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Running title: **Saffron–Biochar Effects in Quails**

Dietary supplementation of saffron petal extract and biochar synergistically improves performance, gut morphology, antioxidant status, and reduces ammonia emission in Japanese quails (*Coturnix coturnix japonica*)

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ABSTRACT

Biochar, owing to its highly porous structure and strong adsorption capacity, has the potential to enhance gut health and nutrient utilization. Saffron (*Crocus sativus L.*) petal extract is rich in phenolic compounds and flavonoids and exhibits pronounced antioxidant and immunomodulatory properties. However, the potential synergistic effects of these two feed additives in poultry nutrition have not been fully elucidated. The present study evaluated the combined effects of dietary biochar and saffron petal extract on growth performance, intestinal morphology, antioxidant status, and lipid metabolism in Japanese quails (*Coturnix coturnix japonica*). A total of 500 quails were randomly allocated to five dietary treatments, each with five replicates of 20 birds per replicate: a control diet, probiotic-supplemented diet, biochar-supplemented diet, saffron petal extract-supplemented diet, and a combined biochar plus saffron petal extract diet. Growth performance indices, serum biochemical parameters, intestinal histomorphology, antioxidant markers, and ammonia emissions were assessed. Combined supplementation with biochar and saffron petal extract significantly improved ($P < 0.05$) body weight gain, FCR and cecal villus height compared with the control and probiotic-fed groups. Moreover, this group showed higher glutathione (GSH) and superoxide dismutase (SOD) compared to other treatments ($P < 0.05$). Additionally, combined diet decreased ammonia emissions at 24 and 48 h, suggesting improved environmental sustainability. The serum lipid profile was also favorably modulated in all additive treatments compared to the control, as evidenced by increased HDL concentrations and reduced triglyceride, LDL and total cholesterol levels. Cecal *Coliforms* and *Salmonella* populations were decreased ($P < 0.05$) in all additive-supplemented groups without any significant changes in total bacterial counts. In conclusion, dietary supplementation with biochar and saffron petal extract exerted synergistic effects on

intestinal morphology, antioxidant defense, and lipid metabolism, ultimately resulting in enhanced growth performance and physiological health in Japanese quails.

Keywords: Biochar, Saffron petal extract, Japanese quail, Gut health, Emission.

INTRODUCTION

The poultry industry plays a critical role in supplying high-quality animal protein to meet the demands of a growing global population (Abudabos et al., 2018). However, increasing concerns regarding antimicrobial resistance and consumer demand for antibiotic-free products have intensified the search for natural alternatives to antibiotic growth promoters in poultry nutrition (Cimrin et al., 2020). Consequently, feed additives that support gut health, immune function, and growth performance without compromising food safety have gained increasing attention.

Among these alternatives, biochar and phytochemical extracts have shown considerable potential. Biochar was introduced as a feed additive in poultry production in recent years and has been proposed as a natural alternative to antibiotics due to its high adsorption capacity, porous structure, and ability to stabilize the gastrointestinal environment (Schmidt et al., 2019; Willson et al., 2019). Biochar can adsorb toxins, reduce antinutritional factors, and promote the growth of beneficial gut microbiota, thereby improving nutrient utilization and feed efficiency (Hien et al., 2018; Man et al., 2021). In addition, biochar has demonstrated the ability to bind mycotoxins such as aflatoxin B1 in the gastrointestinal tract, mitigating their adverse effects on health and performance (Rashidi et al., 2020). These properties support its potential role as a functional feed additive in poultry diets.

Phytogenic feed additives, particularly medicinal plants and their extracts, represent another promising group of antibiotic alternatives. These compounds possess antimicrobial, antioxidant, and immunomodulatory properties and can enhance gut microbial balance without leaving harmful residues in poultry products (Andrew Selaledi et al., 2020). Saffron (*Crocus sativus L.*) is a medicinal plant rich in bioactive compounds, including safranal and carotenoid pigments such as crocin, which exhibit strong antioxidant activity (Cowieson and Kluefer, 2019). Experimental studies have shown that saffron-derived compounds reduce lipid peroxidation and improve oxidative stability in biological tissues (Daneshmand et al., 2012).

