

Tomato pomace as a nontraditional feedstuff: productive and reproductive performance, digestive enzymes, blood metabolites, and the deposition of carotenoids into egg yolk in quail breeders

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ABSTRACT This study aimed to evaluate the inclusion of tomato pomace (TP) into Japanese quail breeders' diet by investigating its effects on digestive enzymes, immune response, antioxidant status, blood biomarkers, productive performance, and the deposition of carotenoids into the egg yolk. A total of 150 mature 8-wk of age Japanese quails (100 females and 50 males) were allocated into 5 treatment groups, with 5 replicates, each of 6 quails (4 females and 2 males). The experimental diets were isoenergetic and isonitrogenous, based on corn and soybean meal, and included 0, 3, 6, 9, and 12% of tomato pomace, respectively. The results showed that dietary supplementation of tomato pomace

up to 12% significantly improved the immune response, antioxidant response, and digestive enzymes of Japanese quail breeders, significantly decreased cholesterol, low-density lipoprotein (LDL), and increased high-density lipoprotein (HDL). Also, TP increased egg weight, egg mass and hatchability, where TP 6% had the greatest egg weight, egg mass and hatchability among other groups. Moreover, tomato pomace inclusion significantly had a positive effect on the deposition of lycopene into the egg yolk and it can be used as a good delivery system to improve human health. Tomato pomace up to 12% could be used as an alternative feedstuff in quail breeders' diets.

Key words: tomato pomace, blood metabolites, egg yolk carotenoids, quail breeders' diets

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INTRODUCTION

Over the last 5 decades, poultry industry grew rapidly (Jha and Berrocso, 2015). Furthermore, the global demand for poultry products has consistently increased over the years, and this demand is expected to continue (FAO, 2013). Feed costs represent almost 70 to 80 % of the total production costs in the poultry production (El-Wardany et al., 2016; Thirumalaisamy et al., 2016). The reduction of the production costs attracted tremendous attention to gain significant benefit from the least production costs (Jha and Mishra, 2021; Alagawany et al., 2021a). Therefore, nutritionists and the animal feed industry look for nonconventional feedstuffs to reduce the feed expenses (Yang et al., 2021). Preferably, the nutritional value of the alternative feedstuff should be

able to replace part of conventional ingredients without compromising productive performance or immunity (Sultana et al., 2016; El-Azeem et al., 2019). Japanese quail is regarded as an important agricultural bird for meat and egg production motivated by nutritional and medicinal benefits in many parts of the world (Jeke et al., 2018). Recently, the attempts to use agroindustrial by-products in poultry feed gained significant attention in the poultry feed industry (Madkour et al., 2008; Madkour et al., 2015; Yang et al., 2021). Tomato pomace (TP) is a source of protein that might reach up to 16% crude protein, and has a nutritional value because it contains many other bioactive compounds such as vitamins, polyphenols, minerals and carotenoids (Marcos et al., 2019; Azabou et al., 2020). In addition, lycopene percentage in TP represents 80 to 90% of total carotenoids (Nour et al., 2018). Previous studies revealed that carotenoids such as lycopene have several health benefits in humans such as antioxidant and inhibitor of cancer cell proliferation (Nahum et al., 2001), immunoprotected activity (Xu et al., 2019). Moreover, tomato juice had hypocholesterolemic effects (Periago et al., 2016). It has been reported that

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increased lipase activity in yellow perch fed with lycopene-enriched diet enhanced the digestion of fat, which could, in turn, explain the better growth rate of yellow perch with a lycopene-enriched diet (El-Gawad et al., 2019). Therefore, increasing lycopene content in egg yolk would provide an extra source of lycopene to improve human health (Olson et al., 2008). It has been reported that dried tomato pulp could be used as an ingredient in growing Japanese quail's diets up to 4 to 6% of the diet (Jouzi et al., 2015). Dietary inclusion of tomato waste in laying hens diet was up to 8% in relation to corn and soybean meal maintained egg weight and egg production (Leke et al., 2015). In broiler diets, TP was included up to 20% in the diet from 29 to 42 d of age without any adverse effect on productive performance (Lira et al., 2010). Generally, tomato pomace is a rich product for many nutritional components like essential amino acids, fatty acids, flavonoids, carotenoids as well as some minerals like Ca, Cu, Mn, Zn, and Se (Azabou et al., 2020). The data concerning the use of tomato pomace in quail diets and its effect on productive and reproductive performance, digestive enzymes, and the deposition of carotenoids into the egg yolk of quail breeders are scanty. Therefore, the objectives of this study were to evaluate the effects of replacing up to 12% of corn and soybean meal content with TP in Japanese quail breeder diets on productive performance, antioxidant status, immune response, blood biomarkers, and digestive enzymes. In addition, unmask the effects of TP on the deposition of carotenoids into the egg yolk of quail breeders.

MATERIALS AND METHODS

Ethical Statement

The research protocol was reviewed and approved by the Faculty of Agriculture, Zagazig University, Egypt (ZU-IACUC/2/F/56/2021).

