Impacts of Purslane (*Portulaca oleracea*) extract supplementation on growing Japanese quails' growth, carcass traits, blood indices, nutrients digestibility and gut microbiota

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ABSTRACT This work aimed to assess how *Portulaca oleracea* extract (**POE**) affected Japanese quail's growth, carcass features, blood parameters, digestibility coefficients, and microbiological aspects. A total of two hundred twenty-five 7-days-old Japanese quails were allotted into 5 experimental groups. Birds were divided as follows: group 1) received only the base diet (control), while groups 2, 3, 4, and 5 received the basal feed supplied with 1.0, 2.0, 3.0, and 4.0 mL POE/kg diet, respectively. The findings cleared those quails' fed diets supplemented with POE had significantly ($P > 0.01$) higher body weight (**BW**) and body weight growth (**BWG**) than the control group. The treatment with POE significantly affected digestive enzymes (amylase and lipase) and digestion coefficients for ether extract (**EE**). The treated groups had decreased serum urea and malonaldehyde (**MDA**) and increased Immunoglobulin G (**IgG**), Immunoglobulin M (**IgM**), superoxide dismutase (**SOD**), total antioxidant capacity (**TAC**), glutathione (**GSH**), and catalase (**CAT**) concentrations than the control. All bacterial counts in dietary cecal samples declined with increasing POE levels. In conclusion, POE supplementation improved quails' performance and nutrient digestibility. Moreover, POE did not harm birds' liver and kidney functions. In addition, this extract promoted the immunity and antioxidant status and minimized the harmful microbial load in quails' intestines, the total bacterial count was decreased by 90% in diet samples supplemented with purslane addition level (4 mL/g), while decreased by 74% in cecal samples supplemented with purslane addition level (4 mL/g) and *Salmonella* don't appear in all addition levels. However, lactic acid bacteria increased by 70%, indicating beneficial of POE in reducing the pathogenic microorganisms.

Key words: blood indices, growth, gut microbiota, purslane extract, quails

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INTRODUCTION

Antibiotics have been extensively utilized in livestock production to treat diseases and enhance animals' performance (Abd El-Hack et al., 2021; Swelum et al., 2021). Because of the constant hazard of current antibiotic resistance mechanisms in various microbes, the European Commission prompted to prohibit its danger as growth promoters in livestock diet (European Union, 2005). Although antibiotics were eliminated from the livestock feed, they negatively affected feed conversion ratio, feed efficiency, and growth enhancement (Sheiha et al., 2020). So, the global society, especially researchers, has a safety trend, which is the natural components like probiotics (Abd El-Hack et al., 2020; El-Saadony et al., 2022a), prebiotics (Yaqoob et al., 2021; Abd El-Hack et al., 2022), essential oils (El-Tarabily et al., 2021), plant extracts (Abou-Kassem et al., 2021), amino acids (Abou-Kassem et al., 2021), green synthesized nanoparticles (Abd El-Ghany et al., 2021), safe pigments (Abdelnour et al., 2020a,b; Ashour et al., 2021), and bioactive medicinal herbs (El-Shall et al., 2022).

Purslane (*Portulaca oleracea*) is a plant defined as Purslane in the United States and Australia, rigla in Egypt, pigweed in England, pourpier in France, and machi-xian in China (Elkhayat et al., 2008). Due to its richness with omega-3 fatty acids and antioxidant qualities, it has nutritional advantages (Palaniswamy et al., 2001;

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Aboulthana et al., 2022), additionally, it displays a wide spectrum of pharmacological activities, like anti-inflammatory, antibacterial, antioxidant, antiulcerogenic, and wound-healing effects .

Moreover, phenolic components in purslane extract demonstrate antioxidant action (Liu et al., 2000). According to Zhao et al. (2013), adding purslane extract (0.2%) to broiler feed enhanced feed conversion ratio and weight gain. Due to their advantageous antibacterial effects and extensive antioxidant activities, medicinal plants have demonstrated greater potential as alternatives to chemical antibiotics . This work aimed to determine the effect of purslane extract on growing Japanese quail's growth performance, carcass traits, blood biochemical measurements, digestion coefficients, and microbiological aspects.

MATERIALS AND METHODS

Purslane Extracts Preparation

Purslane leaves were sliced into bits, dried in an oven at 45°C for 3 d, ground into a fine powder in a grinder, packaged in plastic vacuum containers, and kept in the dark at 4°C until extraction. Purslane leaves powders were extracted by ethanol 80% (v/v) following Saad et al. (2021a) with some modifications. Extraction was performed using 10% of leaves powder and stirring for 1 h and then the ethanolic extract was filtrated by centrifugation at 3,000 rpm for 10 min. The ethanol extract was evaporated at 40°C via a BUCHI rotary evaporator and then freeze-dried.

Total Phenolic Content in Purslane Extract

The total phenolic content of purslane extract was identified following the method following El-Saadony et al. (2022b). 100 μL of diluted Folin−Ciocalteu's reagent and 50 μL of 7.5% Na_2CO_3 were added to 50 μL of purslane extract. The microtiter plate was kept at room temperature for 30 min. The absorbance was read at 765 nm utilizing a plate microtiter reader. The experiment was done in triplicate. Total phenol content (**TPC**) was calculated as mg Gallic acid/g purslane extract.

Assessments of Antioxidant Activity DPPH Scavenging Activity%

Scavenging impacts of the stable 1, 1-diphenyl-2-picrylhydrazyl (**DPPH**) radical were assessed as follow El-Saadony et al. (2022b). Hundred microliter of ethanolic DPPH were supplemented with 50 μL of purslane extract. A control of 100 μL of DPPH in 100 μL ethanol. For 30 min, the microtiter plate was left in the dark for 30 min. The plate microtiter reader was used to evaluate the absorbance at 517 nm. The mean of triplicate results was given as a percentage of DPPH radical scavenging activity.

$$Radical\ scavenging\ activity\ (\%) = \frac{Abs.\ control - Abs.\ sample}{Abs.control}\ x100$$

Evaluation of the Antimicrobial Effect of Purslane Extracts using Disc Diffusion Assay