In poultry, supplementation with saffron petal extract has been associated with improved immune responses and increased relative weights of immune organs, including the bursa of Fabricius and spleen, in Japanese quails (Hosseini-Vashan et al., 2018; Sheikh et al., 2023). These effects have been attributed to the high content of phenolics, flavonoids, tannins, and anthocyanins in saffron petals, which contribute to enhanced antioxidant defense and immune modulation (Srivastava et al., 2010).

Japanese quail (*Coturnix coturnix japonica*) is a well-established experimental model in poultry nutrition and physiology due to its rapid growth, short generation interval, and sensitivity to dietary manipulation. Despite growing evidence supporting the individual benefits of biochar and saffron petal extract, information regarding their combined effects on growth performance, gut morphology, antioxidant status, and lipid metabolism in quails is limited. The potential synergistic interaction between biochar's adsorptive and gut-modulating properties and the bioactive compounds of saffron petals has not been adequately explored.

Therefore, the present study was conducted to test the hypothesis that dietary supplementation with biochar and saffron petal extract, individually or in combination, would

improve growth performance, intestinal morphology, antioxidant capacity, and gas emission through modulation of gut health in Japanese quails.

MATERIALS AND METHODS

Saffron Petal Extraction

Saffron petal extract was prepared according to the method described by Vakili et al. (2022), with standardization of bioactive compounds performed as previously reported. Prior to the experiment, saffron petals containing residual cream and stigma were shade-dried and finely ground. The powdered material was extracted using 50% ethanol at a solid-to-solvent ratio of 1:10 for 2 h. The extract was subsequently concentrated, and the solvent was removed, followed by spray drying, sieving, and packaging. The dried extract was blended with powdered calcium carbonate as a carrier using a Rapid Mill (ceramic pot with ceramic balls) operated at 350 rpm. To ensure uniform distribution, the prepared supplement was initially premixed with 10 kg of the basal diet using a small mixer and then incorporated into the total diet using a horizontal mixer (Kalus et al., 2020). Chemical analysis of the active ingredients of saffron petal extract is reported in Table 1.

Preparation of Pistachio Byproduct Biochar

Pistachio byproduct (PB) was obtained from a pistachio dehulling factory located in Mah Velayat, Khorasan Razavi Province, Iran. The raw material was air-dried and ground to pass through a 2-mm sieve. Biochar was produced via pyrolysis as described by Mirheidari et al. (2020). Briefly, the dried pistachio byproduct was wrapped in aluminum foil to prevent oxygen exposure and placed in an electric furnace. Pyrolysis was conducted at 550°C for 3 h under oxygen-limited

conditions. The elemental composition of the resulting biochar was determined using a carbon–hydrogen–nitrogen–sulfur (CHNS) analyzer (Thermo Finnigan, Flash EA 1112 Series) to ensure compliance with the standards of the European Biochar Institute (2012). The chemical characteristics of the pistachio byproduct biochar are summarized in Table 2.

Experimental Design and Animal Management

All experimental procedures were conducted in accordance with the guidelines of the Iranian Council of Animal Care (1995) and were approved by the Research Animal Ethics Committee of the Saffron Institute, University of Torbat Heydarieh, Iran (approval code: 167788; approved on 14 August 2023). The study was also performed in compliance with the National Institutes of Health and ARRIVE guidelines for animal research (Percie du Sert et al., 2020). Financial support for this research was provided by the Saffron Institute, University of Torbat Heydarieh (grant no. P/167788).

A total of 500 one-day-old Japanese quail chicks were obtained from a commercial hatchery (Pishgaman Belderchin) and randomly allocated to five dietary treatments with five replicates per treatment and 20 birds per replicate. The experiment lasted 35 days. Birds were reared in environmentally controlled facilities under standard management conditions, with ad libitum access to feed and water throughout the experimental period.