Experimental Design

The present study was performed at quail unit, Department of Poultry, Agriculture Faculty, Zagazig University, Egypt. One hundred females and fifty males Japanese quails, 8-wk old, were randomly allocated into five treatment groups based on the level of TP (0, 3, 6, 9, and 12% replacing corn and soybean meal content). Each group had 30 birds with 5 replicates each to include 6 quails (4 females and 2 males). The experimental diets were isoenergetic and isonitrogenous based on corn and soybean meal content (Table 1). The conventional type cages with (50 × 30 × 50 cm³; 1,500 cm² of floor space) were used for rearing quails. Water and feed were provided ad libitum, and the light system was 17 h light: 7 h dark cycle throughout the experimental period (8 wk).

The tomato pomace was obtained from commercial processors (Egyptian International Co. For Food Products (The First Industrial zone, New Borg El Arab, Egypt). It was spread out on a plastic sheet and exposed

Table 1. Ingredients, nutrient contents and chemical analysis of the experimental diets of quail breeders.

Ingredients	Tomato pomace (%)				
	Control	3	6	9	12
Corn	60.20	57.25	56.50	55.53	52.95
Soybean meal 44%	25.00	24.50	20.50	17.00	15.78
Gluten meal	5.70	5.70	7.50	8.88	9.00
Soybean oil	1.50	2.00	1.94	1.94	2.60
Limestone	5.50	5.53	5.55	5.55	5.54
Di-calcium phosphate	1.30	1.20	1.15	1.15	1.13
Salt (NaCl)	0.30	0.30	0.30	0.30	0.30
Premix ¹	0.30	0.30	0.30	0.30	0.30
L-Lysine	0.20	0.20	0.26	0.35	0.40
DL-Methionine	0.00	0.02	0.00	0.00	0.00
TP (19.73% CP)	0.00	3.00	6.00	9.00	12.00
Calculated composition ² (%)					
ME (kcal/kg)	2,918	2,903	2,905	2,903	2,903
Crude protein	20.00	20.05	20.02	19.99	19.99
Calcium	2.50	2.50	2.50	2.50	2.50
Nonphytate P	0.35	0.35	0.35	0.35	0.35
Lysine	1.09	1.08	1.08	1.08	1.09
TSAA ³	0.71	0.70	0.70	0.71	0.71
Analyzed ⁴ (as DM)					
Crude protein	19.99	20.02	19.95	20.09	20.01
Fiber	3.78	4.50	4.74	5.48	6.32
Fat	4.29	4.70	4.87	5.05	5.83

Abbreviation: DM, dry matter.

¹Provides per kg of diet: Vitamin A, 12,000 I.U.; Vitamin D3, 5,000 I.U.; Vitamin E, 130.0 mg; Vitamin K3, 3,605 mg; Vitamin B1 (thiamin), 3.0 mg; Vitamin B2 (riboflavin), 8.0 mg; Vitamin B6, 4,950 mg; Vitamin B12, 17.0 mg; Niacin, 60.0 mg; D-Biotin, 200.0 mg; Calcium D-pantothenate, 18,333 mg; Folic acid, 2,083 mg; manganese, 100.0 mg; iron, 80.0 mg; zinc, 80.0 mg; copper, 8.0 mg; iodine, 2.0 mg; cobalt, 500.0 mg; and selenium, 150.0 mg.

²Calculated according to NRC (1994).

³Total sulfur amino acid.

⁴According to AOAC (2006).

to sunlight to dry. According to Yitbarek (2013) waste particle size is reduced by pounding with a stick and hand crushing. Samples of tomato pomace and diets were analyzed for their content of nutrients according to AOAC (2006). The content of crude protein, crude fiber, ether extract, calcium, and phosphorus of tomato pomace is 20.77, 32.80, 4.01, 0.5 and 0.45%, respectively.

Data Collection

Quail feed intake (**FI**) was recorded after 4 wk of the feeding of the experimental diets. Feed conversion ratio (**FCR**, g feed/g egg) was calculated as the quantity of FI divided by the egg mass. Daily, egg number and egg weight were recorded to calculate the egg mass (egg number × egg weight). Egg shape index, Haugh unit, Shell thickness, and yolk index were used as criteria of egg quality using 15 eggs per replicate (Romanoff and Romanoff, 1949).

Fertility and Hatchability

At 12 and 16 wk of age, eggs from each group (20 females and 10 males) were collected for 5 d and all daily eggs were stored with an average temperature of 15.0 °C and average humidity of 72%. All collected eggs were transferred to the incubation unit. Counted hatching chicks while non-hatched eggs were broken to calculate

the % of fertility and hatchability (Alagawany and Attia, 2015).