The antimicrobial activity of purslane extracts against different bacterial isolates was evaluated by the disc diffusion assay. *Bacillus cereus, Staphylococcus aureus, Listeria monocytogenes, Pseudomonas aeruginosa, Escherichia coli,* and *Salmonella typhi*, and fungal isolates, *Aspergillus niger, Aspergillus flavus, Aspergillus fumigates, Penicillium digitatum, Penicillium notatum,* and *Fusarium oxysporum* were obtained from Department of Agricultural Microbiology, Faculty of Agriculture, Zagazig University, Zagazig, Egypt. Each isolate was incubated at its optimum growth conditions (temperature and time) in optimum growth media (Muller Hinton broth [**MHB**] for bacteria, Potato Dextrose broth [**PDB**] for fungi). The previous isolates were optimized at 10^8 CFU/ml concentrations for bacteria and 10^5 CFU/mL for fungi. Then 100 mL of bacterial inoculum (1×10^8 CFU/mL) was spread on Mueller Hinton agar plates surface (**MHA**), and fungal inoculum (1×10^5 CFU/mL) on Potato Dextrose agar (**PDA**) plates surface. The discs (6 mm) were saturated with different concentrations of the purslane extract (25, 50, 100, 150, 200, 250, and 300 μg/mL) and applied to inoculated petri plates surface. The plates were incubated at 37°C for a day (bacteria) and at 28°C for 5 d (fungi) (El-Saadony et al. 2021; Abdel-Moneim et al., 2022). The experiments were conducted in triplicates to determine significant differences. Sterilized distilled water used as a control. The ruler was used to measure the resultant inhibition zones (mm). The inhibition zones greater than 8 mm witnessed the antibacterial or antifungal activity of purslane extract.

Micro Broth Dilution Method

The Minimum inhibitory concentration (**MIC**) was estimated by the micro-dilution broth method following El-Saadony et al., (2021). Each concentration of tested purslane extract (25, 50, 100, 150, 200, 250, and 300 μg/mL) were added to tubes containing 9 mL of MHB and PDB. Five hundred mocroliter of bacterial (1×10^8 CFU/mL) and standard size of fungal spore suspension (1×10^5 CFU/mL) were added to tubes. Control was MHB and PDB tubes. The tubes were incubated for a day at 37°C (bacteria) and 5 days at 28°C (fungi). The MIC was the least concentration of purslane extract that inhibits the bacterial and fungal growth. The purslane extract with high activity has lower MIC, the lowest concentration that kills the tested bacteria and fungi

called minimum bactericidal concentration (**MBC**) for bacteria and minimum fungicidal concentration (**MFC**) for fungi (El-Saadony et al., 2021). The MBC and MFC were estimated by taking a loopful from each MIC tubes and spread on MHA and PDA then incubated at 37°C for 24 h (bacteria) and 28°C for 5 d (fungi) and observed the bacterial or fungal growth (Saad et al., 2021b).

Animals, Design, and Diets

Two hundred and twenty-five Japanese quails that were 7 d old with an average weight of 27.17 ± 0.075 g was used. Quails were divided among 5 groups at random. Each group contained 45 unsexed chicks (5 replications of 9 birds). Birds were reared in conventional cages that measured 90 × 40 × 40 cm^3.

Ration and drinking water were ad libitum supplied during the study period. The dietary treatments were as follows: the first group received the basal diet (control), whereas the second, third, fourth, and fifth groups received the basal diet in addition to 1.0, 2.0, 3.0, and 4.0 mL POE/kg diet, respectively. According to NRC (1994), the basal diet (Table 1) was performed to satisfy the needs of the quails.

Animal maintenance and care adhered to Zagazig University's (ZU-IACUC/2/F/56/2021) criteria for the care and use of laboratory animals and those of the Egyptian Research Ethics Committee.

Growth Performance and Carcass Traits

Feed consumption and all growth indicators were assessed at 1, 3, and 5 wk of age. At 5 wk, 25 birds (5 per treatment group) were randomly chosen, weighed, and ethically slaughtered for carcass examinations. Before slaughter, the total weight of all edible components was measured and presented as a percentage of the live body weight (**LBW**).

Blood Chemistry

Five quails per treatment were randomly selected post euthanization, and blood samples were taken into heparinized tubes. We employed a centrifuge (Janetzki, T32c, 5000 rpm, Germany) at 2146.56 g for 15 min to separate the plasma for the biochemical parameters. The Biodiagnostic Company's commercial kits measured the biochemical blood parameters (Giza, Egypt).

Digestibility Trials

Six birds were individually housed in metal cages for each treatment. They were weighed before and postcollection to ensure that the birds maintained their weight. A drawer was provided in each cage for the collecting of excreta. A layer of aluminum foil was placed over the drawer to make excreta collecting easier. Fixed containers provided ad libitum access to the experimental diets and water. During excreta collection, it was sprayed with acid to avoid bacterial fermentation on the excreta. Each replicate's and sampling's excreta were homogenized, and a subsample of 100 g was oven dried for 48 h at 70°C.

According to the Association of Official Analytical Chemists (AOAC, 2006), the proximate analysis of experimental feeds and total nitrogen dried excreta was completed using triplicate samples for each nutrient. Nutritive values were calculated and expressed as total digestible nutrients (**TDN**) and metabolizable energy (**ME**). The TDN was calculated using factors 1, 2.25, and 1 for CP, EE, and crude carbohydrates (**CF** and **NFE**), respectively, and metabolizable energy was determined as 4.2 per gram TDN, as proposed by Titus (1961).