The dietary treatments consisted of: (1) a basal diet without additives (control), (2) basal diet supplemented with 1% probiotic (Protexin), (3) basal diet supplemented with 1% pistachio byproduct biochar, (4) basal diet supplemented with 500 mg/kg saffron petal extract, and (5) basal diet supplemented with 1% pistachio byproduct biochar plus 500 mg/kg saffron petal extract. The inclusion level of biochar was selected based on previous findings indicating that moderate inclusion levels (0.5–1.0%) are optimal and prevent excessive binding of essential nutrients

(Schmidt et al., 2019). All diets were formulated to meet or exceed the nutrient requirements of Japanese quails as recommended by the NRC (1994). The ingredient composition of the experimental diets are presented in Table 3. The basal diet was prepared using common feed ingredients, and the experimental treatments were obtained by supplementing the basal diet with biochar or probiotic at a level of 1% of the diet. Biochar and probiotic were added as top-dressing to the basal diet and thoroughly mixed to ensure uniform distribution in the feed.

Measurement and sampling

Representative samples of the experimental diets were collected and analyzed for crude protein (CP; method 990.3, AOAC, 2005) calcium (Ca; method 934.01, AOAC, 2005) and phosphorus (P; method 965.17, AOAC, 2005). Feed intake was recorded daily by weighing the offered feed and refusals. Body weight (BW) was measured weekly, and BW gain was calculated for each replicate. Feed conversion ratio (FCR) was calculated as the ratio of feed intake to body weight gain. Growth performance was evaluated during three periods: starter (1–21 d), grower (21–35 d), and the overall experimental period (1–35 d). Birds had continuous access to feed and water, and daily observations were conducted to monitor health status and mortality, with any losses recorded and accounted for in performance calculations. At the end of the experiment (day 35), five birds per each replicate were euthanized by CO₂ inhalation and the weight of carcass, liver and gizzard organs were measured accordingly.

Humoral immune responses

Humoral immune response was evaluated using sheep red blood cells (SRBC) as the antigen, following the procedure described by Elnaggar et al. (2022a). Fresh blood (20 mL) was collected from two Baluchi sheep into tubes containing EDTA. Red blood cells were washed three

times with phosphate-buffered saline (PBS), and a 7% SRBC suspension was prepared in PBS. At 28 d of age, quails were injected intravenously via the wing vein with 0.005 mL of the 7% SRBC suspension. Seven days post-injection (35 d of age), blood samples (3 mL) were collected from the wing vein, and sera were separated and stored at -4°C until antibody titers were determined.

Blood parameters

At the end of the experimental period, two quails were randomly selected from each replicate, with body weights close to the replicate mean. Birds were euthanized by jugular vein severance, and blood samples were collected into plain tubes. Samples were centrifuged at 3,000 rpm for 10 min to obtain serum. Serum samples were analyzed for biochemical parameters, including glucose, triglycerides, high-density lipoprotein (HDL), and low-density lipoprotein (LDL). Immunoglobulin concentrations (IgM, IgA, and IgG) were determined using commercial ELISA kits (Pars Azmoun, Iran) and read using an ELISA reader (Greiner, Nürtingen, Germany), as described by Elnaggar and El-Kelawy (2018).

Intestinal microbial population

At slaughter (day 35), two quails per replicate were randomly selected, and cecal samples were aseptically collected for microbial analysis. The abdominal surface was disinfected, and ceca were excised using sterile instruments and transferred to sterile nylon bags for laboratory analysis. Cecal contents (0.5 g) were homogenized in sterile saline solution using a 1:10 serial dilution technique (Masouri et al., 2017). Samples were vortexed thoroughly, and appropriate dilutions were plated for bacterial enumeration. *Lactobacillus* spp. were cultured on de Man, Rogosa, and Sharpe (MRS) agar; *Escherichia coli* on eosin methylene blue (EMB) agar; *Campylobacter* spp. on modified charcoal cefoperazone deoxycholate (MCCD) agar; total aerobic bacteria on nutrient

agar; and *coliforms* on MacConkey agar (Gahruie et al., 2020). Results were expressed as \log_{10} colony-forming units (CFU)/g of cecal content.