Blood Biomarkers

At 16-wk-old, six males from each group were randomly sacrificed and blood samples were collected into heparinized at 4,000 rpm for 15 min to obtain plasma (Abd El-Azeem et al., 2014; Madkour et al., 2021; Alagawany et al., 2021b,c). All plasma biomarkers including alanine transaminase (ALT), aspartate transaminase (AST), lactate dehydrogenase (LDH); kidney function, creatinine, urea; triglyceride (TG), total cholesterol (TC), high-density lipoprotein (HDL), low-density lipoprotein (LDL); total protein (TP), globulin (GLOB.), albumin (ALB) were measured using kits from Spectrum Diagnostics & Health Care. Commercial kits from Biodiagnostic Company (Giza, Egypt) were used to measure antioxidants indices levels such as total antioxidant capacity (TAC), glutathione peroxidase (GPX), superoxide dismutase (SOD), catalase (CAT), reduced glutathione (GSH), and malondialdehyde (MDA) and immune functions such as immunoglobulin G (IgG), A (IgA) and M (IgM), Complement 3 (C3), and lysozyme.

Digestive Enzymes

At the end of the experiment (16 wk old), the activity of amylase, protease, and lipase were determined in the ileum of the birds (1 male per replicate). The quail ileum was dissected from the Meckel's diverticulum to 2 cm above the ileocecal junction (Reda, et al., 2021). The ileal contents were aseptically collected in screw-capped sterile specimen vials. The activities of ileal enzymes were assessed according to the method of Najafi et al. (2005).

Carotenoid Analyses in Egg Yolk

Six eggs per group were placed in -20°C for 24 h where egg white was coagulated and was easily

separated from egg yolk. The latter was smashed by a spatula and 1 g of egg yolk was transferred to an amber-colored vial and stirred with 20 mL acetone for 15 min. The mixture was then incubated at -20°C for 48 h to precipitate proteins. After the incubation period, the supernatant was collected and dried under vacuum at 30°C . The dried residue was re-dissolved in 2 mL acetone of high-performance liquid chromatography (HPLC) grade for HPLC analysis (Schierle et al., 2003). The sample was filtered using a $0.22\text{-}\mu\text{m}$ syringe filter. HPLC (Waters 2690 Alliance HPLC system equipped with a Waters 996 photodiode array detector) quantified the concentration of lycopene and lutein. Lutein stock solution of $100\ \mu\text{g}/\text{mL}$ in acetone of HPLC grade was prepared, and 6 serial dilutions were prepared in the concentrations of 70, 60, 50, 40, 30, and $20\ \mu\text{g}/\text{mL}$. Then, the following concentrations were prepared from the previous stock solution: 25, 17.5, 12.5, and $10\ \mu\text{g}/\text{mL}$ in acetone of HPLC grade. Stock solution of $100\ \mu\text{g}/\text{mL}$ was prepared from which the following concentrations were established: 35, 31.5, 28, 24.5, and $17.5\ \mu\text{g}/\text{mL}$ in acetone of HPLC grade (Nimalaratne et al., 2013).

Statistical Analysis

Data of production and reproduction performances, egg quality, plasma biomarkers, digestive enzymes, and yolk carotenoids in quail breeders were analyzed with a generalized linear model (Guide, 2012). The model is:

$$Y_{ij} = +T_i + e_{ij},$$

where Y_{ij} = observation, = overall mean, T_i = Tomato pomace effect, and e_{ij} = random error. The authors used Tukey's test to compare the means among the different groups ($P < 0.05$).

RESULTS

Productive Performance in Quail Breeders

The supplementation of TP maintained egg number ($P > 0.05$) compared with the control group (Table 2).

Table 2. Productive performance of Japanese quail breeders in response to dietary tomato pomace (n = 5).

Items	Tomato pomace (%)					SEM	P value
	0	3	6	9	12		
Egg number/bird							
8–12 wk	25.88	25.20	24.85	24.90	24.24	0.377	0.1157
12–16 wk	23.38	22.70	23.05	22.54	22.03	0.532	0.4917
8–16 wk	24.63	23.95	23.95	23.72	23.13	0.417	0.2334
Egg weight (g)							
8–12 wk	12.22 ^c	12.84 ^b	13.52 ^a	12.68 ^b	12.23 ^c	0.073	<.0001
12–16 wk	12.75 ^d	14.01 ^b	14.45 ^a	13.50 ^c	13.72 ^{bc}	0.089	<.0001
8–16 wk	12.48 ^d	13.42 ^b	13.98 ^a	13.09 ^c	12.98 ^c	0.046	<.0001
Egg mass (g/bird)							
8–12 wk	316.16 ^b	323.48 ^{ab}	335.80 ^a	315.52 ^b	296.44 ^c	4.526	0.0023
12–16 wk	298.05	318.10	332.98	304.46	302.11	8.597	0.0939
8–16 wk	307.42 ^{bc}	321.53 ^{ab}	334.80 ^a	310.45 ^{bc}	300.10 ^c	6.006	0.0185

^{abcd}Means in the same raw with no superscript letters after them or with a common superscript letter following them are not significantly different ($P < 0.05$).