Microbial Count in Diet and Cecal Samples

The feed samples were microbiologically examined at an interval of 0, 7, 14, and 21 d. Ten grams of dietary samples were mixed with 90 mL of sterile saline peptone water (1 g/L peptone and 8.5 g/L NaCl) at the screw bottle and homogenized for 3 min to prepare 10^{-1} dilution. One millilter of the previous dilution was added to 9 mL sterile saline peptone water tube to obtain 10^{-2} dilution, further serial dilutions up to 10^{-7}. One mL of each dilution was placed in sterile one-use petri-dishes. Different media were used to count microorganisms. Total bacterial count (**TBC**) was counted at plate count agar at 30°C for 2 d. The total yeasts and moulds count (**TYMC**) were estimated on Rose Bengal Chloramphenicol agar for 5 d at 25°C. Total coliforms were counted on violet red bile agar after 24 h incubation at 37°C (Harrigen and E., 1976). *Escherichia coli* was counted on eosin methylene blue agar plates after incubation for 24 h at 37°C (Oxoid, 1982). All plates were examined for typical colony types and morphological characteristics associated with each culture medium. On the other hand, the microbial counts in quail caecum were estimated as in diet. Cecal samples (5-replicate) were homogenized in a screw bottle with sterile saline peptone water (1 g/L peptone and 8.5 g/L NaCl). Decimal serial dilutions up to 10^7 were prepared. The different microorganisms were counted on specific media (Reda et al., 2020, 2021). Total bacteria were counted as per Sheiha et al. (2020) and Reda et al. (2020) on plate count agar (**PCA**). Total coliforms were counted on violet red bile agar after 24 h of incubation at 37°C (Harrigen and E., 1976). *Escherichia coli* was counted on eosin methylene blue agar plates after incubation for 24 h at 37°C (Oxoid, 1982; Richard et al., 1986). *Salmonella* spp. was calculated at *Salmonella Shigella* (**SS**) Agar as per Edwards and R.L., 1976; black colonies indicate the exist of *Salmonella* spp. Yeasts and Moulds were enumerated as Kurtzman and Fell (1984). de Man Rogosa and Sharpe (**MRS**) medium was used to count Lactic acid bacteria, according to Argyri et al. (2013). *Enterococcus* spp., was counted in Chromocultfi enterococci agar (Miranda et al., 2005).

Statistical Analysis

Using SAS software, the statistical analyses were performed. A one-way analysis of variance was performed on the data using a normal distribution. The significance of the dietary POE supplementation amounts that were introduced gradually was examined using the post-hoc Tukey's test ($P < 0.05$). The statistical model used was:

$$Y_{ij} = \mu + T_i + e_{ij}$$

where Y_{ij} = observed value; μ = overall mean; T_i = treatment effect (control, and 1−6); and e_{ij} = random error. Differences among recorded means were estimated by the test of Student−Newman−Keuls. The SEM and mean values were reported. The differences between groups are considered significant at $P < 0.05$.

RESULTS

Antioxidant Content

Total phenolic of ethanol extract was significantly increased in a concentration-dependent manner. In contrast, purslane extract (500 μg/mL) consisted of 11% phenolic compounds (Figure 1A). The antioxidant activity of ethanolic purslane extracts was determined by DPPH assay (Figure 1B). DPPH radical inhibition % was significantly high ($P \leq 0.05$) in ethanol extract, recorded at 96% compared to the ascorbic acid with 98%. The Scavenging capacity of 50% of DPPH (SC50) by purslane extract was 0.15 mg/mL

Table 2 shows the inhibition zone diameters of purslane extract as a response to antimicrobial activity against the tested microbial isolates. The purslane concentrations are more potent against Gram-positive bacteria than Gram-negative bacteria. Generally, Purslane is more effective antibacterial than antifungal. Depending on the results, the most sensitive bacteria to purslane extract (300 μg/mL) are *Staphylococcus aureus* and *Escherichia coli,* with IZDs of 32 and 29 mm. In contrast, the resistant bacteria are *Listeria monocytogenes* and *Pseudomonas aeruginosa* with IZDs of 30 and 26 mm. Regarding fungi, *Aspergillus niger, Aspergillus flavus, Aspergillus fumigates,* and *Penicillium notatum* were the resistant isolates to purslane extract concentrations.

The purslane extract inhibited the studied bacteria at concentrations (15−45 μg/mL) while killed at concentrations (25−85 μg/mL). While purslane extract at concentrations (40−45 μg/mL) are required to inhibit the tested fungal isolates, while (70−85 μg/mL) to kill the tested fungi.

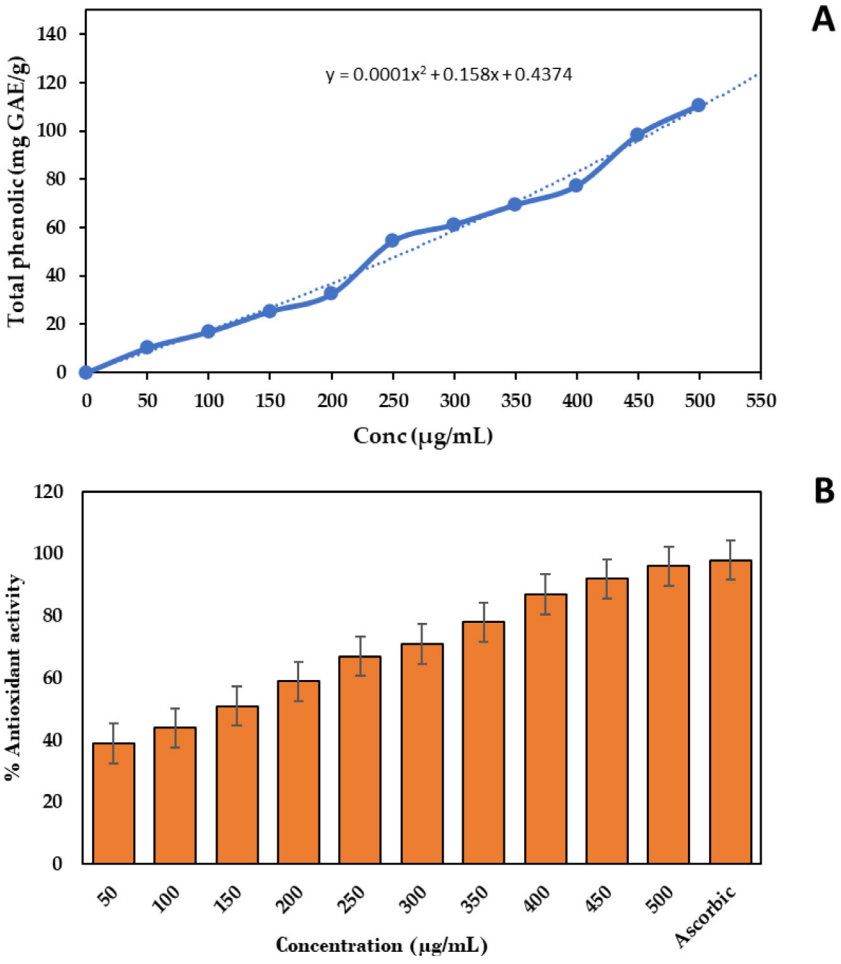

Figure 1. A. total phenolic content mg gallic acid/g in Purslane ethanolic extract; B. % DPPH radical scavenging activity of Purslane ethanolic extract.

