type is an important factor that affects growth traits in Japanese quail (Kumari et al. 2008). Performance parameter measures can provide a

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solid basis for choosing the appropriate egg/meat productive line that suits the desired breeders' demands (Fadhil et al. 2018). One Japanese quail plumage-color mutant has been reported as white (Roberts et al. 1978), while other authors (Somes 1976; Traux and Johnson 1979; Cheng and Kimura 1990; Minvielle et al. 2007) have called it "recessive white." Jessy et al. (2016) reported a significant final BW variation between white and brown quail strains, as brown strain has less body weight than white. White quails possessed the heaviest body weight with the best carcass traits (Mohammed et al. 2017). Most researches focused mainly on studying the inheritance mode of the plumage color variants, but a little number of efforts were arranged to investigate the production performance and the economics of production (Minvielle et al. 2007), as feed cost alone constitutes about 70–80% of the total production cost. Quail's diet consists mainly of feedstuffs like yellow corn and soya bean which are considered as major protein sources. Hence, nutritionists try hard to minimize the cost of feed ingredients by using by-products from various sources and unconventional feed sources (Fadzlin et al. 2020). By-products of fruit processing industry consist mainly of seeds, core, pomace, and peels. Without further processing, these agro-wastes can produce serious environmental pollution (Shalini and Gupta 2010). These by-products have gradually been used more, especially when researchers found that peels possess more promising biological values than other parts of the fruit (Moon and Shibamoto 2009). Pomegranate (*Punica granatum* L.) is a fruit that belongs to the Punicaceae family and contains an inside network of membranes that constitutes about 26–30% of the overall fruit weight (Ismail et al. 2012). Recently, pomegranate is considered to be an effective medicinal plant in Egypt, Greece, and China, grown mainly in arid and semi-arid regions of India, Iran, and Mediterranean countries including Turkey, Egypt, Tunisia, Spain, Morocco, etc. (Ercisli et al. 2011; Newman 2011). By-products of *Punica granatum* L. can modulate gut microbiota and immune function of broiler chickens as well as decrease the odorous gas emissions from excreta (Saeed et al. 2018). Various researches had been done using various medicinal plants, herbs, and their products (extracts and powders) as unconventional feed supplements, functional foods, nutraceuticals, immunomodulators, antimicrobials, and health protective agents in poultry (Ashour et al. 2014). Recently, researchers have stressed on the significant effect of the biological active compounds extracted from by-products of plants, especially those which are rich in phenolic compounds (Akbarian et al. 2013). The PP is a nutritive by-product that is rich in several phytobiotics (Sayed 2014) such as hydrolyzable tannins (ellagitannin, punicalagin, punicalin, and pedunculagin), flavonoids, anthocyanins, and other phenols (Haqqi 2008). The PP also has a therapeutic role in the treatment of several infections, diabetes, diarrhea, and dysentery. It also enhances resistance against intestinal infection and improves digestion and metabolism (Ahmed et al. 2015). Until now, there is limited consideration of PP as a natural supplement in Japanese quail's diet. Therefore, this experiment was designed to detect the response of two strains of Japanese quail to diet containing different levels of PP powder on the productive performance, carcass traits, blood parameters, hepatic gene expression, and the economic profitability of quail production.

Materials and methods

The experiment was conducted during the winter season from February 5 to March 17 in the year 2020 at the Experimental Animal Research Center, Faculty of Veterinary Medicine, Benha University, Egypt.

Birds, housing, and management

In total, 480 1-day-old Japanese quail chicks were divided into two equal strains of white and brown (240 quails/strain). The mean initial weight of quails was about 7.22 ± 0.14 g/quail. Four treatment groups were randomly designed for each strain with 6 replicates each (10 birds/replicate). The experimental house, and feeding and watering troughs were properly cleaned and disinfected before the arrival of the chicks. The quail chicks were identified with wing bands and were housed in separate replicate pens, which were provided with fresh wood shavings (3–4-cm thickness). The dimensions of each replicate pen were $31 \times 76 \times 45$ cm as $W \times L \times H$. Each pen was supplemented with manual plastic feeder and drinker. All birds have free access to water and feed throughout the experiment.

All the housing, managerial, and hygienic conditions were similar for all the different experimental groups. Each experimental room was supplemented with heaters to regulate the room temperature consistently according to the age of chicks. During the first 4 days, standard brooding temperature was applied, then it was gradually decreased from 35 to 28 °C by the end of the second week of age, then it was maintained around 28 °C, and the relative humidity was between 60 and 70%. With 23-h/day light:1-h/day dark, the lighting was used as yellow incandescent light bulbs (100 watts) distributed evenly on the rooms throughout the experimental period.

