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Original Article

Effect of Pistachio Shell (Pistacia vera L.) on Quail Performance, Carcass Traits, Blood Parameters, and Gut Health

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ABSTRACT

The increasing demand for sustainable and cost-effective poultry feeds has accelerated research into alternative sources. This study evaluated the effects of pistachio shell (PS) on the performance, carcass characteristics, oxidative stress markers, lipid profile and gut microbiota of Japanese quails (*Coturnix coturnix japonica*). A total of 240 quails were assigned to diets with 0%, 2%, 4%, or 6% PS for 42 days. Performance metrics (live weight (LW), feed intake (FI), and feed conversion ratio (FCR)) were monitored weekly. Carcass traits, oxidative stress markers (total antioxidant status [TAS], total oxidant status [TOS], oxidative stress index [OSI]), blood lipids, and intestinal microbiota were analyzed. While LW remained unaffected, FCR improved by 5.48% in the 6% PS group due to reduced FI. Abdominal fat weight decreased by 29.17% without affecting carcass weight. Antioxidant capacity improved, with TAS increasing by 36%, and TOS and OSI decreasing by 22.93% and 42.64%, respectively. HDL cholesterol rose significantly in the 6% PS group. PS supplementation also increased caecum pH and beneficial bacteria, while *E. coli* counts declined (p<0.01). The 4% PS group showed the lowest Enterobacter and coliform levels. These findings suggest PS can be a functional feed additive, enhancing feed efficiency, antioxidant status, and gut health in quails.

INTRODUCTION

Increasing global population intensifies the demand for animal protein, and poultry farming reigns supreme as the fastest avenue to meet this need (Daghir *et al.*, 2021). This rapid expansion is fueled by a potent blend of advancements across various key areas. Breeding and genetics play a crucial role in shaping the future of poultry. Poultry farming involves the reproduction of poultry through incubation, along with advancements in breeding, mechanization, automation, and improvements in feed and nutrition conditions. Additionally, improvements in vaccination, health, and protection, coupled with an excellent market network, contribute to the rapid development of the poultry farming sector. Sophisticated breeding techniques help farmers select birds with superior health, productivity, and feed efficiency. This continuous improvement ensures a sustainable and cost-effective protein supply (Wu *et al.*, 2022).

Feed costs are the Achilles' heel of poultry farming, swallowing a staggering portion of total expenses (Mallick *et al.*, 2020). Recognizing this financial pressure, the industry has embarked on a relentless quest for alternative feed sources. Industrial by-products, once considered waste, are emerging as promising alternatives (Brunetti *et al.*, 2022). Pistachio shells, for instance, have shown potential to replace costly conventional ingredients without compromising bird health or performance (Gürbüz *et al.*, 2022). This shift not only offers economic benefits but also promotes sustainability by reducing reliance on resource-intensive crops.

Beyond alternative ingredients, innovative solutions are also being explored within the existing feed framework. Organic acids, naturally occurring compounds like acetic and lactic acid, are making waves as multifunctional feed additives (Brunetti *et al*., 2022). Not only do they act as potent mold inhibitors, effectively extending feed shelf life and minimizing waste, but they also possess antimicrobial properties. By suppressing harmful bacteria like *E. coli* and *Salmonella*, these acids contribute to improved intestinal health and, potentially, enhanced bird performance. Organic acids (*acetic*, *propionic*, *lactic*, *formic*, *sorbic*, *fumaric*, *citric acid*) are also used as poultry feed additives. Some of these acids act as mold inhibitors and positively affect performance by suppressing pathogenic microorganisms such as *E. coli*, *Salmonella*, and *Enterobacteriaceae* (Deliaphan *et al*., 2023).

Pistachio (*Pistachia vera*), especially grown in the Southeast Anatolia Region of Turkey, is consumed as a snack and widely used in the production of baklava and desserts. Pistachios roasted in processing facilities generate soft outer shells as an industrial by-product, often disposed of in nearby areas, leading to environmental pollution. However, pistachio shells have significant nutritional and chemical properties (Ak *et al*., 2016). The soft outer shell of Red Pistachio contains 8.86% protein, 8.66% fat, 12.10% ash, 4.11% carbohydrates, and 14.20% crude cellulose, with an energy value of 129.82 kcal/kg. Similarly, the soft outer shell of Wild Pistachio contains 11.40% protein, 8.86% fat, 8.28% ash, 2.03% carbohydrates, and 21.14% cellulose, with an energy value of 133.46 kcal/kg (Barut, 2019). Additionally, pistachio shell extracts have been found to be rich in phenolic compounds, including anthocyanins, isoflavones, gallic acid, catechin, and epicatechin, which have antioxidant effects. These extracts have also been shown to have cytotoxic effects on cancer cells, indicating a high potential for cancer prevention (Koyuncu, 2018). Pistachio shell also contains a variety of other components, including gallic acid, anacardic acid, oleic acid, linoleic acid, palmitic acid, stearic acid, elaidic acid, myristic acid, margaric acid, flavonoids, sterols, carotenoids, tocopherols, and phenolic compounds such as quercetin, eriodiktiol, luteolin, naringenin, and apigenin (Hürkul, 2021).

The utilization of alternative feed ingredients in poultry diets has garnered increasing interest to enhance performance and health while addressing environmental concerns. One such alternative is pistachio shell (PS), a by-product of the pistachio industry. Pistachio is produced in an area of 410 thousand hectare, with a production amounting to 239 thousand tons in 2022 (Pistachio, 2023). The soft outer shell of pistachios constitutes approximately 18% of the fruit (Demiral *et al*., 2008). Based on this data, around 43 thousand tons of soft outer shells of fresh pistachios were produced in two years. The utilization and economic contribution of this industrial by-product could be very significant. Therefore, this study investigates the effects of incorporating PS into Japanese quail diets and its impact on key performance metrics, carcass characteristics, survival rates, oxidative stress, blood parameters, and intestinal flora.

MATERIALS AND METHODS

Studies were carried out in the chicken farm of Harran University Faculty of Veterinary Medicine in Şanlıurfa (37° 9′ 32.9364 lat., 38° 47′ 48.8724 long.) during October and November, 2021. At the beginning of the study, quail chicks were weighed to obtain live weight averages and evenly distributed among multi-tiered cage systems. Each cage had dimensions of 50*50*50 cm (width, length, height). In the experiment, one control group and three treatment groups were used, with three replicates for each group, each replicate consisting of 20 chicks, totaling 240 one-day-old chicks. At the beginning of the study, the sex of the chicks could not be determined; therefore, they were assigned to the experiment as mixed-sex groups. At the conclusion of the trial, the sex of each bird was identified. However, since the group-by-sex interactions were not statistically significant, all subsequent evaluations were conducted on the combined data from both sexes. This approach ensured a robust analysis while minimizing potential bias associated with sex-based differences. While the control group received a regular quail ration, the treatment groups received this ration added with dried, finely ground soft pistachio shells ground to a size of 1-2 mm at different rates (0%, 2%, 4%, 6%) from day 1 to the end of day 42. Live weights and the amount of consumed feed were determined through weekly measurements. Live weights were taken individually, while feed consumption was recorded as group averages. Feed and water were provided *ad libitum*.

Soft pistachio shells, obtained from a private pistachio processing plant in Şanlıurfa province, Turkey, were used in the study. Table 1 presents the nutritional composition of the soft pistachio shells.

Table 1 – Nutritional composition of the soft pistachio shells.