Table 1. Composition and chemical analysis of the basal diet.

Ingredients	%
Corn	53.03
Soybean meal	38.69
Gluten meal	3.20
Soybean oil	1.67
Di Calcium phostphate	0.81
Vit-min Premix*	0.30
NaCl	0.11
Limestone	0.30
DL-Methionine	0.39
L-Lysine HCl	1.50
Calculated analysis**:	
Crude protein %	24.04
Metabilozable energy Kcal/kg	2903
Calcium %	0.85
Available phosphorous %	0.45
Methionine + Cysteine %	0.88
Lysine %	1.60

*Growth vitamin and Mineral premix Each 2 kg consists of Vit A 12000, 000 IU; Vit D3, 2000, 000 IU; Vit. E. 10g; Vit k3 2 g; Vit B_1, 1000 mg; Vit B_2, 49g; Vit B_6, 105 g; Vit B_{12}, 10 mg; Pantothenic acid, 10 g; Niacin, 20 g, Folic acid, 1000 mg; Biotin, 50 g; Choline Chloride, 500 mg, Fe, 30 g; Mn, 40 g; Cu, 3 g; Co, 200 mg; Si, 100 mg and Zn, 45 g.
**Calculated according to NRC (1994).

Microbial Load in Dietary Samples

Figure 2 shows the diet affected by purslane addition level (1, 2, 3, and 4 mL/kg diet). The microbial count increased with feeding periods, however decreased with increasing the addition level of purslane extract to quail diet. The decrease TBC was 90% by purslane addition level (4 mL/kg diet) comparing control, but increased by 40% with feeding periods. Also, TMYC decreased by 50% in diet samples supplemented with purslane addition level (4 mL/kg diet) compared to control, while the TMYC count increased with feeding period. The coliform and *E. coli* count was decreased by 60 and 80%, respectively in diet samples supplemented with purslane addition level (4 mL/kg diet).

Live Body Weight and Body Weight Gain

The best results were obtained by birds fed 1 and 4 mL POE/ kg diet. Generally, Table 3 showed that BW and BWG were significantly ($P \leq 0.01$) higher for quails fed on feed supplemented with POE levels than in the control group during the study periods. The increase percentages in BW and BWG values were 93.39, and 92.55% at the end of the fifth week of age.

Feed Intake and Feed Conversion Ratio

Table 4 indicates that there were no significant differences among all groups in periods (1−3 wks) and (1−5 wks), but in the period (3−5) weeks, the control group shows the highest significance. All treatments do not reflect any change in birds' FCR.

Carcass Traits

Results tabulated in Table 5 clearly showed insignificant differences in the relative weights of carcass, liver, gizzard, heart, giblets, and dressing of the birds' treated groups compared to the untreated group. In contrast, treatment group 2 and control (untreated group) positively influenced the pH scale for the intestine compared to other treatments, with a percentage rate of 94.13%.

Digestive Enzymes

In Table 6, treatment group 4 appeared to have significant differences in digestive enzymes (amylase and lipase) compared to others. The increases in amylase and lipase levels were 28.95 and 26.09%, respectively. On the other hand, all groups did not significantly affect the protease enzyme.

Table 2. Inhibition zones diameters (mm) of POE against pathogenic bacteria and fungi.

	Concentrations (μg/mL)							Concentrations (μg/mL)	
Microbial isolates	25	50	100	150	200	250	300	MIC	MBC
Inhibition zones (mm)									
Gram-positive bacteria									
Bacillus cereus	12^{ab}	16^{ab}	22^{b}	24^{b}	27^{b}	29^{c}	32^{c}	20^{e}	35^{e}
Listeria monocytogenes	11^{b}	15^{b}	20^{c}	22^{c}	26^{bc}	28^{cd}	30^{d}	25^{d}	45^{d}
Staphylococcus aureus	13^{a}	17^{a}	24^{a}	27^{a}	30^{a}	34^{a}	36^{a}	15^{f}	25^{f}
Gram-negative bacteria									
Escherichia coli	-	14^{bc}	19^{cd}	21^{c}	24^{c}	26^{d}	29^{de}	35^{c}	60^{c}
Pseudomonas aeruginosa	-	11^{d}	15^{e}	17^{f}	21^{de}	23^{f}	26^{f}	45^{a}	85^{a}
Salmonella typhi	-	12^{cd}	17^{d}	20^{d}	22^{d}	24^{e}	28^{e}	40^{b}	75^{b}
Fungi	25	50	100	150	200	250	300	**MIC**	**MFC**
Aspergillus niger	-	10^{e}	14^{f}	15^{g}	19^{e}	24^{e}	27^{ef}	45^{a}	90^{a}
Aspergillus flavus	-	11^{d}	15^{e}	16^{f}	21^{de}	25^{de}	29^{de}	45^{a}	85^{b}
Aspergillus fumigates	-	10^{e}	13^{g}	16^{f}	19^{e}	26^{d}	31^{cd}	45^{a}	90^{a}
Penicillium digitatum	-	12^{cd}	16^{d}	20^{d}	23^{cd}	31^{b}	34^{b}	40^{b}	75^{c}
Penicillium notatum	-	11^{d}	14^{f}	18^{e}	22^{d}	29^{c}	32^{c}	45^{a}	85^{b}
Fusarium oxysporum	-	13^{c}	15^{e}	21^{c}	24^{c}	30^{bc}	33^{b}	40^{b}	70^{d}

Boldface indicates different concentrations of POE.
MIC, minimum inhibitory concentration; MBC, minimum bactericidal concentration; MFC, minimum fungicidal concentration.
$^{a-g}$different lowercase letters in the same column indicate significant differences