Bird, diet, and experimental design

Pomegranate peel (PP) was collected as residues of Qaha Company for food industry, Qaha city, Qalyubia Governorate, Egypt. PP was sun-dried with constant flipping to avoid fungal growth. After complete drying, PP was ground into a powder, tightly packaged in polythene bags, air tightened, and stored at room temperature. Four iso-energetic and iso-nitrogenous diets were formulated and prepared for Japanese quails according to National Research Council (NRC 1994). The experimental diets contained 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 for control, PP-3, PP-6, and PP-9, respectively, as presented in Table 1. The chemical analysis of PP powder percentage (%) of dry matter basis was carried out according to Rabia et al. (2017): 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.

Table 1 Ingredient composition and calculated chemical analysis of the experimental diets

Treatments	PP-0	PP-3	PP-6	PP-9
Feed ingredients (g/kg as fed)				
PP*	0.0	30.0	60.0	90.0
Yellow corn	556.0	535.0	506.0	475.0
Soybean meal (44%CP)	288.0	307.0	298.0	287.0
Corn gluten meal (60% CP)	105.0	98.0	106.0	117.0
Vita. &Min. mix.†	3.0	3.0	3.0	3.0
DL-Methionine	1.0	1.0	1.0	1.0
L-lysine	4.0	3.0	3.0	4.0
Wheat bran	20.0	0.0	0.0	0.0
Limestone	19.0	19.0	19.0	19.0
Salt	4.0	4.0	4.0	4.0
Total	1000	1000	1000	1000
Calculated chemical composition (%)‡				
ME (kcal/kg)	2902.4	2902.3	2901.3	2900.0
CF	3.87	4.09	4.34	4.59
CP	24.01	24.05	24.01	24.06
Na	0.17	0.17	0.17	0.17
Ca	0.82	0.83	0.82	0.82
Available phosphorus	0.41	0.39	0.39	0.38
Methionine	0.56	0.55	0.56	0.56
Lysine	1.39	1.32	1.30	1.37

*PP: pomegranate peel (crude protein 3.97%, crude fiber 12.1%, and metabolizable energy 2825 kcal/kg) (Rabia et al. 2017). †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)

Data collection

The quails were weighed individually on arrival. Throughout the experiment, individual weekly body weight (BW) was recorded. From these measurements, the body weight gain (BWG) was calculated. The data of average feed consumption was determined precisely and calculated as grams per quail all over the experimental period. Unused feed from each experimental group was collected daily, weighed, and taken into consideration for the calculation of feed intake; accordingly, feed conversion ratio (FCR) was calculated weekly for each quail. At day 42 of the experiment, 30 male quails from each group (5/replicate) were randomly selected, weighed, kept separately, and fastened for a period of 12 h, then slaughtered by cutting carotid arteries and jugular veins, and allowing them about 4 min for complete bleeding. Then, they were processed by removing the feather, the viscera, head, neck, shanks, and feet. The eviscerated carcass was weighed and the dressing percentage was calculated according to the formula of Brake et al. (1993):

$$\text{Dressing percentage} = \frac{\text{Eviscerated carcass weight}}{\text{live weight}} \times 100\%$$

Liver, intestine, gizzard, and heart were weighed individually by using digital balance (PGL303, UK), and their percentages in relation to live BW were calculated.

For analysis of messenger ribonucleic acid (mRNA) expression of Insulin Growth Factor-1 (IGF-1) and hepatic Growth Hormone Receptor (GHR) genes, tissue samples of liver were taken from each group and frozen at − 80 °C (Jia et al. 2018). RNA was extracted from the liver tissue using RNeasy® Mini kit (Qiagen, Germany) according to the manufacturer's guidelines. Samples were lysed and then mixed. Ethanol was added to the lysate to provide perfect binding conditions. The lysate was then put onto the RNeasy silica membrane "RNeasy Mini spin column." RNA binds and all pollutants were efficiently washed away. Pure concentrated RNA was eluted in 30 μl water.

According to manufacturer's instructions of complementary DNA (cDNA) Reverse Transcription kits (Applied Bio systems), cDNA was synthesized from the total RNA. Conditions used for this process were 25 °C for 10 min, 37 °C for 120 min, and 85 °C for 5 min. Samples of RNA and cDNA were stored at (− 80 °C).