Nutrient Component	Level
Dry matter (%)	95.09
Crude protein (%)	6.37
Crude fat (%)	7.35
Crude cellulose (%)	14.41
Crude Ash (%)	7.33
Metabolizable energy (kcal/kg)	2900
Nitrogen-Free Extract (%)	59.63

The percentage of dry matter in soft pistachio shells was determined gravimetrically by drying the samples at 105°C for 16 hours. Subsequently, the ash content was quantified by ignition in a muffle furnace at 525°C for 8 hours (Boğa *et al*., 2013). Pistachio shell crude protein content was indirectly determined by measuring nitrogen, using the Kjeldahl method. Following established protocols (AOAC, 1990), the obtained nitrogen value was multiplied by 6.25 to estimate the crude protein content. Nitrogen-Free Extract was calculated using Equation 1, shown below. Crude fat was estimated gravimetrically by the filter bag technique after petroleum ether extraction of the dried sample in an Ankom XT10 extraction system (Ankom, 2009). Crude fiber was determined according to the Weende technique. This method determines the organic residue remaining after digestion with solutions of sulfuric acid and sodium hydroxide, using an Ankom 220 fibre analyser (Ankom, 2008). Metabolizable energy value was determined according to ETEVPF (1989), and calculated using Equation 2.

$$NFE=DM-(CP+CF+CC+CA) \quad (1)$$

$$ME(kcal/kg)=[(\%CPx28.71)+(\%CCx11.96)+(\%CFx74.16)+(\%NFEx33.49)] \quad (2)$$

Where NFE is the nitrogen free extract, DM is dry matter, CP is the crude protein, CF is crude fat, CC is the crude cellulose, CA is the crude ash, and ME is the metabolizable energy.

Performance values

For performance measurements, the live weights of the animals were determined individually by weekly weighing. Live weight gains were calculated by subtracting the live weight of the previous week. Feed consumption was determined as the group average through weekly weighing. The animals had continuous access to feed, and increased feed residues were subtracted to calculate weekly feed consumption. The gender distribution in the groups was balanced, and since no gender interaction effect was observed, the data were presented without distinguishing between males and females. At the end of the experiment, 18 quails (9 males - 9 females) were selected from each group, euthanized using the Cervical Decapitation Method (Berkin & Alçığır, 1999), and subjected to necropsy. Carcass, liver, heart, gizzard, and abdominal fat weights were determined *postmortem*, with weighing performed using an electronic scale with a precision of 0.01g.

The experimental diets were isocaloric (3100 kcal/kg) and isonitrogenic (24% crude protein) across all four treatment groups (control, 2% PS, 4% PS, 6% PS). This means they provided the same amount of energy and protein per kilogram of feed. The main difference between the diets was the inclusion of pistachio shells (PS) at increasing levels. The remaining ingredients, including soybean meal, corn gluten meal, vegetable oil, marble dust, minerals, vitamins, amino acids, and sodium bicarbonate were kept constant across all diets. This ensures that any observed differences in performance or health outcomes can be attributed primarily to the varying levels of pistachio shells (Table 2). The vitamin and mineral mixtures were consistent across all groups, providing essential micronutrients to meet the nutritional requirements of the subjects. The vitamin and mineral mixtures were consistent across all groups, providing essential micronutrients to meet the nutritional requirements of the subjects.

Table 2 – Composition of experimental diets (%).

Ingredients	Treatment Groups			
	Control	2% PS	4% PS	6% PS
Corn	420	420	420	420
Wheat	188	162	137	110
Soybean Meal	223	228	232	237
Corn Gluten Flour	130	130	130	130
Pistachio Shell	0	20	40	60
Vegetable Oil	5	6	7	9
Marble Dust	14	14	14	14
Monocalcium Phosphate	11	11	11	11
Vitamins and Minerals	2.5	2.5	2.5	2.5
Lysine	0.5	0.5	0.5	0.5
Methionine	1.5	1.5	1.5	1.5
NaHCO3	1	1	1	1
Salt	3.5	3.5	3.5	3.5
Total	**1000**	**1000**	**1000**	**1000**
Calculated Nutrient Values				
Crude Protein (%)	24	24	24	24
Metabolizable Energy (kcal/kg)	3100	3100	3100	3100

Vitamin Mixture: A 15.000.000 IU, B1 5.000 mg, B2 8.000 mg, B6 5.000 mg, B12 20 mg, C 100.000 mg, D 6.000.000 IU, K3 3.000 mg, B5 15.000 mg, D-Biotin 200 mg, E 100.000 mg, Folic Acid 2.000 mg, Nicotinic acid 60.000 mg. Mineral Mixture: Cu 16.000 mg, Mn 120.000 mg, Fe 80.000 mg, I 200 mg, Se 300 mg, Zn 80.000 mg; PS: pistachio shell

Biochemical analysis

At the end of the experiment, 18 quails from each group were slaughtered for the analysis of biochemical parameters. 5 ml of blood were taken from each animal, and the plasma was separated by centrifuging the blood at 3500 rpm for 5 minutes. Total oxidative status (TOS), total antioxidant status (TAS), and oxidative stress index (OSI) were analyzed in the plasma of blood samples in the Harran University, Faculty of Medicine Biochemistry Laboratory, according to the Erel method (Erel 2004; 2005). Triglyceride, total cholesterol, and HDL cholesterol levels were measured by the calorimetric method (Rifai *et al*., 1999).

The total antioxidant status (TAS) level was measured in blood samples using Rel Assay Diagnostics kits. The method is based on the principle of quenching the color of the colored ABTS (2,2'-azinobis (3-ethylbenzothiazoline-6-sulfonic acid) cationic radical formed by oxidation of the antioxidant molecule in the samples. Trolox was chosen as the calibrator. The result was expressed as µmol Trolox Equivalent/L (Erel, 2004). Total Oxidative Status (TOS) was determined in blood samples using the Rel Assay Diagnostics kit. The values were measured by oxidizing the ferrous to ferric ion in the oxidant molecules present in the blood samples, and the results were calculated using the colorimetric method. These values were expressed in µmol H2O2 Equivalent/L (Erel, 2005). Oxidative stress index (OSI) is expressed as the percentage of the levels of TOS to the levels of TAS (Eq. 3). When calculating the OSI of the samples, the units of the TAS level were equalized to the units of the TOS level (Ayçiçek *et al*. 2005; 2011). The results are expressed in arbitrary units (AU).

$$OSI = \frac{\text{TOS (µmol H2O2 Eqv/L)}}{\text{TAS (Trolox Eqv/L)}} \times 100 \quad (3)$$

Total cholesterol, HDL cholesterol, and triglyceride levels were measured using Roche Cobas INTEGRA 800 automated analyzers and Roche commercial kits. These parameters were expressed in mg/dL.

Microbiological analysis

Following quail slaughter, conducted aseptically under sterile conditions, 10 grams of each intestinal sample were collected using sterile spatulas and placed in sterile sample bags (Bag Filter, France). Subsequently, each bag containing 10 grams of sample received 90 ml of 0.1% sterile peptone water (LABM) and was homogenized for 1 minute in a stomacher device (Easy Mix, France). To determine the microbial load in each sample, serial dilutions ranging from 10:1 to 10:9 were prepared in tubes containing 9 ml of 0.1% sterile peptone water.

Enumeration of the coliform group bacteria employed the pour plate method. Briefly, 1 ml aliquots from appropriate dilutions were inoculated onto sterile petri dishes, followed by overlaying with 15 ml of Violet Red Bile (VRB, Merck) agar pre-cooled to 45-50°C and homogenized. After solidification, an additional 5 ml of sterile VRB agar was overlaid. Following complete solidification, the plates were incubated at 37°C for 1 day. At the end of incubation, colonies with a diameter of 0.5-2 mm, typically dark red and often surrounded by reddish halos, were enumerated as typical coliforms (Tsola *et al*., 2008)

The ISO 21527-1:2008 procedure was used for yeast and mold count. A 0.1 mL sample was taken from each of the previously prepared dilutions and spread onto a Dichloran Rose Bengal Chloramphenicol (DRBC) agar medium. The petri dishes were then incubated at 25°C for 5 days under aerobic conditions, followed by colony counting (Brooks *et al*., 2012).

For the lactic acid bacteria count, 1 mL was taken from each of a series of previously prepared dilutions and inoculated into sterile petri dishes. Then, De Man Rogosa and Sharpe Agar (MRSA) (Merck) was added to these petri dishes and microbiological seeding was performed using the pour plate method. The petri dishes were then subjected to incubation at 37°C for 24-48 hours under anaerobic conditions. All colonies developed after incubation were counted (Aydın *et al*., 2008).