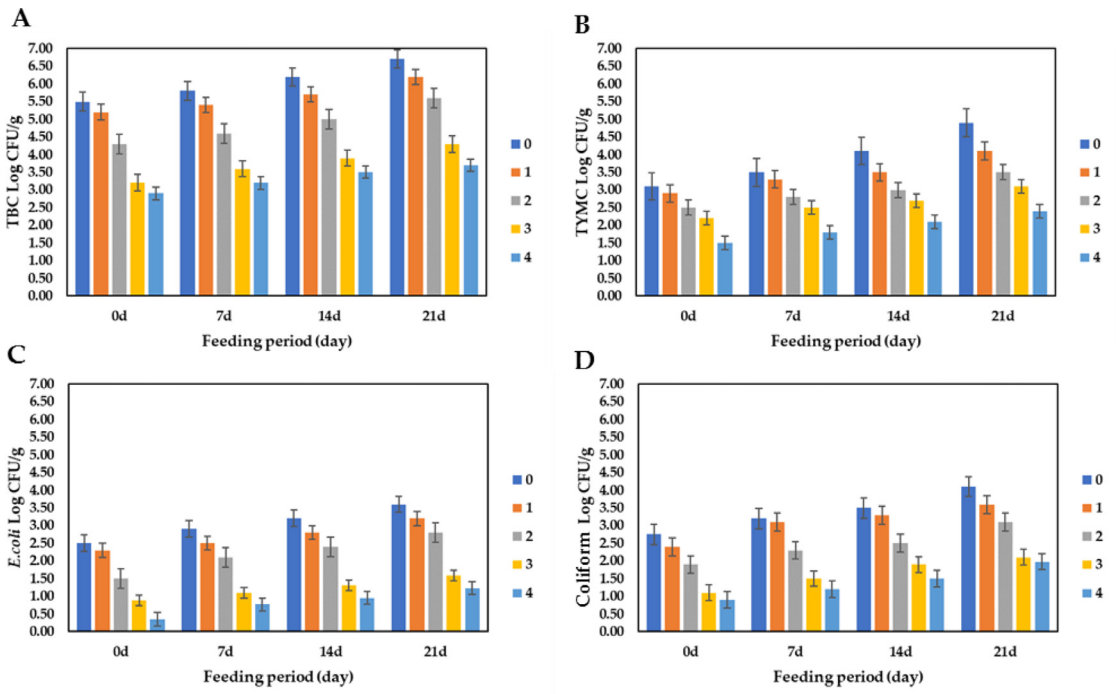

Figure 2. Dietary microbes (TBC, TMYC, *E. coli*, coliform) are represented (Log CFU/g) during feeding as affected by different doses of POE.

Table 3. Effects of dietary POE supplementation on growing Japanese quails' live body weight and body weight gain.

	POE (mL/kg diet)						
Items	0	1	2	3	4	SEM	*P* value
Body weight (g)							
1 wk	28.92	28.96	29.15	29.10	29.04	0.338	0.9901
3 wk	103.32[b]	114.55[a]	108.18[ab]	114.65[a]	113.64[a]	1.883	0.0151
5 wk	209.66[b]	221.07[a]	207.73[b]	217.07[ab]	224.50[a]	2.729	0.0088
Body weight gain (g/day)							
1–3 wk	5.31[b]	6.11[a]	5.65[ab]	6.11[a]	6.04[a]	0.137	0.0144
3–5 wk	7.60	7.61	7.11	7.32	7.92	0.165	0.1212
1–5 wk	6.46[b]	6.86[a]	6.38[b]	6.71[ab]	6.98[a]	0.106	0.0133

POE, *Portulaca oleracea* extract.
[a,b]means in the same row within each classification bearing different letters significantly differ.

Digestion Coefficients

Results tabulated in Table 7 showed insignificant differences in digestion coefficient for CP among the treated and the control (untreated) groups. While treatment group 2 led to significant differences between all groups in the digestion coefficient for EE. Also, the treatment groups (1, 3, and 4), respectively showed the highest significance among the others on digestion coefficient for CF, DM, and OM. Treatment group 4 significantly affected the digestion coefficient for NFE, TDN, and ME compared to the others, with percentage increases of 99.99, 91.85, and 91.84%, respectively.

Table 4. Effects of dietary POE supplementation on feed intake and feed conversion ratio of growing Japanese quails.

	POE (mL/kg diet)						
Items	0	1	2	3	4	SEM	*P* value
Feed intake (g/day)							
1–3 wk	14.39	14.42	14.79	15.17	15.01	0.321	0.5191
3–5 wk	26.11[a]	24.50[ab]	23.86[b]	22.76[b]	24.21[b]	0.567	0.0247
1–5 wk	20.25	19.46	19.33	18.96	19.61	0.444	0.4108
Feed conversion ratio (g feed/g gain)							
1–3 wk	2.72	2.36	2.63	2.49	2.49	0.119	0.4053
3–5 wk	3.44	3.23	3.36	3.11	3.06	0.114	0.2114
1–5 wk	3.14	2.84	3.03	2.83	2.81	0.102	0.1857

POE, *Portulaca oleracea* extract.
[a,b]means in the same row within each classification bearing different letters significantly differ.

Table 5. Effects of dietary POE supplementation on carcass traits and intestinal pH of growing Japanese quails.

	POE (mL/kg diet)						
Items	0	1	2	3	4	SEM	*P* value
Carcass %	70.16	71.48	74.98	73.75	72.63	1.965	0.5330
Liver %	2.18	2.41	2.07	2.16	2.07	0.149	0.5386
Gizzard %	1.94	2.26	2.01	2.18	2.19	0.101	0.2210
Heart %	0.83	0.77	0.94	0.89	0.95	0.065	0.3814
Giblets %	4.95	5.43	5.02	5.22	5.21	0.209	0.6793
Dressing %	75.11	76.91	80.00	78.98	77.83	1.886	0.5149
Intestinal pH	6.82[a]	6.27[b]	6.80[a]	6.42[b]	6.29[b]	0.081	0.0013

POE, *Portulaca oleracea* extract.
[a,b]means in the same row within each classification bearing different letters significantly differ.

Table 6. Effects of dietary POE supplementation on digestive enzyme concentrations in growing Japanese quails.

	POE (mL/kg diet)						
Items	0	1	2	3	4	SEM	*P* value
Amylase	7.12[c]	14.31[b]	7.37[c]	13.54[b]	24.59[a]	1.379	0.0016
Lipase	6.42[d]	21.40[ab]	11.15[cd]	15.39[bc]	24.61[a]	2.276	0.0018
Protease	0.83	1.12	0.96	1.27	1.04	0.148	0.3633

POE, *Portulaca oleracea* extract.
[a-c]means in the same row within each classification bearing different letters significantly differ.