To determine the effect of PP powder on growth performance, expression of hepatic GHR and hepatic IGF-1 genes was analyzed by using the following gene-specific primer sequences (Gasparino et al. 2014): GHR, sense (5′-AACACAGATACCCAACAGCC-3′) and anti-sense (5′-AGAAGTCAGTGTTTGTCAGGG-3′), IGF-1, sense (5′-CACCTAAATCTGCACGCT-3′) and anti-sense (5′-CTTGTGGATGGCATGATCT-3′), and β-actin used as a housekeeping gene, sense(5′-ACCCCAAAGCCAACAGA-3′) and anti-sense (5′- CCAGAGTCCATCACAATACC-3′).

The real-time polymerase chain reaction (RT-PCR) was done according to QuantiTect SYBR Green PCR Kit, Qiagen, and the reactions were done on an Applied Biosystem 7500 Fast RT- PCR detection system (Central Lab, Faculty of Veterinary Medicine, Benha University) under the specific conditions: 95 °C for 10 min and 40 cycles of 95 °C for about 15 s followed finally by 60 °C for 1 min. Alterations in the gene expression of target genes were estimated by the comparative $2-\Delta\Delta Ct$ method (Livak and Schmittgen 2001).

To measure glutathione (GSH) and catalase (CAT) enzymes, a liver tissue sample (250 mg) was mixed in one ml of cold phosphate buffer saline (PBS) solution (pH 7.4) and centrifuged at 4000 rpm for 15 min. The supernatant was removed for assaying. CAT level was determined according to Aebi (1984), and GSH activity was measured as described by Beutler et al. (1963) using a commercial kit (bio-diagnostic, Egypt).

Heparinized blood samples were collected for hematological picture. Total leukocytic count (TLC) and red blood cells (RBC) were performed in a Neubauer hemocytometer by using a 1:200 dilution with Natt and Herrick solution. Hemoglobin (Hb) concentration was measured as described by Campbell (1995). Blood samples were collected in plain tubes and centrifuged at 3000 rpm for 10 min to harvest serum. Analyses of triglycerides (TG), total protein (TP), albumin (Alb), and cholesterol were performed using commercial kits (Centronic, Germany) on Chem 7, USA.

The economic evaluation of the experimental groups was measured via calculation of production costs, total returns (TR), and net return (NR) values according to Fardos et al. (2017). Total feed intake cost was calculated for each quail chick in each experimental group during the experimental period by multiplying the total feed intake per quail × cost of 1-kg diet. NR was calculated according to Kareem and Karwan (2019) by subtracting TC from TR.

Statistical analysis

The data were analyzed by ANOVA using the GLM procedures of the SPSS/PC "version 16" (SPSS 2004) after verifying normality using Shapiro–Wilk's test and homogeneity of variance components between experimental groups by Levene's test. The model included the fixed effects of the genetic type of Japanese quail (two strains: white and brown), the dietary treatment (four levels: control, PP-3%, PP-6%, and PP-9%), and their interaction. Multiple mean comparisons were done using Duncan multiple range test (Duncan 1955). Moreover, polynomial contrasts test was applied on productive traits, carcass traits, and the economic evaluation parameters.

Results

Growth performance

The effects of genetic type and dietary treatment with different levels of PP powder on growth traits are illustrated in Table 2. The initial body weight of the quail chicks of both plumages color did not differ significantly ($P = 0.577$). FCR had no significant differences in both strains as well. On the other hand, the final BW, total feed intake (TFI), and BWG of white growing quails showed significant ($P < 0.001$) increase over the brown strain. Referring to quails fed diet containing PP powder, the growth traits significantly ($P < 0.05$) improved over the control. Quail group fed with 6% PP powder had the highest ($P < 0.001$) average final BW and BWG ($P < 0.001$). Final BW increased by 43.7 g/quail in quails fed with 6% PP powder as compared with the control

Table 2 Effect of genetic type and dietary treatments on final body weight, body weight gain, total feed intake, and feed conversion ratio of Japanese quail

Parameter	Genetic type (G)		Dietary pomegranate peel powder (PP)				RSD	*P-values*			
								G	PP		G×PP
	White	Brown	PP-0	PP-3	PP-6	PP-9			Linear	Quadratic	
Initial BW (g)	7.24	7.21	7.28	7.20	7.22	7.20	0.13	0.577	0.366	0.599	0.154
Final BW (g)	225.5	203.1	186.7^{d}	216.1^{c}	230.4^{a}	224.2^{b}	3.41	< 0.001	< 0.001	< 0.001	< 0.001
BWG (g)	218.3	195.9	179.4^{d}	208.9^{c}	223.2^{a}	217.1^{b}	3.41	< 0.001	< 0.001	< 0.001	< 0.001
TFI (g)	872.4	778.6	860.1^{a}	813.9^{ab}	839.4^{ab}	788.5^{b}	44.1	< 0.001	0.032	0.898	0.213
FCR (g/g)	4.07	4.01	4.84^{a}	3.91^{b}	3.78^{b}	3.64^{b}	0.22	0.526	< 0.001	0.001	0.121