For the total mesophilic aerobic bacteria count (Total bacteria), dilutions were prepared and poured into sterile petri dishes containing Plate Count Agar (PCA) (Merck 1.05463, Germany). The petri dishes were incubated at 30±1°C for 3 days, and petri dishes with colonies were evaluated. The colonies grown in PCA were counted and multiplied by the dilution factor to calculate the Total Aerobic Mesophilic Bacteria (TAMB) count (Alçay, 2019).

For the *Enterobacteriaceae* count, dilutions were prepared and seeded onto Violet Red Bile Glucose Agar (Merck 1.10275, Germany). The petri dishes were incubated at 30±1°C for 48 hours. At the end of incubation, the 1-2 mm diameter red colonies with a halo around them were subjected to the oxidase test (Merck 1.13300, Germany). The typical colonies with a negative result were counted (Harrigan, 1976).

Tryptone Bile X-Glucuronide Agar (LAB-M HAL003) medium was used for the *E. coli* count in accordance with the ISO 16649-1:2018 procedure (ISO 2018). The plates were incubated at 44°C for 24 hours, and opaque bluish-green colonies were counted and evaluated.

Statistical assessments

The effect of PS inclusion into the diets of Japanase quail on key performance metrics, slaughter characteristics, survival rates, oxidative stress, blood parameters, and intestinal flora was assessed using a one-way analysis of variance (ANOVA). The gender distribution across the experimental groups was equally distributed, and no significant interaction effect between gender and treatment was observed. Consequently, the data were analyzed and presented without distinguishing between males and females. Given that the experimental groups were structured with linearly increasing doses, orthogonal contrasts—including linear, quadratic, and cubic effects—were assessed when the F-test indicated statistical significance. If ANOVA indicated a significant effect, the Duncan test was used to determine the significance of the difference between the treatment groups. The SPSS software was used for all statistical evaluations (SPSS, 1999).

RESULTS

The study evaluated the effects of pistachio shell (PS) inclusion in quail diets on key performance metrics over different weeks. The results, presented in Table 3, highlight significant variations in live weight (LW), feed intake (FI), and feed conversion ratio (FCR) across various treatments. During the first 3-week period, a significant difference (p<0.01) was found between the control and treatment groups in terms of LW, FI, and FCR. With the increase in pistachio shell ratio in the diet, LW and FCR increased, while FI decreased gradually (Table 3). The highest LW and FCR were obtained in the 4% and 6% PS groups, with the lowest FI in the 6% PS group. In the initial 0-3 weeks, quails receiving 6% PS exhibited a remarkable increase of approximately 8.27% in LW compared to the control group. In the 4–6-weeks period, a significant difference (p<0.01) was observed between the control and treatment groups in terms of LW, FI, and FCR. Between weeks 4 and 6, the group fed a diet containing 6% PS exhibited a significant decrease in LW of approximately 3.46% compared to the control group. Feed consumption in the 6% PS group increased by 2% compared to the control group. Correspondingly, a significant increase of approximately 10.8% was observed in the FCR of the 6% PS group. Cumulatively over weeks 0-6, while no significant difference was found in LW among treatments, the quails fed with 6% PS exhibited consistent improvement in FCR, with a reduction of approximately 5.48% compared to the control group. A gradual decrease in FI accompanied the increasing inclusion of PS in the diet, with a corresponding decrease in weight gain, resulting in a higher FCR. The lowest FI and the highest FCR were obtained in PS groups. These findings suggest that including 6% PS in quail diets positively influences performance, leading to increased LW and improved FCR, particularly during specific development phases.

Since the performance values in Table 3 were determined to be significant, their linear, quadratic, and cubic effects were analyzed. The results indicated statistically significant linear, quadratic, and cubic effects during both the 0–3 week and 4–6 week periods. However, in the overall 0–6 week period, LW was not significantly influenced by PS levels in any of these terms, whereas FI and FCR exhibited significant effects (p<0.01).

Table 3 – Effects of pistachio shell levels on quail live weight (LW), feed intake (FI), and feed conversion ratio (FCR).

Weeks	Trait	Control	PS 2%	PS 4%	PS 6%	L	Q	C
0-3	LW	107.90±2.32^{b}	107.07±2.45^{b}	118.05±2.40^{a}	116.82±2.38^{a}	<0.01	<0.01	<0.01
	FI	274.00±0.66^{a}	254.00±0.87^{b}	226.69±2.04^{c}	215.67±1.38^{d}	<0.01	<0.01	<0.01
	FCR	2.57±0.08^{a}	2.43±0.08^{a}	2.07±0.03^{b}	2.02±0.05^{b}	<0.01	<0.01	<0.01
4-6	LW	160.84±1.80^{a}	157.38±1.16^{ab}	150.35±1.52^{c}	155.27±1.34^{b}	<0.01	<0.01	<0.01
	FI	552.57±1.44^{b}	525.59±2.40^{c}	563.74±2.48^{a}	563.66±2.11^{a}	<0.01	<0.01	<0.01
	FCR	3.43±0.07^{b}	3.22±0.06^{c}	3.74±0.07^{a}	3.80±0.05^{a}	<0.01	<0.01	<0.01
0-6	LW	269.98±3.74	265.95±2.90	269.60±3.31	272.80±2.69	NS	NS	NS
	FI	826.53±2.11^{a}	779.71±3.51^{c}	790.07±3.56^{b}	779.34±3.51^{c}	<0.01	<0.01	<0.01
	FCR	3.10±0.05^{a}	3.02±0.03^{ab}	3.00±0.04^{ab}	2.93±0.03^{b}	<0.01	<0.01	0.05

The difference between letters bearing the same symbols in the same row is not significant (p<0.05). The gender distribution across the experimental groups was equally distributed, and no significant interaction effect between gender and treatment was observed. PS: pistachio shell, NS - Non-significant; L – Linear; Q – Quadratic; C - Cubic.

The results of variance analysis indicating the effects of different ratios of added PS in quail diets on carcass, liver, heart, gizzard, and abdominal fat weights are presented in Table 4. Carcass weight was significantly influenced by PS supplementation, as indicated by a quadratic effect (p<0.01) and a cubic effect (p=0.05), while the linear effect was not significant. Carcass weight was highest in the control (203.50 g) and 6% PS (204.06 g) groups, whereas the 2% and 4% PS groups exhibited significantly lower values (192.94 g and 193.89 g, respectively). The significant quadratic trend suggests that carcass weight initially declined with moderate PS supplementation, but recovered at higher levels, implying a threshold effect where lower doses may negatively impact growth, while higher doses mitigate this effect. The presence of a cubic effect indicates additional complexity in the dose-response relationship, likely due to interactive metabolic factors influencing carcass deposition. Abdominal fat content exhibited a strong response to PS supplementation, with significant linear (p<0.01), quadratic (p<0.01), and cubic (p<0.01) effects. Compared to the control (3.12 g), abdominal fat was significantly reduced at all PS supplementation levels, with the lowest value observed at 4% PS (1.87 g). The significant linear effect indicates a general reduction in abdominal fat as PS levels increased, whereas the quadratic effect suggests that fat deposition reached its lowest point at moderate supplementation before slightly increasing at the highest PS level. The presence of a cubic effect further implies a complex, non-linear response, possibly due to metabolic adaptations or changes in lipid metabolism associated with PS inclusion. Liver, heart and gizzard weights were not significantly affected by PS levels, with no statistical differences among the groups.

Table 4 – Effects of Pistachio Shell Supplementation on the Carcass Characteristics and Internal Organ Weights of Quails.

Trait	Control	PS 2%	PS 4%	PS 6%	L	Q	C
Carcass (g)	203.50±2.91[a]	192.94±2.77[b]	193.89±4.04[b]	204.06±3.03[a]	NS	<0.01	0.05
Liver (g)	7.84±0.41	8.02±0.47	7.42±0.58	7.65±0.43	NS	NS	NS
Heart (g)	2.53±0.11	2.56±0.09	2.55±0.07	2.61±0.07	NS	NS	NS
Gizzard (g)	4.74±0.17	4.97±0.16	4.86±0.18	4.99±0.26	NS	NS	NS
Abd. fat (g)	3.12±0.33[a]	2.17±0.14[b]	1.87±0.24[b]	2.21±0.23[b]	<0.01	<0.01	<0.01

The difference between letters bearing the same symbols in the same row is not significant. The gender distribution across the experimental groups was equally distributed, and no significant interaction effect between gender and treatment was observed. PS: pistachio shell; NS - Non-significant; L – Linear; Q – Quadratic; C - Cubic.