Evaluation of Antioxidant Status and Ammonia Emission

Assessment of Oxidative Stress Biomarkers

At the end of the experimental period, blood samples were collected via brachial venipuncture into plain tubes and centrifuged at $3,000 \times g$ for 10 min at 4°C to obtain serum (Pourmollaie et al., 2025). Serum samples were transferred into microtubes and stored at -80°C until analysis.

Oxidative stress biomarkers were evaluated by measuring malondialdehyde (MDA), total antioxidant capacity (TAC), glutathione (GSH), and superoxide dismutase (SOD) activity using commercial colorimetric assay kits (ZellBio GmbH, Germany), according to the manufacturer's instructions (Tomsič et al., 2016). Lipid peroxidation was assessed using the thiobarbituric acid reactive substances (TBARS) method, with absorbance read at 532 nm and results expressed as $\mu\text{mol/L}$ MDA equivalents (Placer et al., 1966).

Total antioxidant capacity was determined using the ferric reducing antioxidant power (FRAP) assay and expressed as $\mu\text{mol Trolox equivalents/L}$. Glutathione concentration was measured using the DTNB recycling method at 412 nm, and SOD activity was determined based on inhibition of superoxide radicals generated by the xanthine oxidase system, expressed as U/mL. All analyses were performed in duplicate, and inter- and intra-assay coefficients of variation were maintained below 10%.

Ammonia Emission Measurement

Ammonia (NH₃) emission from excreta was evaluated during the final week of the experiment following the method described by Kalus et al. (2020), with minor modifications. Fresh excreta samples (approximately 200 g) were collected immediately after defecation from each replicate and placed into airtight plastic zipper bags. Each bag was subsequently enclosed in a 1-L sealed plastic container equipped with two openings: one fitted with a gas-permeable membrane to equalize pressure and the other used for gas sampling.

Samples were incubated at room temperature ($25 \pm 1^\circ\text{C}$), and ammonia concentration was measured at 0 h (baseline) and 24 h and 48 h using a hand-operated gas sampling pump (AP-20; Gastec Corp., Kitagawa, Japan) connected to ammonia detector tubes (3LA, 3M). Ammonia concentrations were read directly from the detector tube scale and expressed as parts per million (ppm) per 100 mL of headspace air. All measurements were performed in triplicate for each dietary treatment, and emission data were used to assess treatment effects on nitrogen volatilization and environmental impact.

Histological Processing and H&E Staining

Cecal and ileal samples were collected, gently flushed with 0.1 M PBS (pH 7.2) to remove digesta, and immediately fixed in 10% neutral-buffered formalin for 24–48 h. Fixed tissues were dehydrated through a graded ethanol series, cleared in xylene, and embedded in paraffin wax. Sections of 5 μm thickness were prepared using a rotary microtome and stained with hematoxylin and eosin (H&E) following standard histological protocols.

Histological slides were examined under a light microscope (Olympus BX53, Japan) equipped with an image analysis system (Motic Images Plus 2.0). Morphometric parameters, including villus height (measured from the villus tip to the villus–crypt junction), villus width (measured at the midpoint), crypt depth (measured from the base of the crypt to the villus–crypt

junction), and villus height to crypt depth (VH:CD) ratio, were determined. For each sample, at least ten well oriented villi and their associated crypts were measured to obtain representative mean values, as described by Cui et al. (2005).

Statistical analysis

All data were analyzed using the General Linear Model (GLM) procedure of SAS software (version 9.1; SAS Institute Inc., Cary, NC, USA). The experiment followed a completely randomized design with dietary treatment as the fixed effect. Differences among treatment means were evaluated using one way analysis of variance (ANOVA). When a significant treatment effect was detected, means were separated using Tukey's honestly significant difference (HSD) test. The statistical model used was:

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

Where, Y_{ij} is the observed value, μ is the overall mean, T_i is the fixed effect of dietary treatment, and e_{ij} is the residual error.

Statistical significance was declared at $P < 0.05$. Graphical representations were generated using GraphPad Prism software (version 9.0; GraphPad Software Inc., San Diego, CA, USA), and results are presented as means \pm standard error of the mean (SEM).