Liver and Kidney Functions

In Table 8, insignificant differences were detected among all tested groups involving the liver enzyme control (**AST**) control. The values of AST and ALT reflect a good nutritional status of birds and healthy liver. There was a significant effect ($P < 0.05$) on the ALT enzyme for treated birds in groups 1 and 2. Treatment group 3 showed a significant increase ($P < 0.05$) in TP and GLOP, but these elevations were still within normal ranges, as indicated by the nonsign of toxicity. All treated and untreated (control) groups did not affect ALP and A/G. Creatinine and urea indicate kidney functions; the data showed a nonsignificant difference among treated birds and the control group in serum creatinine. The treated groups decreased serum urea compared to the control group, and the percentage decreased by 43.84% compared to the control group.

Lipid Profile

The data in Table 9 showed that treatment groups 2, 3, and 4 had lower TC, TG, LDL, and VLDL levels than those from the control group and treatment group 1, with decreased percentages of 64.26, 32.71, 59.15, and 32.7 %, respectively. At the same time, the supplementation did not affect the HDL levels, and no significant change was noted.

Immunity and Antioxidant Status

Table 10 shows that the treated groups caused an increase in serum IgG and IgM levels more than the control group. This increase promotes and induces earlier maturation of humoral immune responses. There were significant differences in complement three levels between the groups with an increasing percentage of 41.99%. These differences were caused by Purslane supplementation. On the other hand, this supplementation did not significantly influence the lysozyme enzyme contrasted to the untreated group. The treatments showed a significant effect with increased SOD, TAC, GSH, and CAT levels. In contrast, the treated groups revealed a lowering in MDA levels contrasted to the control group,

Table 7. Effects of dietary POE supplementation on the digestion coefficient for various nutrients and nutritive values of growing Japanese quails.

	POE (mL/kg diet)						
Items	0	1	2	3	4	SEM	*P* value
CP	86.07	87.43	86.23	87.03	88.17	0.802	0.4005
EE	72.61[c]	81.00[a]	73.68[c]	78.11[b]	80.16[ab]	0.859	0.0001
CF	26.10[c]	29.24[a]	26.93[bc]	28.62[ab]	29.85[a]	0.564	0.0042
NFE	78.45[c]	81.90[b]	79.21[c]	82.17[ab]	84.36[a]	0.683	0.0008
DM	78.44[b]	81.57[a]	78.79[b]	82.38[a]	83.20[a]	0.746	0.0051
OM	80.77[b]	83.44[a]	80.89[b]	84.01[a]	85.28[a]	0.736	0.0066
TDN	72.11[d]	76.63[ab]	73.08[cd]	75.30[bc]	78.51[a]	0.761	0.0011
ME	3029[d]	3219[ab]	3069[cd]	3163[bc]	3298[a]	31.949	0.0011

CF, crude fibers; CP, crude protein; DM, dry matter; EE, ether extract; ME, metabolizable energy; NFE, nitrogen-free extract; OM, organic matter; POE, *Portulaca oleracea* extract; TDN, total digested nutrients.
[a-d]means in the same row within each classification bearing different letters significantly differ.

Table 8. Effects of dietary POE supplementation on liver and kidney functions in growing Japanese quails.

Items	POE (mL/kg diet) 0	1	2	3	4	SEM	*P* value
TP (g/dL)	3.01[c]	3.36[cb]	3.63[ab]	3.98[a]	3.55[ab]	0.123	0.0052
ALB (g/dL)	1.67	1.69	2.01	2.13	1.86	0.174	0.3730
GLOB (g/dL)	1.34[c]	1.67[b]	1.62[b]	1.85[a]	1.70[ab]	0.051	0.0007
A/G (%)	1.25	1.02	1.25	1.15	1.11	0.142	0.7600
AST (IU/L)	151.70	141.05	123.95	136.40	155.9	10.335	0.2982
ALT (IU/L)	8.15[ab]	9.35[a]	5.81[bc]	4.30[c]	9.23[a]	0.812	0.0057
Creatinine (mg/dL)	0.63	0.53	0.57	0.59	0.46	0.049	0.3914
Urea (mg/dL)	4.95[a]	2.81[b]	3.06[b]	3.21[b]	2.17[b]	0.386	0.0149

A/G, albumin/globulin ratio; ALT, alanine transaminase; AST, aspartate aminotransferase; POE, *Portulaca oleracea* extract; TP, total protein; ALB, albumin; GLOB, globulin.
[a-c]means in the same row within each classification bearing different letters significantly differ.

Table 9. Effects of dietary POE supplementation on lipid profile of growing Japanese quails.

Items	POE (mL/kg diet) 0	1	2	3	4	SEM	*P* value
TC (mg/dL)	282.39[a]	294.75[a]	206.02[b]	223.45[b]	189.40[b]	10.775	0.0002
TG (mg/dL)	247.85[a]	254.40[a]	166.85[b]	81.07[d]	105.51[c]	7.490	<0.0001
HDL (mg/dL)	34.07	41.12	36.50	47.92	50.74	4.155	0.0759
LDL (mg/dL)	198.76[a]	202.75[a]	136.15[bc]	159.32[b]	117.56[c]	9.578	0.0003
VLDL (mg/dL)	49.57[a]	50.88[a]	33.37[b]	16.21[d]	21.10[c]	1.498	<0.0001

HDL, high-density lipoproteins; LDL, low-density lipoproteins; POE, *Portulaca oleracea* extract; TC, total cholesterol; TG, triglycerides; VLDL, very low-density lipoproteins.
[a-d]means in the same row within each classification bearing different letters significantly differ.

thus reflecting the positive ability of birds' bodies to scavenge free radicals.

Intestinal Microbial Load

Table 11 shows that all bacterial counts in cecal samples reduced with increasing the purslane addition level (1, 2, 3, and 4 mL/kg diet). The reductions were 74, 66, 45, 49, and 63% for TBC, TYMC, *E. coli*, Coliform, and *Enterococcus* spp., respectively, compared to control. No *Salmonella* count was detected in purslane treatments. However, the LAB count increased by 70% compared to the control.