Liver weight remained similar across all groups, indicating no impact of PS addition on liver health and function. Heart weight followed the same pattern as liver weight, showing no significant changes with increasing PS inclusion. Like liver and heart, gizzard weight remained consistent across all groups, implying no influence of PS on muscular development or digestive capabilities. These findings collectively indicate that while there are subtle changes in carcass weight, the most notable impact is on abdominal fat, pointing towards a potential avenue for further exploration in optimizing quail diets for improved health and productivity.

Records of deceased animals were kept during the trial, and survival rates were calculated at its conclusion. The variance analysis results indicating the effects of feeds with different PS ratios on the survival rate (%) parameter of quails are presented in Table 5. The study involved 60 animals in each treatment group. Remarkably, the mortality rate was reduced to zero in the groups where 4% and 6% PS was included in the feed, indicating a 100% survival rate. In contrast, the control group and the treatment group with 2% PS maintained a high survival rate of 98.33%, with only one dead animal in each group, resulting in a mortality rate of 1.67%. This suggests that the inclusion of PS in the diet, especially at higher proportions, positively influences the survival rate of quails, demonstrating its potential as a beneficial dietary component.

Table 5 – The survival rate (%) parameters of quails.

Traits	Control	PS 2%	PS 4%	PS 6%
Number of animals	60	60	60	60
Number of died animals	1	1	0	0
Mortality rate %	1.67	1.67	0	0
Survival rate (%)	98.33	98.33	100	100

PS: pistachio shell

The variance analysis results regarding the effects of feeds with different pistachio shell ratios on oxidative stress and blood parameters in quail blood plasma are provided in Table 6. The total antioxidant status (TAS) exhibited a significant increase (p<0.01) with the inclusion of PS, showing a progressive increase from 1.30 μmol trolox Eqv/L in the control group to 1.77 μmol trolox Eqv/L in the PS 6% group. Conversely, total oxidant status (TOS) demonstrated a significant decrease (p<0.01), declining from 16.75 μmol in the control group to 12.91 μmol in the PS 6% group. The oxidative stress index (OSI) exhibited a notable decline (*p*<0.01) with increasing PS ratios, reaching its lowest value of 0.74 in the PS 6% group. Regarding blood parameters, triglyceride levels showed a decreasing trend with the inclusion of PS, dropping from 258.62 mg/dL in the control group to 213.69 mg/dL in the 6% PS group. High-density lipoprotein (HDL) cholesterol had an increasing pattern, with the highest value of 162.17 mg/dL in the 6% PS group.

The oxidative stress parameters and lipid profiles showed varying responses to different PS supplementation levels, showing dose-dependent trends. TAS increased consistently with PS levels, reaching its highest value at 6%, suggesting a potent antioxidant effect of PS. Similarly, TOS and OSI decreased significantly with increasing PS levels, particularly at 6%, indicating a reduction in oxidative stress. This trend underscores the role of PS in enhancing the oxidative balance, likely due to its bioactive compounds. While triglyceride levels were lowest at 4% PS, a slight increase at 6% suggests a possible metabolic threshold where higher doses may lose efficacy. Cholesterol levels did not show significant trends, but fluctuations hint at individual variability in response. HDL cholesterol, however, displayed a clear linear increase, peaking at 6% PS, which may point to a beneficial effect on lipid metabolism. These findings demonstrate that while PS supplementation offers substantial benefits in oxidative stress management, the optimal dose may vary depending on the parameter, with 4% and 6% PS generally yielding the most favorable outcomes.

Table 6 – Effects of Pistachio Shell Levels on Oxidative Stress and Blood Parameters.

Trait	Control	PS 2%	PS 4%	PS 6%	L	Q	C
Oxidative stress parameters							
TAS (µmol Trolox Eqv/L)	1.30±0.02c	1.47±0.04b	1.59±0.05b	1.77±0.06a	<0.01	<0.01	<0.01
TOS (µmol)	16.75±0.21a	14.51±0.43b	13.74±0.35bc	12.91±0.29c	<0.01	<0.01	<0.01
OSI	1.29±0.02a	1.00±0.04b	0.88±0.04c	0.74±0.02d	<0.01	<0.01	<0.01
Blood Parameters							
Triglyceride (mg/dL)	258.62±25.59	202.46±22.09	191.08±19.13	213.69±12.33	NS	NS	NS
Cholesterol (mg/dL)	222.33±13.10	245.33±18.03	216.94±13.40	241.56±19.73	NS	NS	NS
HDL Cholesterol (mg/dL)	125.67±6.32	144.39±10.92	144.06±9.27	162.17±11.98	0.05	NS	NS

The difference between letters bearing the same symbols in the same row is not significant (*p*<0.05). The gender distribution across the experimental groups was equally distributed, and no significant interaction effect between gender and treatment was observed. TAS: Total antioxidant status, TOS: Total oxidant status, OSI: Oxidative stress index, HDL: High-Density Lipoprotein, PS: pistachio shell, NS - Non-significant; L – Linear; Q – Quadratic; C - Cubic.

The results of the variance analysis on the effects of feeds with different PS ratios on quail intestinal flora parameters are presented in Table 7. The pH levels in the small intestine and caecum were significantly affected by the PS ratios (*p*<0.01). In the small intestine, the pH reached its highest values at the 2% PS and 4% PS levels, following a quadratic trend. This quadratic relationship indicates that the response of the small intestine pH to increasing PS levels is non-linear, with an initial rise to a peak at intermediate doses, followed by a subsequent decline at higher concentrations. In contrast, the caecum pH peaked at 2% PS and then dropped sharply, indicating a cubic effect. The concentrations of acetic acid and propionic acid in the caecum showed varying trends with different PS ratios, with significant differences observed (*p*<0.01). However, the inclusion of 6% PS led to a substantial decrease in acetic acid and propionic acid compared to control and other PS groups. In the 6% treatment compared to the control, there was observed a decrease of 49.21% in acetic acid content and a decrease of 79.09% in propionic acid content. The analysis of acetic acid and propionic acid concentrations in Table 7 reveals a similar nonlinear dose-response pattern. Both acids increased from the control, reaching a maximum at 2% PS, followed by a decline at higher PS levels. Specifically, acetic acid concentrations rose from 2.52 g/kg in the control to 2.89 g/kg at 2% PS, then decreased slightly to 2.60 g/kg at 4% PS, and finally dropped sharply to 1.28 g/kg at 6% PS. Similarly, propionic acid levels increased from 5.50 g/kg in the control to a peak of 7.59 g/kg at 2% PS, then decreased modestly to 7.29 g/kg at 4% PS, and ultimately declined markedly to 1.15 g/kg at 6% PS. Both traits exhibited significant linear, quadratic, and cubic effects-with the quadratic (*p*<0.01 for both) and cubic (*p*<0.01 for both) components being highly significant, and the linear effect for propionic acid being marginally significant (*p*=0.05). This suggests that the initial increase in concentrations with increasing PS levels follows a positive linear trend; however, the subsequent decline is best explained by a quadratic relationship. The additional significant cubic effects indicate further complexity in the dose-response patterns, possibly reflecting inflection points or threshold effects at higher doses. The results indicated significant variations in lactic acid concentration among the treatments ($p<0.01$). The PS 4% treatment demonstrated a significant increase (6.31%) compared to the control, suggesting a potential stimulatory effect of this plant substance concentration on lactic acid production. Conversely, the PS 6% treatment exhibited a substantial decrease (41.49%) in lactic acid concentration, indicating a possible inhibitory effect at higher concentrations. In the control group, lactic acid levels were 16.90 g/kg. Following PS supplementation, values slightly decreased to 16.62 g/kg at 2% PS, then peaked at 17.97 g/kg at 4% PS, before dropping sharply to 9.88 g/kg at 6% PS. Statistical analysis indicated significant linear ($p<0.01$) and quadratic ($p<0.01$) effects, with the quadratic component best capturing the initial increase and subsequent decline in lactic acid concentration, while the cubic effect was not significant. These findings suggest that moderate PS supplementation (around 4%) optimizes lactic acid production, whereas higher levels (6%) may exert a detrimental effect, underscoring the importance of dosage optimization. These findings highlight the importance of optimizing plant substance concentrations in the diet to modulate lactic acid production effectively.