RESULTS

Performance and carcass attributes

The effects of dietary supplementation with saffron petal extract and biochar on growth performance of Japanese quails are presented in Table 4. Dietary treatments significantly affected BW

gain and FCR over the entire period ($P < 0.05$). Body weight gain improved in quails fed saffron petal extract- and biochar plus saffron petal extract-diets compared to other treatments without affecting feed intake and FCR during 1-21 days of old. However, feed intake, growth performance and FCR were not affected by additives inclusion during 21-35 days. At the age of 21-35 days, quails fed the combined diet containing 1% PB biochar + 500 mg/kg saffron petal extract exhibited greater body weight gain (273 g) and improved FCR (2.75) compared with the control and probiotic-fed groups ($P < 0.05$). Other single additive-supplements improved BW gain and FCR compared to the control group during the 1-35 days.

Relative carcass yield and organ weights are summarized in Table 5. Dietary treatments did not significantly affect the relative weights of the carcass, liver, or gizzard ($P > 0.05$). In contrast, intestinal digesta pH was significantly reduced in birds receiving diets containing saffron petal extract or biochar alone compared to the control and probiotic-fed groups ($P < 0.05$).

Humoral immune response

Humoral immune parameters are presented in Table 6. Dietary treatment significantly influenced serum immunoglobulin concentrations ($P < 0.05$). Additive supplementation significantly increased serum IgG and IgM levels compared to the control with most marked in birds fed the combined biochar and saffron petal extract diet ($P < 0.05$). Conversely, serum IgA concentration was highest in the control group ($P < 0.05$).

Blood parameters

Serum biochemical indices are reported in Table 7. All dietary additive supplementation significantly increased serum total protein and albumin, concentrations compared with the control group, while serum urea concentration was reduced ($P < 0.05$).

Creatinine concentration was significantly lower in quails fed the probiotic-supplemented diet compared with other treatments ($P < 0.05$). The albumin to globulin (A/G) ratio differed significantly among dietary treatments, with the lowest value recorded in the biochar plus saffron petal extract group ($P < 0.05$).

Serum Lipid Profile

Serum lipid parameters are presented in Table 8. Dietary treatments significantly affected all lipid indices in Japanese quails ($P < 0.0001$). Birds receiving diets supplemented with probiotic, saffron petal extract and/or biochar exhibited significantly lower serum concentrations of total cholesterol, triglycerides, LDL, and VLDL compared with the control group ($P < 0.05$). In contrast, HDL concentrations were significantly higher in all additive-supplemented groups relative to the control ($p < 0.05$).

Intestinal microbial population

The effects of dietary treatments on cecal microbial populations are shown in Figure 1. All experimental diets containing biochar and/or saffron petal extract significantly reduced *coliforms*, and *Salmonella* populations, compared with the control group ($P < 0.05$). Compared to the control group, the probiotic-, biochar-, and saffron petal extract-supplemented groups exhibited the higher counts of *Lactobacillus* ($P < 0.05$). *E. coli* populations was also decreased in birds fed probiotic or combined supplementation with biochar and saffron petal extract ($P < 0.05$).

Antioxidant enzymes

Serum antioxidant indices, including TAC, MDA, GSH, and SOD, are illustrated in Figure 2. Quails fed diets supplemented with PB biochar combined with saffron petal extract exhibited significantly higher GSH and SOD activities compared with the control group ($P < 0.01$). Birds

receiving biochar and/or saffron petal extract showed a trend ($P < 0.07$) for higher TAC, compared with the control.

Ammonia Emission

Ammonia emission measured after 24 and 48 h is presented in Figure 3. Dietary biochar plus saffron petal extract supplementation significantly reduced ammonia release at both time points compared with the control ($P < 0.05$).

Intestinal Morphology

Ileal and cecal morphometric parameters, including villus height, villus width, crypt depth, and VH:CD ratio, are presented in Figure 4. Birds receiving diets supplemented with biochar plus saffron petal extract exhibited significantly increased cecal villus height compared with the control group ($P < 0.05$). A trend for deeper cecal crypt depth was also observed in quails fed a combined additives compared with the control ($P < 0.10$). However, ileal and cecal villus width and VH:CD were not affected by dietary treatments ($P > 0.05$). The saffron petal extract-supplemented group showed a tendency for improvement in ileal villus height and crypt depth compared with the control ($P < 0.10$).