DISCUSSION

Plants include various substances, including phenolic compounds and flavonoids (phenolic acids, lignins, stilbenes, and terpenoids) (El-Saadony et al., 2022c). The phenolic content was predicted to increase to 26% by increasing the concentration to 1 mg/mL (Figure 1A) compared to 4% in the study of Cai et al. (2004). At the same time, Uddin et al. (2012) observed that ethanol extract of Purslane is rich in phenolic contents while the content of total phenolics in Purslane was (0.7g/100 g) flavonoids (0.6 g/100 g) in the investigation of Alu'datt et al., 2019.

Table 10. Effects of dietary POE supplementation on immunity and antioxidant status of growing Japanese quails.

Items	POE (mL/kg diet) 0	1	2	3	4	SEM	*P* value
Immunity							
IgM (mg/dl)	0.61[c]	0.82[b]	0.84[b]	1.01[a]	0.86[b]	0.043	0.0018
IgA (mg/dl)	0.72[b]	0.88[a]	0.92[a]	0.99[a]	0.85[a]	0.039	0.0102
Complement 3	77.71[c]	58.23[c]	109.77[b]	132.40[a]	138.65[a]	6.825	<0.0001
Lysozyme	0.28	0.30	0.32	0.40	0.22	0.034	0.0570
Antioxidants							
SOD (U/mL)	0.42[c]	0.48[c]	0.51[bc]	0.60[b]	0.81[a]	0.032	0.0001
MDA (nmol/mL)	0.36[a]	0.29[ab]	0.25[b]	0.15[c]	0.16[c]	0.025	0.0008
TAC (ng/ml)	0.28[c]	0.41[bc]	0.31[c]	0.55[b]	0.75[a]	0.046	0.0002
GSH (ng/ml)	0.43[b]	0.38[b]	0.42[b]	0.52[ab]	0.62[a]	0.044	0.0250
CAT (ng/ml)	0.27[c]	0.42[b]	0.45[ab]	0.54[a]	0.37[bc]	0.036	0.0039

IgA, Immunoglobulin A; IgM, Immunoglobulin M; CAT, catalase; GSH, Glutathione; MDA, malondialdehyde; POE, *Portulaca oleracea* extract; SOD, superoxide dismutase; TAC, total antioxidant capacity.
[a-c]means in the same row within each classification bearing different letters significantly differ.

Table 11. Cecal microbiota in Japanese quail (Log CFU/g) as affected by different purslane treatments.

	Log CFU/mL						
POE (mL/Kg diet)	TBC	TYMC	*E. coli*	Coliform	*Salmonella*	*Enterococcus*	LAB
0	8.7[a]	3.5[a]	5.5[a]	6.1[a]	1.2	5.2[a]	4.3[c]
1	7.5[b]	3[b]	5[b]	5.8[b]	ND	4.8[b]	5.1[b]
2	6.4[c]	2.8[c]	4.4[c]	5.3[c]	ND	4.2[c]	6.2[ab]
3	5.9[d]	2.4[d]	4.1[d]	4.9[d]	ND	3.8[d]	6.8[b]
4	5.0[e]	2.1[e]	3.8[e]	4.1[e]	ND	3.2[e]	7.2[a]

Boldface indicates the following, LAB, lactic acid bacteria count; TBC, total bacterial count; TYMC, total yeasts and moulds count.
Purslane extract (POE).
[a-e]different lowercase letters in the same column indicate significant differences.

The antioxidant effect of Purslane is because of its high phenolic compounds (Youssef and S.M., 2014). Purslane extract (**PE**) had the maximum level of DPPH and ABTS radical scavenging activity, following Wang et al. (2021). The IC_{50} values for DPPH and ABTS radicals were 5.112 and 12.607 g/ mL, respectively. Purslane hexane extract showed considerable antimicrobial activity versus *Bacillus subtilis, Candida albicans, Escherichia coli, Enterobacter cloacae, Shigella dysenteriae, Salmonella typhi,* and *Staphylococcus aureus* with average IZDs of 25 to 28 mm, also exhibited a high level of minimum inhibition concentration (125−250 μg/mL) against *Bacillus subtilis, Candida albicans, Enterobacter cloacae, Salmonella typhi, Shigella dysenteriae, Escherichia coli, Streptococcus agalactiae*, and *Staphylococcus aureus* except for *Klebsiella Pneumonia, Micrococcus luteus*, and *Pseudomonas aeruginosa* (Ojah et al., 2021).

According to Table 3, POE increased quail BW and BWG. This improvement may be attributable to certain polyphenols found in Purslane, which control key metabolic processes for glucose homeostasis in the liver and carbohydrate metabolism, including glycolysis, glycogenesis, and gluconeogenesis, typically compromised in diabetes. Also, Purslane is a good source of omega-3 fatty acids, which greatly affects the development and avoidance of different cardiovascular disorders and keeps the immune organs healthy (Uddin et al., 2014). In our study, Purslane supplementation promoted and improved BWG. These conflicts with Kartikasari et al. (2017) found that the chickens' BW was not affected by Purslane supplementation. At the same time, Zhao et al. (2013) illustrated that adding 2% to broilers' diet improved BWG and FCR. Ghorbani et al. (2014) proposed a different view and revealed that the broiler chicks fed on diets supplemented with purslane extract did not improve FCR. Also, Shehata and Raga E.Abd, 2011 showed that FI recorded the highest values in hens fed 10% dietary purslane leaves.

The increase in FI may be due to enhanced palatability and physical characteristics of the purslane leave diets. The beneficial effect of POE on birds' BWG and may be due to altered postabsorptive metabolic use of nutrients or enhanced nutritional digestibility and absorption (Habibian et al., 2019). While FI and the FCR of broiler chickens supplied on a diet with 2,500 or 7,500 mg/kg of dried Purslane powder reduced without affecting broiler chickens' BWG during the period (0−43) days of age (Sadeghi et al., 2016). On the other hand, Elhussein et al. (2015) showed a substantial decrease in FI and BWG in broiler chickens fed a ration including 20,000 mg/kg of dried purslane powder than the control group during the period (1−42) days of age. This difference can be caused by variations in the chemical makeup of purslane samples and varying dosing levels. Other elements must also be considered, like bird age and sex, dietary contents, genetic background, and experimental circumstances (Habibian et al., 2019).