Table 7 – Effects of Different Levels of Pistachio Shell (PS) on Intestinal pH, Fermentation Products, and Microbial Populations.

Trait	Control	PS 2%	PS 4%	PS 6%	L	Q	C
Small Intestine pH	6.30±0.012^{c}	6.46±0.023^{a}	6.46±0.012^{a}	6.38±0.017^{b}	NS	<0.01	NS
Caecum pH	6.00±0.000^{d}	7.20±0.000^{a}	6.53±0.000^{b}	6.11±0.012^{c}	NS	NS	<0.01
Lactic acid (g kg^{-1})	16.90±0.006^{b}	16.62±0.012^{c}	17.97±0.012^{a}	9.88±0.058^{d}	<0.01	<0.01	NS
Acedic acid (g kg^{-1})	2.52±0.006^{c}	2.89±0.017^{a}	2.60±0.023^{b}	1.28±0.017^{d}	<0.01	<0.01	<0.01
Propionic acid (g kg^{-1})	5.50±0.116^{c}	7.59±0.018^{a}	7.29±0.023^{b}	1.15±0.029^{d}	0.05	<0.01	<0.01
Butiric acid (g kg^{-1})	-----	-----	-----	-----	-----	-----	-----
Lactic acid bact. (g kg^{-1})	8.03±0.026^{b}	8.34±0.012^{a}	7.39±0.036^{c}	8.42±0.010^{a}	NS	NS	<0.01
Total Bacteria (CFU g^{-1})	9.38±0.010^{d}	9.83±0.004^{b}	9.55±0.015^{c}	10.18±0.017^{a}	<0.01	0.05	<0.01
Enterobacter (CFU g^{-1})	8.94±0.003^{b}	8.48±0.005^{c}	6.71±0.003^{d}	8.99±0.002^{a}	NS	0.05	<0.01
Coliform Bacteria (CFU g^{-1})	8.31±0.007^{c}	8.46±0.005^{b}	6.31±0.007^{d}	8.56±0.007^{a}	NS	NS	<0.01
E. Coli (CFU g^{-1})	7.98±0.003^{a}	7.70±0.003^{b}	5.44±0.005^{c}	5.01±0.014^{d}	<0.01	<0.01	<0.01
Yeast (CFU g^{-1})	3.53±0.004^{b}	4.34±0.012^{a}	2.32±0.007^{d}	2.46±0.005^{c}	<0.01	0.05	<0.01
Mold (CFU g^{-1})	3.00±0.000^{a}	2.78±0.002^{c}	2.91±0.002^{b}	2.49±0.005^{d}	<0.01	<0.01	<0.01

The difference between letters bearing the same symbols in the same row is not significant ($p<0.05$). CFU g-1 is the number of colony-forming units per gram. The gender distribution across the experimental groups was equally distributed, and no significant interaction effect between gender and treatment was observed; PS: pistachio shell, NS - Non-significant; L – Linear; Q – Quadratic; C - Cubic.

Regarding microbial populations, the number of lactic acid bacteria (LAB), total bacteria, Enterobacter, and coliform bacteria were significantly influenced by the PS addition ratios ($p<0.01$). The introduction of 2% and 6% of PS led to the highest counts for LAB, while 6% of PS led to the highest total bacterial count (Table 7). Analysis of lactic acid bacteria (LAB) concentrations indicates a complex, non-linear response to increasing PS supplementation levels. In the control group, LAB counts were approximately 8.03 g/kg; with 2% PS supplementation, the counts increased slightly to around 8.34 g/kg, then decreased to about 7.39 g/kg at 4% PS, and finally increased again to roughly 8.42 g/kg at 6% PS. While the linear and quadratic effects were not statistically significant, the cubic component was highly significant ($p<0.01$), suggesting that the response of LAB levels to PS follows an intricate dose-response relationship with multiple inflection points. The lowest number of Enterobacter (6.71) was observed in the 4% PS group, while the highest number of Enterobacter (8.99) was detected in the 6% PS group, and coliform bacteria showed a similar trend. In the control group, Enterobacter counts were approximately 8.94 CFU/g, decreasing to around 8.48 CFU/g at 2% PS and further dropping to about 6.71 CFU/g at 4% PS. However, at 6% PS, Enterobacter levels rebounded to approximately 8.99 CFU/g, surpassing the control value. Although the linear trend was not statistically significant, the significant quadratic ($p=0.05$) and highly significant cubic effects ($p<0.01$) indicate that the response follows a multi-phasic pattern with multiple inflection points. The initial count of *E. coli* in the control group was recorded as 7.98 CFU mL-1. Upon the addition of PS at concentrations of 2%, 4%, and 6%, the count decreased to 7.70, 5.44, and 5.01 CFU mL-1, respectively. The response of *E. coli* to increasing PS levels followed a distinct non-linear pattern, as evidenced by significant linear ($p<0.01$), quadratic ($p<0.01$), and cubic ($p<0.01$) effects. Initially, *E. coli* counts declined with moderate PS supplementation, suggesting a suppressive effect. However, as the PS level increased further, the reduction became more pronounced, indicating a potential threshold beyond which microbial balance was significantly disrupted. The presence of a strong cubic effect suggests that the relationship between PS supplementation and *E. coli* counts is not a simple linear decline but rather a complex dose-response interaction with multiple inflection points. The counts of yeast and mold also exhibited significant variations with PS inclusion, demonstrating the intricate impact on the intestinal microbial environment. The highest mean yeast value was observed in the 2% PS group (4.34 CFU g-1) compared to the control (3.53 CFU g-1), representing a 23% increase. However, in the subsequent PS groups (4% and 6%), yeast levels decreased to 2.32 CFU g-1 and 2.46 CFU g-1, respectively, representing decreases of 33.3% and 30.3% compared to the control. The yeast population exhibited a non-linear response to increasing PS levels, as indicated by significant linear (p<0.01), quadratic (p=0.05), and cubic (p<0.01) effects. While a moderate PS level appeared to promote yeast growth, higher doses led to a substantial decline, suggesting a threshold beyond which PS exerts an inhibitory effect. The significant cubic trend implies a more complex dose-response relationship, where yeast counts fluctuate rather than following a straightforward increasing or decreasing pattern. This suggests that while lower PS levels may support yeast proliferation, excessive supplementation could disrupt microbial equilibrium, emphasizing the importance of precise dosing strategies for maintaining a balanced intestinal microbiota. Conversely, mold levels showed a significant decrease (p<0.01) as the PS ratio increased in the diet. The lowest mold values were obtained in the 2%, 4%, and 6% PS groups (2.78, 2.91, and 2.49 CFU g-1, respectively). Compared to the control group (3.00 CFU g-1), this represented decreases of 7.3%, 3.0%, and 17.0%, respectively (Table 7). The mold population demonstrated a clear sensitivity to increasing PS levels, showing a statistically significant decline driven by linear (p<0.01), quadratic (p<0.01), and cubic (p<0.01) effects. The data suggest that even at low PS concentrations, mold counts begin to decrease, with the reduction becoming more pronounced as the PS level increases. The cubic effect further indicates that this decline does not follow a simple downward trend, but rather involves fluctuations, likely influenced by microbial competition or environmental adaptation within the intestinal ecosystem. These results suggest that PS supplementation has a strong inhibitory effect on mold populations, reinforcing its potential role in modulating fungal balance in the gut microbiota.