Table 3. Feed intake and feed conversion of Japanese quail breeders in response to dietary tomato pomace (n = 5).

Items	Tomato pomace (%)					SEM	P value
	0	3	6	9	12		
Feed intake (g/bird)							
8–12 wk	983.00 ^b	1,017.00 ^b	1,077.50 ^a	987.50 ^b	1,060.50 ^a	11.027	0.0013
12–16 wk	1,057.50 ^c	1,136.50 ^{bc}	1,171.50 ^{ab}	1,137.50 ^{bc}	1,232.00 ^a	22.748	0.0066
8–16 wk	1,020.25 ^c	1,076.75 ^{bc}	1,124.50 ^{ab}	1,062.50 ^c	1,146.25 ^a	15.964	0.0040
Feed conversion ratio (g feed/g egg)							
8–12 wk	3.11 ^b	3.14 ^b	3.21 ^b	3.13 ^b	3.58 ^a	0.043	0.0004
12–16 wk	3.55 ^b	3.57 ^b	3.52 ^b	3.74 ^b	4.08 ^a	0.058	0.0008
8–16 wk	3.32 ^b	3.35 ^b	3.36 ^b	3.43 ^b	3.82 ^a	0.038	<.0001

^{abc}Means in the same raw with no superscript letters after them or with a common superscript letter following them are not significantly different ($P < 0.05$).

Table 4. Fertility and hatchability performance of Japanese quail breeders in response to dietary tomato pomace (n = 5).

Items	Tomato pomace (%)					SEM	P value
	0	3	6	9	12		
Fertility %							
8–12 wk	82.61	84.00	89.89	90.04	85.08	2.055	0.1039
12–16 wk	86.90	93.45	96.99	94.65	90.71	2.166	0.0675
8–16 wk	84.76 ^b	88.72 ^{ab}	93.44 ^a	92.34 ^a	87.90 ^{ab}	1.617	0.0363
Hatchability %							
8–12 wk	76.37 ^b	76.89 ^b	87.42 ^a	74.74 ^b	78.12 ^b	2.354	0.0287
12–16 wk	77.62 ^c	79.56 ^{bc}	86.91 ^{ab}	87.79 ^{ab}	89.17 ^a	2.743	0.0464
8–16 wk	77.00 ^b	78.22 ^b	87.17 ^a	81.26 ^{ab}	83.64 ^{ab}	2.083	0.0446

^{ab}Means in the same raw with no superscript letters after them or with a common superscript letter following them are not significantly different ($P < 0.05$).

TP increased egg weight and egg mass where TP 6% had the greatest egg weight and egg mass among other groups ($P < 0.05$; Table 2). The inclusion of TP 6 and 12% increased FI compared with the other groups ($P < 0.05$; Table 3). Whereas, the worst value of FCR was recorded in 12% TP when compared with the other groups ($P < 0.05$; Table 3).

Reproductive Performance and Egg Quality in Quail Breeders

The results presented in Table 4 showed TP 6 and 12% improved fertility ($P < 0.05$). In the same line, TP 6% enhanced hatchability ($P < 0.05$; Table 5). Results showed that TP had no effects on egg quality parameters such as albumin %, yolk %, shell %, yolk index, Haugh unit, shell thickness, and egg shape index ($P > 0.05$; Table 5).

Table 5. Egg quality of Japanese quail breeders in response to dietary tomato pomace (n = 5).

Items	Tomato pomace (%)					SEM	P value
	0	3	6	9	12		
Albumin %	52.58	53.46	53.10	52.95	53.24	1.338	0.9927
Yolk %	31.64	32.47	32.33	32.16	31.73	1.392	0.9941
Shell %	15.29	14.08	14.57	14.90	15.04	2.449	0.9971
Shell thickness (mm)	0.23	0.20	0.21	0.21	0.23	0.012	0.4075
Egg shape index (%)	75.21	77.32	77.31	74.08	75.33	2.078	0.7892
Yolk index	46.53	48.80	49.08	48.19	48.76	2.251	0.9295
Haugh unit	82.81	84.02	84.64	82.11	83.18	1.315	0.6957

Means in the same raw with no superscript letters after them or with a common superscript letter following them are not significantly different ($P < 0.05$).

Liver and Kidney Functions in Quail Breeders

Results presented in Table 6 showed that dietary addition of TP 6% improved total protein, albumin, and globulin concentration in plasma. TP 12% enhanced liver functions as indicated by ALT and AST concentration in plasma (Table 6). Also, dietary TP 6% improved kidney function through decreased creatinine levels in the plasma (Table 6). However, TP 9% reduced urea level in the plasma (Table 6).

Lipid Metabolism in Quail Breeders

Dietary administration of 3 and 12% doses of TP (Table 7) reduced cholesterol levels in the plasma of quail breeders. Moreover, TP supplementation reduced triglycerides and the best level was 9% compared to other levels and the control group (Table 7). In addition,

Table 6. Liver and kidney functions of Japanese quail breeders in response to dietary tomato pomace (n = 6).