FI, feed intake; *BWG,* body weight gain; *FCR,* feed conversion ratio

PP-0, 0% pomegranate peel powder; *PP-3*, 3% pomegranate peel powder; *PP-6*, 6% pomegranate peel powder; *PP-9*, 9% pomegranate peel powder; *TFI*, total feed intake; *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 $P < 0.05$

group. The dietary PP powder at different levels significantly ($P=0.032$) decreased TFI. PP powder 9% decreased TFI (−71.6 g/quail) compared with the control group. FCR was significantly ($P<0.001$) better in all quail groups fed with PP powder than the control group for the total grow-out period (1–42 days). A significant ($P<0.001$) interaction between genetic type and dietary treatment was found in final BW and BWG all over the experimental periods.

Carcass traits

All carcass traits including preslaughter BW, carcass weight, intestine percentage, and gizzard percentage of white quails were significantly ($P<0.05$) higher than that of brown strain, while there were no significant differences between the two strains for dressing percentage, liver percentage, and heart percentage (Table 3). Regardless of the effect of strain, the dietary PP powder at different levels had no significant effect in carcass traits except for the liver percentage, which was significantly ($P=0.011$) higher for quail group fed with 3% PP powder and control group, and the lowest liver percentage was for quail group fed with 9% PP powder. The interaction effects between genetic type and dietary treatment were non-significant for carcass traits.

Blood, antioxidant, and biochemical indices

Table 4 indicated significant differences ($P<0.001$) of hematological and biochemical parameter values of brown and white quails. Brown quails had higher values in RBC and TLC as well as heterophils and lymphocytes than white strain. On the contrary, white quails had higher values in Hb, albumin, total protein, cholesterol, and triglycerides than brown quails. Concerning the dietary treatments, it was found that quails fed diet 9% PP powder had higher significant ($P<0.001$) TLC, heterophils, and lymphocytes than other experimental groups, and they had the lowest significant ($P<0.001$) level of cholesterol and triglycerides. Concerning the effect of strain type on the values of the antioxidant enzyme, catalase results revealed no significant ($P=0.836$) difference between white and brown quails while glutathione values were significantly ($P<0.035$) higher for the white quails. Regardless of the effect of strain, the dietary PP powder at different levels had a higher significant ($P<0.001$) increase in catalase and glutathione values than control group: the highest values were for quails fed diet 9% PP powder, while the lowest values were for the control group. A significant ($P<0.05$) interaction between genetic type and dietary treatment was found in Hb, TLC, heterophils, lymphocytes, albumin, and cholesterol.

Gene expression

Figures 1 and 2 illustrate the interaction effect of genetic type and dietary treatments on hepatic Growth Hormone Receptor (GHR) and Insulin Growth Factor-1 (IGF-1) expression of Japanese quails. A significant ($P<0.05$) interaction between genetic type and dietary treatment was found in the expression of hepatic GHR and IGF-1 genes. White quails fed diet 3% PP powder had the greatest expression of hepatic GHR gene. The expression of hepatic IGF-1 gene in brown quails fed diet 6% and 9% PP powder was significantly ($P<0.05$) greater than other groups.

Economic evaluation

In Table 5, the SR, TR, and TC of white growing quails showed significant ($P<0.001$) increase over the brown strain, while there were no significant differences in TFI cost and NR between the two strains. Regardless of the effect of strain, the dietary PP powder at different levels significantly ($P=0.002$) decreased TFI cost and TC compared

Table 3 Effect of genetic type and dietary treatments on carcass traits of Japanese quail

Parameter	Genetic type (G)		Dietary pomegranate peel powder (PP)				RSD	*P-values*			
								G	PP		G×PP
	White	Brown	PP-0	PP-3	PP-6	PP-9			Linear	Quadratic	
Slaughter BW (g)	254.3	203.2	236.6	228.0	217.9	214.7	25.7	<0.001	0.056	0.745	0.474
Carcass weight (g)	184.2	151.6	176.4	169.4	163.7	162.4	17.6	<0.001	0.065	0.609	0.570
Dressing perc. (%)	75.4	74.6	75.0	74.6	75.1	75.6	4.6	0.601	0.718	0.773	0.971
Liver perc. (%)	2.27	2.24	2.44[a]	2.48[a]	2.19[ab]	1.90[b]	0.5	0.865	0.011	0.311	0.305
Intestine perc. (%)	4.56	4.06	4.44	4.39	4.11	4.29	0.75	0.046	0.515	0.633	0.460
Gizzard perc. (%)	3.93	3.06	3.21	3.62	3.65	3.48	0.47	<0.001	0.233	0.074	0.162
Heart perc. (%)	0.90	0.92	0.92	0.85	0.94	0.93	0.14	0.691	0.591	0.565	0.364