DISCUSSION

Live weight (LW), feed intake (FI) and feed conversion ratio (FCR)

The results of this study indicated that the addition of PS at 2%, 4%, and 6% levels to quail diets has no negative effects on live weight, but it can significantly reduce feed consumption and improve FCR. The lowest FI (779.34g) and the highest LW (272.80g), and consequently the best FCR (2.93) values, were recorded in the 6% PS group. This suggests that PS could be a potential feed additive for quail production, as it could improve performance and reduce feed costs. Our findings align with Abdelaziz *et al*. (2015), who achieved the lowest FI and the highest FCR when adding 250 mg/kg thyme oil to Hubbard broiler chick diets. Additionally, Ahmed *et al*. (2015) reported that the addition of 10g/kg dried basil (*Ocimum basilicum* L.), 10g/kg dried chamomile, and 5g dried basil + 5g dried chamomile to broiler chick diets did not affect LW in the basil and basil+chamomile groups. The researchers indicated a reduction in FI, an improvement in FCR, and consequently, improved performance. The observed reduction in feed consumption and improvement in FCR with PS addition may be due to several factors. One possibility is that PS may act as a prebiotic, stimulating the growth of beneficial bacteria in the gut (Singh & Kim, 2021). These beneficial bacteria can improve nutrient absorption and utilization, which could lead to reduced feed consumption and improved performance. This finding aligns with previous research by Abou-Kassem *et al*. (2021), who reported a similar decrease in FI but no significant impact on FCR when probiotics were added to quail diets. While the reduced FI in our study resulted in a decrease in live weight compared to the control group, it is important to consider the potential benefits of PS on gut health. Another possibility is that PS may increase the digestibility of nutrients in the diet. PS is a good source of fiber, which can help to improve the digestibility of other nutrients in the diet (Tejeda & Kim, 2021). This could also contribute to the observed reduction in feed consumption and improvement in feed conversion ratio.

Plant-based feed additives like PS have received increased interest due to their potential to improve poultry performance and promote gut health. This study adds to the growing body of research exploring the benefits of such additives. Similar positive effects on feed conversion ratio and feed intake have been observed with the addition of basil leaves (Çetin *et al*., 2018) and artichoke leaves (Effati *et al*., 2014) to broiler diets, suggesting a potential common mechanism of action related to improved nutrient utilization or prebiotic effects. Studies focusing on essential oils like thyme and garlic (Vogt & Rauch, 1991) or blends containing fennel and sage (Çabuk *et al*., 2006) also highlight the promise of natural additives for animal feed. Interestingly, while Radwan *et al*. (2007) reported no negative effects of artichoke leaves on layer hens, El-Deek *et al*. (2012) observed increased feed intake in pepper-fed broiler chickens. This suggests that different additives may have varying impacts on feed consumption, warranting further investigation within specific species and contexts.

The use of by-products like pistachio shells, pepper seeds, and pomegranate peel in poultry diets has generated mixed results in terms of performance parameters like carcass yield, feed intake, and feed conversion ratio. This variability can be attributed to several factors, including processing methods, dietary composition, and even species differences. Tannins and other anti-nutritional factors present in some by-products can influence palatability and digestibility, potentially leading to reduced feed intake and decreased performance (Priolo *et al*., 2000; Ordouny *et al*., 2017). However, studies like that of Yosefi *et al*. (2018) demonstrate that processing techniques can mitigate these negative effects. Our results align with Zanganeh *et al*. (2017), who reported no negative effects on performance with moderate inclusion of pistachio leaf extract. These findings highlight the importance of investigating optimal processing methods and dosage levels for specific by-products and poultry species to unlock their potential benefits in feed formulations.

Poultry species, including broilers and potentially quails, demonstrate remarkable adaptability to dietary changes and tolerance for some plant products containing potentially adverse factors. This adaptability has been observed from their early stages of life. For example, Çetin (1996) reported that White Leghorns adapted to common vetch in their diets without significant impacts on feed consumption or egg production, despite a slight decrease in live weight. The author suggests that birds may develop mechanisms to overcome the negative effects of certain compounds over time. Similarly, Çetin *et al*. (2019) found that broiler chicks tolerated diets containing varying levels of wormwood, with feed consumption initially increasing at low doses and then decreasing as the wormwood concentration rose. These findings raise intriguing questions about potential adaptation mechanisms and their species-specificity. Quails undergo a certain adaptation process, which can be understood through linear, quadratic, and cubic effects. In the context of our research on PS in quail diets, it's crucial to consider whether quails exhibit similar adaptation capabilities and how dosage might influence their response to potentially unfavorable components of PS.

The observed differences in survival rates among treatment groups highlight the potential benefits of pistachio shell supplementation. The absence of mortality in the groups with 4% and 6% pistachio shell signifies a substantial improvement in the overall survival of quails. These results underscore the importance of considering dietary components, such as pistachio shell, in optimizing the overall well-being and survival of quail populations in agricultural settings.

Slaughter characteristics

Inclusion of PS at 2%, 4%, and 6% in quail diets appears to have minimal effects on overall body weight, organ function, and digestive capacity. Abdominal fat deposition decreased with increasing PS levels, suggesting potential for dietary manipulation with PS to regulate fat content in quail production. Compared to the control group, abdominal fat weight was lower in all PS groups. This suggests that the addition of fiber-rich PS, which has lower digestibility compared to the control diet, may lead to a reduction in fat accumulation.

The current study demonstrated that dietary inclusion of PS at levels of 2%, 4%, and 6% for 6 weeks had no significant adverse effects on vital organ weights or carcass yield in quails. Importantly, abdominal fat weight was significantly lower in the PS-fed groups compared to the control group. These findings align with previous research on plant-based feed additives in poultry. Ahmadi-Kohanali *et al*. (2022) similarly observed a decrease in abdominal fat and no negative impact on carcass weight when adding PS to broiler diets. Consistent with our findings, other studies have reported reduced abdominal fat in broilers fed chili pepper (El-Deek *et al*., 2012) and wormwood (Lee *et al*., 2006; Kostadinovic *et al*., 2015). Overall, these observations suggest that various plant-based additives hold potential for modulating fat deposition in poultry.

The mechanisms underlying PS's fat-lowering effects remain to be elucidated, but potential pathways include altered lipid metabolism (Flees *et al*., 2021), appetite modulation (Chuang *et al*., 2020), or intestinal microbiota changes (Fu *et al*., 2023). Further research is warranted to investigate these mechanisms, as well as the long-term effects of PS inclusion, the specific bioactive compounds responsible for the observed effects, and the potential for synergistic interactions with other feed additives. Our observation indicated that PS at levels of 2%, 4%, and 6% did not affect carcass yield aligns with several studies on other plant additives in poultry diets. Similar findings were reported by Abbas (2010) in broilers fed fenugreek, parsley, or sweet basil seed at 3 g/kg each; and by Daş *et al*. (2020) in quails receiving 0.1%, 0.2%, or 0.3% mint oil. These studies suggest that certain plant additives, including PS, can be safely incorporated into poultry diets without compromising important carcass parameters like yield and dressing percentage. This is beneficial for both producers and consumers, as it contributes to improved meat production efficiency and potentially higher quality meat cuts. While some plant additives like PS and mint oil appear neutral towards organ weights, others may exert more nuanced effects. For example, Çetin *et al*. (2019) observed no significant differences in carcass, heart, gizzard, or abdominal fat weights in broilers fed varying levels of wormwood. However, they did report increased liver and pancreas weights in the group receiving the lowest wormwood dosage (11.76 g/kg). This highlights the importance of investigating dosage-dependent effects and potential organ-specific responses when introducing new plant additives.

Blood paramaters

The TAS increased progressively with PS levels, reaching a substantial 36% elevation in the 6% PS group compared to the control. This suggests potentially enhanced overall antioxidant capacity with increasing PS inclusion. The TOS level of quail blood plasma decreased steadily with increasing PS levels, dropping by 22.93% in the 6% PS group. This decline potentially indicates a reduced oxidative activity and improved protection against free radical damage. This finding indicates that PS has a high antioxidant value. Therefore, the increase in TAS value is crucial for strengthening the immune system (Hosseini-Vashan & Raei-Moghadam, 2019). The OSI followed a similar trend to that of TOS, showing a significant 42.64% decrease in the 6% PS group compared to the control, further supporting the potential for improved oxidative balance with PS addition. This is important for reducing oxidizing agents and stress, especially considering that stress is a critical factor negatively impacting the performance of poultry (Surai *et al*., 2019).