Items	Tomato pomace (%)					SEM	P value
	0	3	6	9	12		
TP (g/dL)	3.52 ^b	2.65 ^b	4.75 ^a	3.10 ^b	2.85 ^b	0.263	0.0032
ALB (g/dL)	1.88 ^b	1.81 ^b	2.46 ^a	1.74 ^b	1.40 ^b	0.146	0.0102
GLOB (g/dL)	1.64 ^b	0.84 ^c	2.29 ^a	1.36 ^b	1.45 ^b	0.142	0.0011
A/G (%)	1.16 ^b	2.19 ^a	1.09 ^b	1.29 ^b	0.95 ^b	0.090	<0.0001
ALT (IU/L)	17.65 ^a	11.63 ^{ab}	6.56 ^b	16.35 ^a	5.54 ^b	1.797	0.0030
AST (IU/L)	386.75 ^a	349.20 ^a	257.50 ^b	323.90 ^a	182.45 ^c	18.66	0.0002
LDH (IU/L)	113.30 ^b	157.88 ^b	408.83 ^a	174.05 ^b	465.45 ^a	41.084	0.0008
Creatinine (mg/dL)	0.59 ^a	0.56 ^a	0.32 ^c	0.45 ^b	0.38 ^{bc}	0.026	0.0002
Urea (mg/dL)	5.92 ^a	4.94 ^{ab}	5.92 ^a	4.04 ^b	4.97 ^{ab}	0.259	0.0314

Abbreviations: A/G, albumin/globulin; ALT, alanine transaminase; ALB, albumin; AST, aspartate transaminase; GLOB, globulin; LDH, lactate dehydrogenase; TP, total protein.

^{abc}Means in the same raw with no superscript letters after them or with a common superscript letter following them are not significantly different ($P < 0.05$).

Table 7. Plasma lipid profile of Japanese quail breeders in response to dietary tomato pomace (n = 6).

Items	Tomato pomace (%)					SEM	P value
	0	3	6	9	12		
TC (mg/dL)	431.25 ^a	348.30 ^b	397.15 ^a	409.65 ^a	345.65 ^b	11.997	0.0021
TG (mg/dL)	335.69 ^a	185.70 ^c	245.51 ^b	142.30 ^d	162.15 ^{cd}	9.020	<0.0001
HDL (mg/dL)	43.57 ^b	50.29 ^a	43.58 ^b	48.18 ^a	48.62 ^a	1.122	0.0088
LDL (mg/dL)	320.55 ^a	260.87 ^b	304.47 ^{ab}	333.02 ^a	264.61 ^b	13.148	0.0131
VLDL (mg/dL)	67.14 ^a	37.14 ^c	49.10 ^b	28.46 ^d	32.43 ^{cd}	1.804	<0.0001
Free fatty acids	0.79 ^a	0.49 ^b	0.61 ^b	0.46 ^b	0.50 ^b	0.041	0.0039

Abbreviations: HDL, high-density lipoproteins; LDL, low-density lipoproteins; TC, total cholesterol, TG, triglyceride; VLDL, very low-density lipoproteins.

^{abcd}Means in the same raw with no superscript letters after them or with a common superscript letter following them are not significantly different ($P < 0.05$).

TP reduced LDL, VLDL, and free fatty acids (Table 7). In contrast, dietary supplementation of TP increased HDL (Table 7).

Immune Response and Antioxidant Status in Quail Breeders

Results presented in Table 8 indicated that TP has an immune stimulator effect through the improvement of complement 3 and IgM, with the best level

was TP 6% among treatment groups and control groups. In addition, TP improved antioxidant status through increase SOD, TAC, and GSH activities while reduced lipid peroxidation (MDA; Table 8). However, TP showed no effects on CAT and GPX (Table 8).

Digestive Enzymes in Quail Breeders

Interestingly, dietary administration of TP (Table 9) enhanced ileal amylase and Lipase activities especially

Table 8. Immune response and antioxidants status of Japanese quail breeders in response to dietary tomato pomace (n = 6).

Items	Tomato pomace (%)					SEM	P value
	0	3	6	9	12		
Immunity							
Complement 3	122.50 ^c	12.00 ^c	193.50 ^a	186.50 ^a	153.00 ^b	6.755	<0.0001
Lysozyme (U/mL)	0.34	0.23	0.23	0.15	0.28	0.033	0.0813
IgM (mg/dL)	0.60 ^b	0.76 ^{ab}	1.00 ^a	0.91 ^{ab}	1.04 ^a	0.078	0.0424
IgG (mg/dL)	0.40	0.59	0.65	0.78	0.66	0.079	0.1870
IgA (mg/dL)	0.48	0.77	0.81	0.95	0.74	0.120	0.3887
Antioxidants							
SOD (U/mL)	0.32 ^c	0.46 ^{ab}	0.48 ^{ab}	0.39 ^{bc}	0.51 ^a	0.023	0.0130
MDA (nmol/mL)	0.54 ^a	0.29 ^b	0.36 ^b	0.40 ^b	0.29 ^b	0.030	0.0022
TAC (ng/mL)	0.32 ^b	0.45 ^a	0.47 ^a	0.40 ^{ab}	0.49 ^a	0.030	0.0278
GSH (mmol/L)	0.34 ^b	0.22 ^c	0.21 ^c	0.15 ^d	0.53 ^a	0.013	<0.0001
CAT (U / mL)	0.30	0.39	0.37	0.31	0.42	0.049	0.3624
GPX (mU / mL)	0.23	0.22	0.20	0.15	0.27	0.032	0.3608