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 4 Effect of genetic type and dietary treatments on hematological, biochemical indices, and hepatic antioxidant status of Japanese quail

Parameter	Genetic type (G)		Dietary pomegranate peel powder (PP)				RSD	*P-values*		
	White	Brown	PP-0	PP-3	PP-6	PP-9		G	PP	G × PP
RBC (× 10^6/µl)	2.76	5.35	4.20	4.22	3.76	4.03	0.30	< 0.001	0.062	0.836
Hb (g/dl)	15.65	14.36	14.5	15.1	15.3	15.1	0.72	< 0.001	0.383	0.017
TLC (× 10^3/µl)	2.87	9.47	5.25[c]	6.55[a]	5.95[b]	6.92[a]	0.53	< 0.001	< 0.001	< 0.001
Hetero. (× 10^3/µl)	1.29	4.88	2.57[c]	3.31[a]	3.06[b]	3.38[a]	1.11	< 0.001	< 0.001	0.002
Lym. (× 10^3/µl)	0.98	4.10	2.05[d]	2.71[b]	2.41[c]	3.00[a]	0.73	< 0.001	< 0.001	< 0.001
Alb. (g/dl)	2.63	1.47	2.51[a]	1.64[d]	1.92[c]	2.13[b]	0.90	< 0.001	< 0.001	< 0.001
TP (g/dl)	4.73	3.23	4.32	3.40	3.90	4.30	1.29	0.006	0.490	0.359
Chol. (mg/dl)	171.3	153.9	182.5[a]	170.1[b]	153.3[c]	144.6[d]	20.3	< 0.001	< 0.001	< 0.001
TG (mg/dl)	330.2	269.6	321.8[a]	294.8[c]	306.1[b]	277.3[d]	6.75	< 0.001	< 0.001	0.31
CAT (U/gm)	4.07	4.02	2.76[c]	3.90[b]	4.56[a]	4.98[a]	0.52	0.836	< 0.001	0.502
GSH (nmol/gm)	5.65	5.23	4.43[c]	5.23[b]	5.85[a]	6.26[a]	0.44	0.035	< 0.001	0.759

RBC, red blood cells; *Hb,* hemoglobin; *TLC,* total leucocytic count; *Hetero.,* heterophils; *Lym.,* lymphocytes; *Alb.,* albumin; *TP,* total protein; *Chol.,* cholesterol; *TG,* triglycerides; *CAT,* catalase ; *GSH,* glutathione

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 $P < 0.05$

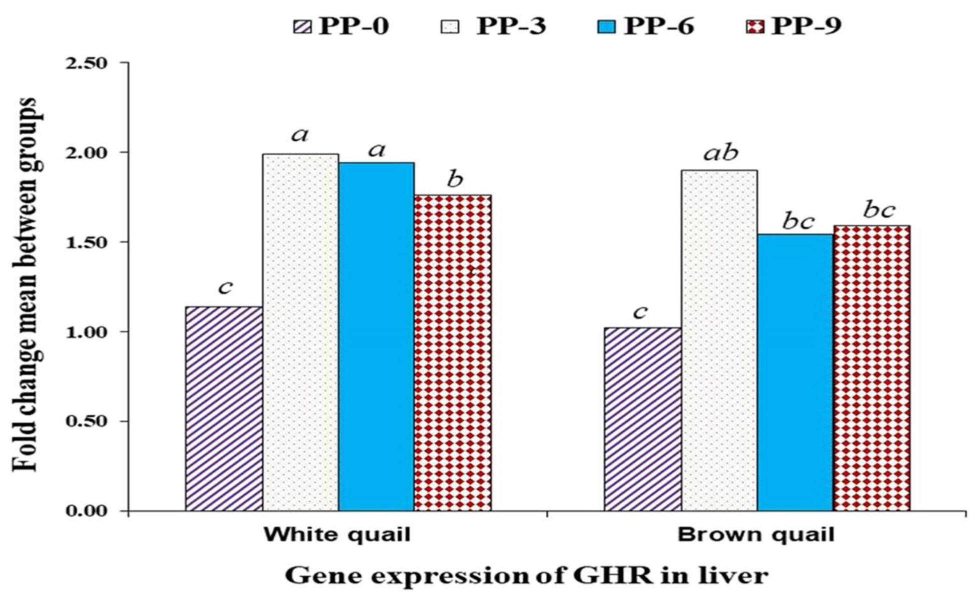

Fig. 1 Effect of genetic type × dietary treatments interactions on hepatic Growth Hormone Receptor (GHR) expression of Japanese quails. Values marked with the different letters on the bars are significantly different ($P < 0.05$)