Adequate levels of total antioxidants can delay the oxidation of target molecules or eliminate oxidizing agents, preventing potential damage (Pisoschi *et al*., 2021). The OSI is defined as the balance mechanism between TAS and TOS and is reported to contribute to cellular and molecular tissue damage in disease conditions (Yavuz *et al*., 2023). Therefore, addition of natural antioxidants, such as PS, to poultry diets can assist in reducing oxidative stress and minimizing these adverse effects. Our findings on the use of PS in quail diets add to the evidence supporting the use of natural additives as antioxidants in poultry feed. Interestingly, while some additives like peppermint oil show clear antioxidant effects, others may exhibit more nuanced responses. For example, Çetin *et al*. (2019) reported no significant differences in TAS, TOS, or OSI with varying levels of wormwood supplementation in broilers. However, they observed reduced triglyceride levels in the groups receiving wormwood, suggesting other potential metabolic benefits beyond direct antioxidant action.

The insignificant differences among groups in plasma triglyceride, cholesterol, and HDL cholesterol values suggest that the addition of PS to the quail diet does not have a significant effect on these parameters (Table 6). The parabolic response of triglycerides, initially decreasing with PS but slightly rising at higher levels, suggests potential complexities in its impact on lipid metabolism. This aligns with Çetin *et al*. (2019) who observed reduced triglyceride levels in broilers fed wormwood, possibly indicating diverse mechanisms affecting fat metabolism across different additives. Although the differences in HDL cholesterol levels were not statistically significant among the groups, the overall trend suggests potential positive effects with PS supplementation. The increase in HDL cholesterol, a marker of "good" cholesterol, with PS inclusion is particularly promising. This finding aligns with observations in broilers fed chili pepper (El-Deek *et al*., 2012) and wormwood (Lee *et al*., 2006; Kostadinovic *et al*., 2015), suggesting potential for improved cardiovascular health through dietary modifications.

Further research is crucial to elucidate the specific mechanisms behind the observed antioxidant effects of different plant additives like PS. Investigating their bioavailability, potential impact on specific antioxidant enzymes, and interactions with other dietary components can provide valuable insights. Additionally, exploring dosage optimization and potential synergistic effects when combining certain additives could further enhance their antioxidant potential.

Intestinal flora

Maintaining optimal intestinal health in quails is crucial for their well-being, performance, and resistance to disease. Dietary fiber plays a significant role in shaping the intestinal microbiome, influencing the types and activity of intestinal bacteria (Reda *et al*., 2020). This study explored the effects of incorporating PS, which is rich in fiber, into quail diets on intestinal pH, microbial populations, and potential prebiotic properties. The study indicates that the inclusion of PS in quail diets has significant effects on pH levels and microbial populations in the small intestine and caecum. The addition of PS increased the pH of both the small intestine and cecum compared to the control group. Compared to the control group, PS inclusion at 2%, 4% and 6% increased caecum pH by 20%, 8.8% and 1.8% respectively, suggesting potential shifts in fermentation patterns. This alteration in gut pH could influence the types and activity of gut bacteria, potentially impacting the overall health and digestive processes of quails (Rehman *et al*., 2008). This suggests potential shifts in fermentation patterns, possibly due to a decrease in fiber fermenting bacteria or the influence of tannins (Brutti *et al*., 2023). Tannins in PS may lead to a reduction in the number of cellulolytic bacteria in the rumen. Ghasemi *et al*. (2012) noted that when PS was used as the sole roughage in sheep, rumen pH increased, and total VFA concentration decreased. However, contrary results have been also reported in some studies. Zanganeh *et al*. (2017) reported that when adding 0.1, 0.2, 0.3 g/kg of wild pistachio leaf extract (WPLE) to broiler diets, the pH of the small intestine significantly decreased in the 0.2% and 0.3% WPLE groups ($p<0.05$). As the dietary fiber (PS) ratio increased, the content of acetic acid (49.2%) and propionic acid (79.1%) significantly decreased. Conversely, the counts of LAB increased by 4.9% and the total bacterial count went up by 8.5%. Lactic acid, a marker of carbohydrate fermentation, displayed a significant decrease of 41.5% at 6% PS. Similarly, short-chain fatty acids (SCFAs) such as acetic acid and propionic acid also showed significant reductions. This suggests a modulation of microbial metabolic pathways, favoring SCFA production.

Several studies support the observed fluctuations in microbial populations and organic acid levels in response to increasing doses. The initial rise in lactic acid, acetic acid, and propionic acid, as well as microbial counts, aligns with findings from Kristensen *et al*. (2010), who demonstrated that microbial inoculants in silage fermentation lead to an initial increase in lactic acid bacteria and acid production before reaching an inhibitory threshold (Kristensen *et al*., 2010). This early increase is likely due to an abundance of nutrients and the activation of microbial metabolism. The subsequent decline in acid levels and microbial counts corresponds with studies highlighting acid-induced stress and metabolic inhibition. Trček *et al*. (2015) reported that bacteria experience growth suppression upon exposure to high concentrations of acetic acid, triggering adaptive stress responses and altering cellular function (Trček *et al*., 2015). Additionally, Vázquez *et al*. (2011) demonstrated that organic acids such as formic, acetic, and propionic acids can inhibit microbial growth at high concentrations due to their ability to lower intracellular pH and disrupt metabolic pathways (Vázquez *et al*., 2011). Following this decline, the final stage of increased acid production and microbial resurgence suggests an adaptive response, where microbial communities develop tolerance mechanisms. Sakata *et al*. (1999) showed that probiotic bacteria, when exposed to increasing doses of organic acids, adjust their metabolism and develop acid resistance, leading to a restoration of acid production and microbial growth (Sakata *et al*., 1999). This adaptive phase is also supported by Silva *et al*. (2016), who found that lactic acid bacteria in silage fermentation initially struggled with high acid levels, but later re-established growth as they adapted to the acidic environment (Silva *et al*., 2016).

Bacterial populations, including LAB, total bacteria, and Enterobacter responded positively to PS addition, indicating a potential increase in beneficial bacteria associated with SCFA production. The highest counts of LAB, total bacteria, and Enterobacter were observed in the 6% PS group. Total LAB and total bacterial counts increased slightly but significantly at 6% PS, indicating a potential increase in beneficial bacteria associated with SCFA production. Research findings on the impact of feed additives on intestinal flora may present conflicting results. The increase in metabolic activity of Lactobacilli is known to result in the production of end products such as lactate and succinate, along with bacterial biomass and SCFAs, including acetic acid, propionic acid, and butyric acid

(Gunal *et al*., 2006). In our study, where PS was used as an additive, an increase in short-chain acids such as acetic and propionic acid was observed in the caecum and intestinal of quails. However, Olnood *et al*. (2015) reported that the probiotics used in the feed additives did not affect the pH, SCFAs, and lactic acid concentrations in both ileum and ceca. Abdelaziz *et al*. (2015) investigated the effects of adding 250 mg/kg peppermint oil, 250 mg/kg thyme oil, 1g/kg antitoxin (Mycofix Plus), and 20 g/kg rice bran to the diet on the intestinal flora of Hubbard broiler chicks. The researchers obtained the highest Lactic Acid Bacteria (LAB) count (5.23) in the antitoxin group (Mycofix Plus 1g/kg), the lowest total bacteria count (6.40) in the group fed with 250 mg/kg thyme oil, and the lowest coliform bacteria count (6.70) in the group fed with 20 g/kg rice bran. In our study, the results obtained with PS were found to be more effective in terms of antitoxin compared to Abdelaziz *et al*. (2015) results, and the LAB count increased significantly (8.42).