Our results demonstrated that purslane supplementation did not affect birds' carcass trait weight. These results follow Konca et al. (2015), who illustrated that slaughter weight, carcass, and carcass cut yields of quails were not affected by supplementation of 10% purslane seed. In addition, carcass characteristics were not influenced in broiler chickens fed on a diet containing dried purslane powder (Sadeghi et al., 2016). On the contrary, El-Hashash et al. (2020) showed a significant lowering in rats' hepatic and renal weight. The considerable effect in the current study on pH intestine conflicts with Zhao et al. (2013) found that purslane extract did not change pH values of ileum and cecum contents.

The positive significance of some digestion coefficients, especially in treatment group 4, may be due to the raising of the digestive enzymes (amylase and lipase) in the treated groups. Intestinal development, pancreatic digestive enzyme activity, intestinal nutrient digestion, and growth rate in broiler chickens are all favorably correlated with intestinal mucosal antioxidant capacity, according to Wu et al. (2016). These conclusions were confirmed in the current study, where POE-supplemented birds showed more notable BWG and antioxidant capacity improvements than untreated birds.

The current study shows that AST levels were not affected while ALT levels are affected and raised by POE supplementation. Kamel et al. (2017) proposed a different view, who stated that purslane seeds extract reduced liver enzyme activity. Also, Shanshan and EL Bushuty (Shanshan and D.H.E., 2020) found that rats treated with Purslane seeds powder had lower levels of AST and ALT enzymes. Furthermore, according to Soo-Jung et al. (2011), the Purslane powder-treated groups showed significantly lower AST, ALT, and ALP activity.

The antioxidant factors rising from feeding quail birds a diet supplemented with POE might decrease birds' serum urea. Purslane constituents such as flavonoids (quercetin), ascorbic acid, omega-3, B-carotene and glutathione have antioxidant activity (Ghahramani et al., 2016). These findings follow the results of Shirwaikar et al. (2003) illustrated that the reduced uric acid levels in the *Portulaca oleracea*-treated animals might be because of its antioxidant effects.

The POE supplementation reduced the harmful levels of TC, TG, LDL, and VLDL. This may be due to the findings obtained by Habibian et al. (2019). Purslane's lipid-lowering properties are most likely due to synergism between its active ingredients or a simple combination of each component's actions. Also, there was found that Melatonin in purslane extract plays a role in the noted anti-obesity and antidiabetic. In addition, omega-3 fatty acids in Purslane can lower the thickness of the blood and may be beneficial in the treatment of vascular disorders (Liu et al., 2000). These findings partially run parallel with Habibian et al. (2019), who observed that purslane powder, aqueous extract, or methanolic purslane extract to broilers diet led to lower plasma concentrations of TC, LDL, and TG and higher plasma levels of HDL. In addition, a significant reduction was recorded in serum TC of rabbits fed on purslane extract (Movahedian et al., 2007).

This study refers to improving birds' immunity treated with Purslane seed extract. While others have recommended that other substances like saponins, proteins, amino acids, melatonin, vitamin C, vitamin E, and trace mineral contents may also donate to the antioxidant effect of this plant, Purslane contains phenolic constituents involving flavonoids, phenolic acids, and alkaloids, which are related to the antioxidant promoters (Uddin et al., 2012). Glutathione and coenzyme Q10 are also abundant in Purslane (Okafor et al., 2014). The stomach absorbs the feed's glutathione in its whole, which serves as a substrate for GPx in animal cells (Simopoulos et al., 1992). As a crucial element of the respiratory chain in the inner mitochondrial membrane, coenzyme Q10 not only serves as an electron and proton carrier and fuels ATP synthesis, but it can also act as an important antioxidant in its reduced form (ubiquinol) to prevent the buildup of free radicals, especially reactive oxygen intermediates, and diminish the per-oxidative hazard to the body (Geng and Y.M., 2005).

These data concur with those of Habibian et al. (2017). They discovered that purslane supplementation boosted liver SOD, CAT, and GPx activities and decreased plasma and hepatic MDA levels close to control levels at 24 and 49 d of the trial. Ghorbani et al. (2014) offered an alternative perspective, claiming that supplementing with purslane extract did not affect broiler immunity. A significant appositive effect on antioxidants (SOD, TAC, GSH, and CAT) is a good indicator for birds' healthy status due to antioxidants status improvement. These findings follow the results of many researchers who stated that purslane inclusion in the diet has hypolipidemic properties (Movahedian et al., 2007) and can improve broiler chicken antioxidant status (Ghorbani et al., 2013; Habibian et al., 2019).

The current study showed that POE supplementation significantly affected the microbial intestinal load. This might be a mirrored image of enhancing the birds' growth performance. Minimizing the antibacterial load reflects the scientific fact that Chan et al. (2000) stated that Purslane has antibacterial, antifungal, and anti-inflammatory properties. These findings are the same opinion of Zhao et al. (2013) noticed that using *Portulaca oleracea* decreased *E. coli* count in gastrointestinal tract compared to the control group. This reason results in an improvement in intestinal microecology. Also, Wang et al, (2021) found that the gut microbiota of Sanhuang broilers was largely consisted of *Gallibacterium, Bacteroides,* and *Escherichia-Shigella*, among other bacteria, according to microbial diversity study. *Lactobacillus* counts increased considerably as purslane concentration increased, whereas *Escherichia-Shigella* counts decreased. The counts of Bacteroides, caecigallinarum, Lachnospiraceae, Lactobacillales, and Firmicutes differed significantly from the control group. These findings show that consuming purslane may increase the amount of *Lactobacillus* in the colon, alter the habitat of gut microbiota, and accelerate glucose metabolism, all of which may boost growth performance.

CONCLUSIONS

The *Portulaca oleracea* extract (**POE**) supplementation improved quails' performance and nutritive values for some substances (CF, DM, OM, NFE, TDN, and ME). Moreover, POE did not cause any harmful effects on birds' liver and kidney functions. In addition, this extract promotes the immunity and antioxidant status and minimizes the harmful microbial load in the quails' intestines, so we recommend using POE as dietary supplementation.

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DISCLOSURES

The authors declare that there is no conflict of interest in this paper.

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