with the control group. PP powder 9% decreased TFI cost (− 0.039 $/quail) compared with the control group. SR, TR, and NR values were significantly ($P < 0.001$) higher for all quails fed PP powder at different levels compared with the control group. Quails fed with 6% PP powder diet significantly exhibited the highest TR value. Moreover, quails that received 6% and 9% PP powder significantly ($P < 0.001$) recorded higher NR (0.279 and 0.274 $/quail, respectively) compared with the control group. The effects of interactions between the genetic type and dietary PP powder levels were significant ($P < 0.05$) for SR, TR, and NR**.**

Discussion

In the present study, there was no significant difference of the initial BW among the quails of the two plumage colors while white strain had higher final BW than brown strain. These results are supported by the findings of Jessy et al. (2016) who detected no significant variation of BW among white and brown quails at 0 day. Also, the strain effect on BW was reported by Mohammed et al. (2017) who detected a significant difference on BW of quails

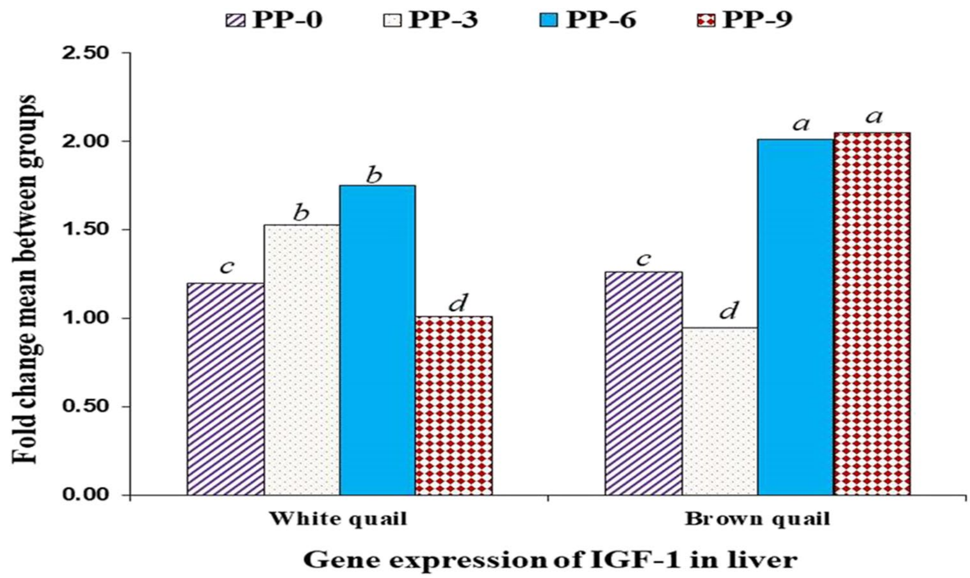

Fig. 2 Effect of genetic type × dietary treatments interactions on hepatic Insulin Growth Factor-1 (IGF-1) expression of Japanese quails. Values marked with the different letters on the bars are significantly different ($P < 0.05$)

Table 5 Effect of genetic type and dietary treatments on economic evaluation parameters of Japanese quails

Parameter	Genetic type (G)		Dietary pomegranate peel powder (PP)				RSD	*P-values*			
								G	PP		G×PP
	White	Brown	PP-0	PP-3	PP-6	PP-9			Linear	Quadratic	
TFI cost ($/bird)	0.323	0.288	0.327[a]	0.301[b]	0.307[ab]	0.288[b]	0.01	0.294	0.002	0.619	0.214
SR ($/bird)	0.874	0.780	0.733[d]	0.830[c]	0.885[a]	0.862[b]	0.01	<0.001	<0.001	<0.001	<0.001
TR ($/bird)	0.906	0.812	0.765[d]	0.862[c]	0.917[a]	0.894[b]	0.02	<0.001	<0.001	<0.001	<0.001
TC ($/bird)	0.686	0.587	0.658[a]	0.632[ab]	0.638[ab]	0.619[b]	0.01	<0.001	0.002	0.619	0.213
NR ($/bird)	0.220	0.225	0.107[c]	0.230[b]	0.279[a]	0.274[a]	0.03	0.611	<0.001	<0.001	0.04

TFI cost, total feed intake cost; *SR,* selling return ; *TR,* total return; *TC,* total cost; *NR,* net return