The findings of Missotten *et al*. (2013) suggested that increasing the population of LAB through lactic acid fermentation could positively influence the gut microbiome of chickens, potentially leading to enhanced resistance against infectious diseases. The lowest counts of Enterobacter (6.71 CFU g-1) and coliform bacteria (6.31 CFU g-1) were obtained in the group fed with 4% PS. Compared to the control group, the 4% PS group showed a significant decrease: 24.94% for Enterobacter and 24.07% for coliform bacteria. This decrease is likely due to the potential prebiotic effect of PS, which may stimulate the growth of beneficial gut bacteria such as LAB. Increased LAB populations can compete with and inhibit the growth of pathogenic bacteria like enterobacteria and coliforms (Abou-Kassem *et al*., 2021). These findings suggest that diets rich in fermentable substrates, like PS, can promote gut health in quails by reducing the risk of pathogenic bacterial overgrowth. Similar to our study, Abou-Kassem *et al*. (2021) reported a significant decrease in total coliform counts when two probiotic strains (*Bacillus toyonensis* and *Bifidobacterium bifidum*) were added to quail diets. This further supports the idea that promoting beneficial gut microbiota can suppress pathogenic bacteria in birds. Contrary to our findings, Olnood *et al*. (2015), who investigated the intestinal microbial profile of four *Lactobacillus* strains (*Lactobacillus johnsonii*, *Lactobacillus crispatus*, *Lactobacillus salivarius* and an unidentified *Lactobacillus* sp.), demonstrated that all four probiotics tended to reduce the number of Enterobacteria in the ileum compared to control treatments. The decrease in the number of Enterobacteria, which are LAB, has been attributed to the antimicrobial effect of the probiotics added to the diet.

The findings also suggest that the addition of PS can increase the population of beneficial bacteria in the cecal content while reducing pathogenic bacteria. Indeed, a decrease in the numbers of pathogenic bacteria and molds is observed with the addition of pistachio shell. Hosseini-Vashan *et al*. (2020) observed a decrease in the total intestinal microbial population when they added 200-400 mg/kg of PS extract to broiler diets. Ahmadi-Kohanali *et al*. (2022) indicated that the addition of green PS extract to broiler diets infected with Staphylococcus aureus resulted in improved growth performance and a reduction in microbial population in the gastrointestinal system.

Pistachio shell-supplemented diets in quails led to significant decrease in the number of *E. coli* (Table 7). This suggests that quails fed with diets containing PS supplementation showed increased resistance to the pathogenic effects of *E. coli* in their intestinal flora. Zanganeh *et al*. (2017) reported that the addition of 0.1, 0.2, and 0.3 g/kg wild pistachio leaf extract (WPLE) to broiler diets did not affect the Coliform bacterial population in the cecum, increased *Lactobacillus*, and significantly reduced *E. coli* ($p<0.05$). Plant extracts, particularly those utilizing phenolic compounds, are crucial in enhancing beneficial (*Lactobacillus*) bacterial populations and reducing pathogenic bacteria in the cecum through the action of microorganisms.

Despite a limited understanding of the role of yeast within the complex poultry gut microflora (Delavenne *et al*., 2011), evidence suggests its potential for both pathogen control and immune system stimulation (Byrd *et al*., 2017; Adhikari *et al*., 2023). In this study, the effect of PS on intestinal yeast populations in quails was also investigated. A statistically significant difference ($p<0.01$) was observed amongst treatment groups, with the highest yeast value (4.34) recorded in the 2% PS group. Additionally, dietary supplementation with 2% PS promoted intestinal yeast colonization in quails, potentially contributing to enhanced pathogen resistance through immune system modulation. Yeast showed a significant increase at the 2% PS group, but declined at the 4% one, hinting at a complex dynamic between different microbial groups in response to PS inclusion.

Intestinal mold levels differed significantly among treatment groups ($p<0.01$), exhibiting an inverse relationship with dietary PS inclusion. Despite an initial increase in mold level occurring at the 2% PS level, mold quantity progressively decreased with higher PS levels, coinciding with the lowest *E. coli* levels. This indicates that supplementing quail diets with 6% PS effectively suppresses mold growth within the intestinal microflora, potentially exerting an antimicrobial effect. Supporting these findings, Abdel-Hack *et al*. (2018) demonstrated the antifungal properties of plant-derived compounds like thyme, anise, cinnamon, and mint, specifically their ability to reduce aflatoxins.

The study suggests that PS supplementation in quail diets influences gut pH, microbial populations, and fermentation patterns, contributing to improved gut health, increased beneficial bacteria, and reduced pathogenic bacteria. In addition, the findings suggest that PS exerts a prebiotic-like effect in quails, potentially promoting beneficial bacteria, enhancing SCFA production, and suppressing potential pathogens. Further research exploring the specific bacterial species affected and the potential mechanisms of action would be valuable for refining PS use as a gut health promoter in quail production.

CONCLUSION

The comprehensive evaluation of pistachio shell (PS) inclusion in quail diets has provided valuable insights into its multifaceted impact on key performance metrics, health parameters, and antioxidant status. The study, conducted over different weeks, revealed significant variations in live weight (LW), feed intake (FI), and feed conversion ratio (FCR) across various treatments. Notably, the inclusion of 6% PS emerged as a pivotal factor, positively influencing quail performance, leading to increased LW and improved FCR during specific developmental phases.

Slaughter characteristics, including carcass weights, organ health, and abdominal fat deposition demonstrated minimal effects with PS inclusion, emphasizing its potential as a balanced dietary component for quail production. The reduction in abdominal fat, particularly evident with 6% PS, suggests a promising avenue for dietary manipulation to regulate fat content in quail, contributing to both economic and nutritional considerations.

Survival rate analysis indicated a remarkable improvement in overall quail survival with 4% and 6% PS inclusion, highlighting its positive influence on the well-being of the quail population. This underscores the importance of considering dietary components, such as PS, in optimizing overall health and survival in agricultural settings.

The exploration of oxidative stress and blood parameters revealed a significant increase in total antioxidant status (TAS) and a simultaneous decrease in total oxidant status (TOS) with increasing PS levels. This trend, along with a notable decline in oxidative stress index (OSI), signifies a potential enhancement of the overall antioxidant capacity in quails fed PS-supplemented diets. The favorable modulation of blood parameters, such as triglyceride levels and HDL cholesterol, further suggests potential cardiovascular benefits associated with PS inclusion.

The investigation into intestinal flora parameters unveiled significant shifts in microbial populations and fermentation patterns with PS supplementation. The increase in beneficial bacteria, including Lactic Acid Bacteria (LAB) and total bacteria, coupled with a reduction in pathogenic bacteria such as Enterobacter and *E. coli*, highlights the prebiotic-like effects of PS. Additionally, the observed decrease in intestinal yeast and mold populations, particularly at 6% PS, suggests potential antimicrobial effects, contributing to improved gut health.

In summary, the findings of this study collectively indicate that the inclusion of pistachio shell in quail diets at varying proportions positively influences performance, health, antioxidant status, and gut microbiota. These outcomes underscore the potential of PS as a functional feed additive in optimizing quail production systems. However, further research is warranted to delve into the specific mechanisms underlying these effects, optimize dose levels, and assess long-term implications, ultimately enhancing our understanding of the broader applications of PS in poultry nutrition.

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AUTHOR CONTRIBUTIONS

This study is derived from the master's thesis completed by Mustafa Apaydın under the supervision of Dr. Mehmet Cetin. The following statements should be used, followed by the author's initials: Conceptualization, MC and MA; methodology, MC; software, MC; validation, MC; formal analysis, MC; investigation, MC and MA; resources, MC and MA; data curation, MA; writing—original draft preparation,

MC; writing—review and editing, MC; visualization, MC and MA; supervision, MC; project administration, MC and MA; funding acquisition, MC. All authors have read and agreed to the published version of the manuscript.

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AUTHOR STATEMENT

All animal procedures and protocols used in this experiment were reviewed and approved by the Harran University Animal Research Ethics Board (AREB) (HRU AREB Document Date and Number: April 24, 2021-32561), with the Animal Experiments Local Ethics Committee.

DATA AVAILABILITY STATEMENT

The data supporting the findings are available from the corresponding author upon reasonable request.

CONFLICT OF INTEREST

The authors declares that they do not have any known financial interests or personal relationships that could be perceived as influencing the work reported in this paper.

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