Abbreviations: CAT, catalase; GPX, glutathione peroxidase; GSH, glutathione reduced; Immunoglobulin M: (IgM), G (IgG), and A (IgA); MDA, malondialdehyde; SOD, superoxide dismutase; TAC, total antioxidant capacity.

^{abcd}Means in the same raw with no superscript letters after them or with a common superscript letter following them are not significantly different ($P < 0.05$).

Table 9. Digestive enzymes of Japanese quail breeders as affected by dietary tomato waste meal (n = 5).

Items	Tomato pomace (%)					SEM	P value
	0	3	6	9	12		
Protease (U/L)	0.42	0.31	0.28	0.26	0.33	0.074	0.6110
Amylase(U/L)	9.60 ^c	14.10 ^b	18.20 ^a	12.80 ^b	17.60 ^a	0.531	<0.0001
Lipase (U/L)	3.41 ^d	3.43 ^d	6.45 ^c	8.40 ^b	10.41 ^a	0.365	<0.0001

^{abcd}Means in the same raw with no superscript letters after them or with a common superscript letter following them are not significantly different ($P < 0.05$).

Table 10. Carotenoid content in egg yolk of Japanese quail breeders as affected by dietary tomato waste meal (n = 6).

Items	Tomato pomace (%)					SEM	P value
	0	3	6	9	12		
Lutein (mg/g)	0.11 ^b	0.20 ^a	0.16 ^{ab}	0.10 ^b	0.19 ^a	0.022	0.0392
Lycopene (mg/g)	0.014 ^c	0.019 ^{ab}	0.021 ^{ab}	0.016 ^{bc}	0.022 ^a	0.001	0.0181

^{abc}Means in the same raw with no superscript letters after them or with a common superscript letter following them are not significantly different ($P < 0.05$).

the % level was the most effective in this manner. However, supplementing TP to quail breeder diets had no effects on ileal protease activity (Table 9).

The Concentration of Lutein and Lycopene in Egg Yolk of Quail Laying Hens

The concentrations of lutein and lycopene in egg yolks are shown in Table 10. Supplementing TP to quail breeder diets increased lutein and lycopene concentration in egg yolk (Table 10). The highest lutein concentration in the egg yolk was achieved with levels of 3 and 12% TP. Regarding lycopene concentration, the 12% TP achieved the highest lycopene concentration in egg yolk of quail laying hens (Table 10).

DISCUSSION

The main target of the present study was to verify the possible inclusion of tomato pomace into quail breeder diets and its effect on the productive performance, antioxidant status, immune response, blood biomarkers, and digestive enzymes of Japanese quail breeders. The present findings regarding productive performance are consistent with those of Akdemir et al. (2012) who found that linear increases in egg production, egg weight, and feed intake while linear decreases in feed conversion were observed with tomato powder supplementation in laying hens diets. Also, dietary inclusion of 17 g of tomato paste per kg of diet in laying hens improved egg production but there were no significant differences in feed intake (An et al., 2019). Tomato waste could be included in laying hens diet up to 8% with no adverse impact on egg weight and egg production (Leke et al., 2015). The effect of tomato by-products on growth performance and egg production is a controversial issue (Panaite et al., 2019). In contrast to our results, Panaite et al. (2019) found that dried tomato waste at 5 and 7.5% decreased daily feed consumption and had no differences in egg weight and their components. On the

same context, a previous study found that dietary addition of 50, 100, and 150 kg/t dried tomato product in Hy-line W36 layers did not influence daily feed consumption and body weight (Jafari et al., 2006). The discrepancies between results may be due to tomato by-product sources or components. Generally, tomato pomace is a rich product for many nutritional components like essential amino acids, fatty acids, flavonoids, carotenoids, as well as some minerals like Ca, Cu, Mn, Zn, and Se (Azabou et al., 2020).

To best our knowledge, no studies evaluated the effects of TP on fertility and hatchability in quail breeders. Our results showed that increased levels of TP were accompanied by increased lycopene and lutein concentrations in egg yolk and that may be improved egg quality via improved antioxidant status accompanied by reduced yolk lipid peroxidation (Akdemir et al., 2012; Akdemir et al., 2012; FAO, 2013; Saed et al., 2018).