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 $P < 0.05$

with different plumage color except at 0 day. The current results are also in line with those of Vali et al. (2005) who reported significant final BW variation in the two quail strains, and Ojo et al. (2014) who found that the BW of white plumage color quails surpassed the brown quails at 2 and 4 weeks of age. On the contrary, to the present results, Fadhil et al. (2018) reported that brown strain had higher BW than white strain. This variation in BW of both strains might be attributed to the difference in the genetic development of flocks reared in different areas and ecological regions (Darden and Marks 1988; Marks 1971), and might be due to the recessive gene action which has a recessive depressive effect on quail BW that was more obvious in brown quails (Minvielle et al. 2007). It might also be due to better FCR for the white strain (Islam et al. 2014). Concerning the effect of dietary PP powder at different levels, the growth traits results were significantly improved for all quails fed PP powder over the control. Quail group fed with 6% PP powder had the highest average final BW and BWG. Also, significant interaction results were found between genetic type and dietary treatment in final BW and BWG all over the experimental periods. These results are in agreement with Saeed et al. (2018) who informed that there is a growing interest among scientists in improving the performance of avian species and increasing feed utilization by supplementing a pomegranate by-product to the feed. The PP is a nutritive by-product that is rich in several phytobiotics (Sayed 2014), such as hydrolyzable tannins (ellagitannin, punicalagin, punicalin, and pedunculagin), flavonoids, anthocyanins, and other phenols (Haqqi 2008). So, the current result of improving the BW and BWG of quails fed with 6% PP powder might be attributed to the antioxidative and antimicrobial properties of PP powder. Some investigations showed that phenolic compounds of pomegranate are responsible for its antimicrobial activity due to its ability in the disruption of the bacteria cell membrane (Kanatt et al. 2010). Also, the improvement in quail productive performance upon addition of PP is attributed to the positive effect via stimulation of gastro-intestinal enzymatic activity, and thus enhancing nutrient digestibility and absorption (Banerjee et al. 2013). The current findings are in consent with those got by Baset et al. (2020) who found that the addition of PP powder significantly ($P < 0.05$) improved the broiler chickens' body weight and BWG. Moreover, Rajaian et al. (2013) stated that PP had an antibacterial activity, reducing the pathogens in the intestine. Consequently, more nutrient is available in the intestinal lumen for absorption to convert into body mass. The dietary PP powder at different levels significantly decreases TFI. Quails fed PP powder 9% recorded the lowest TFI value compared with the control group. FCR was significantly better in all quail groups fed with PP powder than the control group. These results were in the same line with those reported by Ahmed et al. (2015) who showed that the supplementation of Pomegranate by-products at 0.5 and 1.0% in the broiler diet reduced the average daily feed intake and improved FCR values. In contrast, Cho et al. (2014) mentioned that PP supplementation to the broiler feed had no significant effect on FI or FCR compared with the control group, and Bostami et al. (2015) revealed that daily feed intake and feed conversion percent remain unaffected ($P > 0.05$) among the dietary pomegranate groups.

Higher growth performance of white quails over the brown was reflected on weights of live BW at slaughtering, carcass weight, intestine percentage, and gizzard percentage. This result of the white strain of Japanese quails is an indicator for their higher efficiency and ability for meat production. This finding is in agreement with Fadhil et al. (2018) who observed that color variation had a significant effect on several carcass traits, as the white line had shown highly significant values ($P < 0.01$) in the breast, thigh, and back than brown lines. These results indicate the effective role for the strain type in such characteristics (Mohammed et al. 2017).

Concerning the effect of the dietary PP powder at different levels, it was found that it had no significant effect in carcass traits except for the liver. Quail group fed with 9% PP powder recorded the lowest value of liver weight percentage. In the line of this result came Sharifian et al. (2019) who stated that dietary PP extract supplementation did not affect the relative weights of carcass, breast, thigh, and internal organs of broiler chickens. Also, Marzoni et al. (2014) proved that the supplementation of a mixture of natural antioxidants did not cause any influence on slaughter yields from broiler chickens. Moreover, Baset et al. (2020) stated that broilers ate diets filled up with PP powder generated the lowest ($P < 0.05$) value of liver percentage compared to the control. These observations somewhat coincided with those noticed by Fawzia et al. (2020) who stated that the dietary supplementation of pomegranate extract at 100, 150, and 200 mg did not significantly affect all carcass traits. Conversely, carcass traits of broiler chickens fed diet supplemented with PP extract was found better than control group according to Hamady et al. (2015). Another study Hamad and Kareem (2019) found that addition of 1% and 1.5% PP powder improved carcass quality of Japanese quail. Differences in values of blood indices between brown and white quails were in the normal range. Dietary PP powder at different levels had significant improvement in some hematological and biochemical parameters compared with the control group. It was found that quails fed diet 3% and 9% PP powder had higher significant immune cells count, such as TLC, heterophils, and lymphocytes, than other experimental groups, and they had the lowest level of cholesterol and triglycerides. This result agreed with Saeed et al. (2018) who found that the *P. granatum L.* by-products improved immunity. As previously described by other studies (Sarica and Urkmez 2016; Yaseen et al. 2016), PP inclusion in the diet reduced serum cholesterol through the hypocholesterolemic and hypolipidemic effects and through its interference with lipid digestion, absorption, and metabolism. Also, Fawzia et al. (2020) reported that pomegranate may have a cholesterol-lowering activity; they also found that feeding rabbits on diets supplemented with PP extract at levels 100-, 150-, and 200-mg/kg diet increased ($P < 0.05$) albumin and globulin compared to the control group, and decreased levels of glucose, total lipids, triglycerides, and total cholesterol than those of the control group. Also, Afaq et al. (2005) observed that PP significantly decreases the levels of total cholesterol (TC) and triglyceride (TG).