Representative H&E micrographs

Intestinal Morphology

Representative hematoxylin and eosin (H&E)-stained ileal sections from quails fed the experimental diets are presented in Figure 5. Histological examination revealed clear differences in intestinal structure among dietary treatments. Quails in the control group exhibited shorter, irregularly arranged villi. Birds fed diets containing PB biochar and saffron petal extract, either alone or in combination, displayed more pronounced improvements in ileal morphology,

characterized by taller and more slender villi. The combined biochar plus saffron petal extract treatment exhibited the most developed villus structure, with greater villus height and a higher VH:CD ratio compared with the control and other treatments.

DISCUSSION

The enhancement of growth efficiency observed with combined supplementation likely reflects improved nutrient utilization rather than simple stimulation of feed intake. Phytogetic compounds derived from saffron petals contain biologically active constituents such as crocin, flavonoids, and phenolic acids, which have been associated with improved digestive function, metabolic efficiency, and antioxidant protection in poultry (Elnaggar et al., 2022b; Sheikh et al., 2023). Notably, responses to phytogetic additives are not always mediated through changes in feed intake. For example, Tufarelli et al. (2023) reported that dietary peppermint powder did not influence feed intake in broiler chickens, suggesting that improvements in performance parameters associated with phytogetic supplementation may result primarily from enhanced nutrient digestibility, metabolic modulation, or gut health rather than increased consumption. On the other hand, a combination of the peppermint essential oil and the probiotics linearly reduced feed intake in broilers (Gahruie et al., 2020; Hosseini-Vashan and Piray, 2021). Variability in growth responses to phytogetic additives reported in the literature (Amprako et al., 2018; Hosseini-Vashan and Piray, 2021) further emphasizes the importance of botanical source, inclusion level, and dietary context.

Biochar may exert complementary effects through physicochemical mechanisms. Due to its porous structure and high adsorption capacity, biochar can bind luminal toxins, excess nitrogen compounds, and microbial metabolites, thereby stabilizing intestinal conditions and reducing

metabolic burden (Schmidt et al., 2017). Previous studies have demonstrated that dietary biochar can support growth performance, particularly during early developmental stages or under dietary challenges (Fouladi et al., 2018; Zahed et al., 2025). The functional properties of biochar, however, depend on factors such as pyrolysis temperature, mineral composition, and ash content (Giannenas et al., 2011), which may explain inconsistencies among published findings. In the present context, the complementary metabolic effects of saffron bioactives and the detoxifying properties of PB biochar likely contributed to the observed improvements in growth efficiency.

The absence of detrimental effects on relative organ weights supports the safety of these additives at the tested inclusion levels. Similar findings have been reported in birds receiving antioxidant rich phytochemical compounds or low level biochar supplementation (Sheikh et al., 2023; Bagherzadeh Kasmani et al., 2025; Zahed et al., 2025). Maintenance of organ integrity is particularly important in evaluating novel feed additives, as structural alterations in digestive tissues may indicate metabolic stress or impaired nutrient handling. The present findings therefore reinforce the feasibility of incorporating agricultural byproduct-derived additives into practical poultry diets.

Immune modulation represents another key dimension of phytochemical and carbon-based feed additives. Following antigen presentation, B lymphocytes generate immunoglobulins including IgM, IgG, and IgA, which form the cornerstone of humoral immunity in poultry (Ochsenbein and Zinkernagel, 2000). Enhanced systemic immunoglobulin production in supplemented groups suggests improved immune competence and adaptive responsiveness. Phytochemical antioxidants may support lymphocyte proliferation and antibody synthesis by reducing oxidative stress and preserving cellular integrity (Schmidt et al., 2017). Saffron petals derived

polyphenols have previously been associated with immunomodulatory effects in broilers (Sheikh et al., 2023), and improved nutrient availability may further facilitate immune protein synthesis.