Inclusion of TP 6% improved total protein, albumin, and globulin. Inclusion of 15% dried tomato pomace into growing rabbit diets increased plasmic total protein, albumin, and globulin contents (Elazab et al., 2011). However, Jouzi et al. (2015) found that dietary supplement up to 24% TP had no significant effect on total protein, albumin, and globulin in Japanese quail. Moreover, TP with levels 6 and 12% improved liver and kidney function. Lycopene, the prominent compound in tomato pomace, displays powerful antioxidant properties and improved liver function in laying hens (An et al., 2019; Orhan et al., 2021). Lycopene improved liver and kidney and reverse the harmful impacts of aflatoxin in Peckin ducklin (El-Sheshtawy et al., 2021). Also, dietary lycopene (100 mg/kg) in broilers diets reduced the ALT (Mezban et al., 2019). Moreover, lycopene has a hepatoprotective effect in rats (Jiang et al., 2016).

One of the most important results obtained in the current study, the suppression effect of tomato pomace on cholesterol, triglycerides, and LDL levels. In agreement with our results, dietary addition up to 7% of tomato

waste had a beneficial role in modulating lipid metabolism via reducing serum total cholesterol, LDL, and triglycerides levels in broiler chickens (Mahata et al., 2016). TP 5% in broiler diets from 1 to 28 d decreased triglycerides and increased HDL levels in the serum (Hosseini-Vashan et al., 2016). Lycopene supplementation, either as a purified form or in tomato paste into laying hens diets reduced serum and egg cholesterol via adjustment some genes that related to lipid metabolism (Orhan et al., 2021). Dietary tomato powder up to 2% in growing rabbit diets decreased cholesterol, LDL, VLDL triglycerides, while increased HDL (Elwan et al., 2019). Also, tomato pomace inclusion into laying hens diets reduced triglycerides under heat stress conditions (Saed et al., 2018). Dietary supplementation of TP extract reduced cholesterol, LDL, while increased HDL in growing rabbits (Hassan et al., 2021). Tomato juice had hypocholesterolemic effects via suppressing 3-hydroxy-3-methylglutaryl coenzyme A reductase (HMGCR) activity that serving as the rate-limiting enzyme of cholesterol synthesis (Periago et al., 2016) then adjust the low-density lipoprotein receptor (LDL-R) and suppress the activity of acyl-coenzyme A: cholesterol acyltransferase (ACAT) (Palozza et al., 2012). Maternal supplementation of lycopene or in ovo feeding increased serum HDL cholesterol in broiler chicks (Sun, et al., 2015). In line with previously mentioned studies, dietary lycopene (100 mg/kg) reduced serum total cholesterol, triglycerides, and LDL levels in broiler chickens by activating the AMPK signaling pathway, thereby regulating lipid metabolism through increasing the mRNA abundance of adenosine monophosphate activated protein kinase α (AMPK- α), whereas decreasing the mRNA abundance of sterol regulatory element-binding protein 1, FAS, and ACC compared to the control group (Wan et al., 2021). Lycopene supplementation with 200 mg/kg in Japanese quail diets increased the HDL concentration whereas VLDL and LDL concentrations reduced (Sahin et al., 2006). Also, dietary inclusion of lycopene (100 mg/kg) reduced plasma cholesterol, triglycerides, and VLDL levels in broiler chicks, while increased HDL level (Mezbani et al., 2019).

The current findings showed that 6% TP was the best level as an immune stimulator in quail breeders. In ovo or maternal supplementation of lycopene increased immune organ index in broiler chicks (Sun et al., 2015). Lycopene (200 and 400 mg/kg diet) supplementation could enhance the immune response in yellow perch (El-Gawad et al., 2019). Tomato by-products are an excellent source of carotenoids, that serve as natural antioxidants and lycopene represents approximately 80 to 90% of the total carotenoids, while β -carotene represents (7–10%) (Nour et al., 2018). It has been reported that lycopene, the major carotenoid component in tomato waste, has an immunoprotective activity (Xu et al., 2019). Inclusion of tomato powder up to 2% into growing rabbit diets enhanced immune response via increasing the immunoglobulins (IgM, IgG, and IgA), phagocytosis, and chemotaxis (Elwan et al., 2019)

Moreover, dietary lycopene up to 80 mg/kg diet increased immune organ index of breeding hens and alleviate lipopolysaccharide (LPS) - induced stress (Sun et al., 2014).