Increased GSH and CAT in the liver tissue were also recorded by PP dietary inclusion in quail diets. The highest values were for quails fed diet 6% and 9% PP powder. This improvement suggests that natural polyphenols present in PP can stimulate antioxidant defense system (Al-Shammari et al. 2019; Ghosh et al. 2020). Moreover, CAT inhibits the lipid peroxidation of hydrogen and peroxide toxicity at cellular level (Oloruntola et al. 2018). This result is in accordance with Fawzia et al. (2020) and Sharifiyan et al. (2016) who revealed that rabbits fed on diet supplementing with PBE recorded higher ($P < 0.05$) catalase concentration than the control group.

The growth and metabolism in animals and poultry are mainly based on growth hormone (GH) (Jiang and Lucy 2001). The actions of GH are mediated through the growth hormone receptors (GHR). These receptors are present in liver (Herington et al. 1976). GH released to the circulation has a direct effect on the liver which gives a signal for releasing and synthesis of IGF-I (Switonski 2002). The expression of IGF-1 and GH genes was significantly upregulated in the liver of the quails fed with the dietary PP powder at different levels. White quails fed diet 3% and 6% PP powder had greater expression of hepatic GHR gene. The expression of hepatic IGF-1 gene was higher for brown quails fed diet 6% and 9% PP powder. This agrees with the results of the phenotype, as quails fed diet 6% PP powder had the highest final BW. So, the current results agree with Beckman (2011) and Clemmons (2004) who stated that hepatic IGF-1 level is synchronous with growth rate; thus, feeding level can be considered as a key factor that can promote nutrient usage and growth performance. Also, Gasparino et al. (2014) showed that birds with higher growth rates have higher levels of GHR expression in the liver and muscles than birds with low growth rates. GH and IGF-1 are anabolic hormones with the key roles in metabolism and growth (Vahid et al. 2020). Therefore, it can be deducted that the upregulation of the expression of IGF-1 and GH genes observed in the liver was the reason for improved quail's growth performance.

Higher growth performance of white quails over the brown was also reflected on the SR and TR. Regarding the effect of dietary PP powder at different levels on economic return, the current results revealed that the addition of PP powder reduced the TFI cost at all dietary levels, saving about $ 4.47, 14.08, and 14.72/ton for PP_3, PP_6, and PP_9, respectively. Meanwhile, the highest TFI cost/ton was found for the control diet. Quails fed 6% and 9% PP powder showed the least cost–benefit, declaring that it is the best level from the economic point of view, as they had higher NR value. 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, final BW, TFI, BWG, and dressed weight of carcass of white growing Japanese quails were significantly higher than the brown strain. Quail group fed with 6% PP powder had the highest ($P<0.001$) average final BW and BWG ($P<0.001$). Dietary inclusion of PP powder at levels up to 9% for quails improved growth performance, increased antioxidant properties, enhanced hepatic gene expression, but did not show any adverse influence on carcass quality or blood indices. Economically, white quails recorded the highest value of TR. Moreover, quails fed 6% and 9% PP powder had higher NR value.

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Author contribution 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 first draft of the manuscript was written by Eman Ramadan Kamel and Fathy Attia Ismaiel Abdelfattah. All authors read and approved the final manuscript.

Availability of data and material Data will be made available on reasonable request.

Declarations

Ethical 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, Number BUFVM 03–04-2020. Sampling procedures and management of the experimental birds were according to the guide lines of Federation Animal Science Societies (Societies 1999).

Conflict of interest The authors declare no competing interests.

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