Due to its content of ascorbic acid and the antioxidant minerals especially Zn and Se, tomato pomace may be used as antioxidant supplements (Elbadrawy and Sello, 2016) the current findings showed that the antioxidants status had improved through enhanced SOD, TAC, and GSH activity and at the time reduced lipid peroxidation (MDA). It has been reported that tomato waste juice could be used as an antioxidant source in broiler diets to maintain internal organ development and preserve normal health (Madkour et al., 2020; Orhan et al., 2021). Dietary tomato paste improved antioxidant capacity by increasing lycopene serum concentrations and decreased MDA in laying hens (Orhan et al., 2021). Moreover, lycopene as a powerful antioxidant compound reduced MDA concentration and reactive oxygen species (ROS) levels, while improving the total antioxidant capacity (T-AOC) and antioxidant enzyme activities in mice (Xu, et al., 2019) and rabbits (Hassan et al., 2021). Tomato paste or lycopene supplementation to laying hens reduced serum and egg yolk MDA (An et al., 2019). In ovo lycopene improved the antioxidant status of chicks and increased hepatic T-AOC, GSH/GSSG, and GSH-Px activity while decreased hepatic MDA (Sun et al., 2015). Dietary lycopene up to 300 mg/kg in Japanese quail diets improved Superoxide dismutase in the muscle and liver (Amer et al., 2020) Decreased MDA level in the present study may be due to the ability of lycopene in quenching free radical anions and increasing the number of conjugated double-bonds (Alshatwi et al., 2010). The improvement in the antioxidant status in the current study might attribute to existing of multiple bioactive molecules with antioxidant activities like carotenes, phenolic compounds, lycopene, flavonoids, vitamin A, and ascorbic acid in tomato by-products (Amer et al., 2020).

As regards plant extracts rich in polyphenols, it has been shown that digestive secretions, such as saliva and digestive enzymes, increase the absorption and utilization of nutrients which, in turn increase the growth of animals (Elwan et al., 2019). Tomato by-products are rich sources of amino acids especially lysine, pigments such as β -carotene and lycopene, and ascorbic acid, vitamin E and micronutrients Interestingly dietary administration of TP enhanced ileum Amylase and Lipase activities (Amer et al., 2020). The intestinal enzyme activities have a significant role in the life activities and physiological processes of the animal (Cho et al., 2012). It has been reported that increased lipase activity in yellow perch fed with lycopene-enriched diet enhanced the digestion of fat, which could, in turn, explain the better growth rate of yellow perch with a lycopene-enriched diet (El-Gawad et al., 2019). To the best of our knowledge, there was no study on the impact of tomato pomace on digestive enzymes activities in Japanese quail. Due to the anti-inflammatory effect, supplementation of lycopene improved gastrointestinal parameters and

brush border enzymes activities in mucositis rats (Kuchay et al., 2015).

In human studies, there is a relationship between consuming tomato or lycopene-rich products and decreased LDL then decreased blood pressure (SBP) risk that reflects on improved the marker of cardiac risk, flow-mediated vasodilatation of the brachial artery (FMD) (Li and Xu, 2013; Cheng et al., 2017).

One of the main objectives of the current study was to investigate the possibility to incorporate carotenoids especially lycopene into the egg yolk and use the egg as a good delivery system to improve human health. It has been reported that tomato powder supplementation was an effective way to transfer carotenoids into egg yolk in laying hens (Akdemir et al., 2012). The present findings showed that increased levels of TP in quail diets were accompanied by increased lycopene and lutein in egg yolk. Our results agree with studies that suggested that tomato pomace is a significant source of functional food components such as lycopene, β -carotene, and phenolic acids (Yitbarek, 2013; Borycka, 2017) In this respect, it has been hypothesized that lycopene-enriched yolks, even at low levels, might be a significant source of lycopene than tomato products, however, this hypothesis needs further studies to confirm (Olson et al., 2008). Other studies also noted that supplementation of tomato powder into a diet of quail or laying hens increased the incorporation of lycopene and other carotenoids such as lutein, or zeaxanthin into egg yolk (Karadas et al., 2006; An et al., 2019). Moreover, dietary lycopene, either as a tomato pomace or purified form, reduced serum and egg yolk cholesterol concentrations and increased the concentrations of serum and egg yolk lycopene (Akdemir et al., 2012; Orhan et al., 2021).

Another point of view related to carotenoids incorporation into the egg yolk increased lycopene and other carotenoids into the egg yolk was associated with decreased egg yolk MDA concentrations (Akdemir et al., 2012). The reduction in MDA in egg yolk due to tomato supplementation is considered as an effective strategy to enhance oxidative stability of fresh eggs (Saed et al., 2018; An et al., 2019; Panaite et al., 2019) and meat quality in rabbits (Hassan et al., 2021), so we could conclude that in the current study the improvement in the percentage of fertility and hatchability may be due to increase the oxidative stability of the egg yolk.

CONCLUSIONS

From the previous results, we conclude that dietary inclusion of tomato pomace up to 12% improved the productive performance of Japanese quail breeders, decreased cholesterol, LDL, and increased HDL. Also, tomato pomace improved the immune response, antioxidant status, and digestive enzymes of Japanese quail breeders. finally, dietary tomato pomace had a positive effect on the deposition of lycopene into the egg yolk and it can be used as a good delivery system to improve human health, and tomato pomace up to 12% could be used as an alternative feedstuff in quail breeders diets.

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DISCLOSURES

There is no conflict of interest.

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