Alterations in immunoglobulin profiles may reflect redistribution of immune activity between systemic and mucosal compartments. Because mucosal immunity is closely linked to gut microbial ecology and epithelial integrity, the interplay between dietary additives, microbiota composition, and immune function warrants further mechanistic investigation. Nonetheless, the immunological responses observed in the present study are consistent with the hypothesis that antioxidant rich phytochemical compounds and detoxifying carbon matrices can jointly enhance immune resilience.

Serum biochemical parameters provide additional insight into metabolic regulation. Elevated globulin concentrations are commonly interpreted as indicators of enhanced immune protein synthesis, whereas reduced urea levels suggest improved nitrogen utilization efficiency and decreased protein catabolism (Mousa et al., 2023). Efficient nitrogen metabolism not only supports growth but may also reduce nitrogen excretion and subsequent ammonia volatilization (Schmidt et al., 2017). Improvements in albumin concentration may further reflect enhanced hepatic synthetic function (Dibner and Ivey, 1999). Collectively, these biochemical shifts are consistent with improved metabolic balance in supplemented birds.

The modulation of lipid metabolism by phytochemical additives has been documented in multiple studies.

Consistent with our results, addition of saffron flower or petal extract to the diet of broilers (Hosseini-Vashan and Piray, 2021) or laying hens (Vakili et al., 2025) decreased the plasma concentration of cholesterol. Dietary flavonoids and other plant-derived polyphenols have been

shown to modulate lipid metabolism in poultry by down-regulating hepatic lipogenic enzymes (e.g., fatty acid synthase and acetyl-CoA carboxylase), enhancing lipid oxidation, and interacting with bile acid synthesis pathways (e.g., CYP7A1) and gut microbiota, thereby influencing cholesterol homeostasis and fat deposition (Chambers et al., 2019; Tan et al., 2022). However, responses to essential oils and plant extracts are influenced by genotype, age, sex, and dietary formulation (Hussein et al., 2023), which may explain variability across experimental conditions. The present findings align with the broader literature suggesting that phytochemical compounds contribute to improved lipid homeostasis, potentially enhancing cardiovascular and hepatic health in poultry. Moreover, Biochar supplementation may attenuate circulating cholesterol concentrations through sequestration of bile acids in the intestinal lumen, thereby interrupting enterohepatic recirculation and stimulating hepatic cholesterol catabolism (Neuvonen et al., 1989). Reduced bile acid reabsorption enhances the hepatic conversion of cholesterol into bile acids, ultimately contributing to decreased serum cholesterol levels (Elghalid et al., 2022; Kramer and Glombic, 2006). Collectively, these hypolipidemic effects suggest that PB biochar may serve as a promising functional feed additive to support metabolic health in poultry.

The gastrointestinal microbiota constitutes a central regulator of nutrient digestion, immune signaling, and pathogen resistance. Probiotics improve microbial balance through competitive exclusion, production of organic acids and bacteriocins, and modulation of host immunity (Bermúdez-Humarán et al., 2024). Similarly, phytochemical extracts may selectively suppress pathogenic bacteria while supporting beneficial microbial populations. Mahfuz et al. (2020) demonstrated favorable microbial shifts in broilers receiving mushroom extracts, including reductions in *Salmonella* populations. In the present study, saffron polyphenols likely exerted antimicrobial and antioxidant effects within the intestinal lumen, while biochar may have provided

a porous matrix capable of adsorbing harmful metabolites and stabilizing luminal pH. The combination of these mechanisms likely contributed to improved microbial homeostasis.

Intestinal morphology represents a structural indicator of nutrient absorption capacity and mucosal health. Villus height and the villus height to crypt depth ratio are widely recognized as markers of epithelial renewal efficiency and absorptive surface area (Cui et al., 2005). Oxidative stress impairs enterocyte proliferation and increases epithelial turnover, whereas antioxidant support preserves mucosal integrity (Liu et al., 2022). Enhanced antioxidant enzyme activity in phytochemical-fed poultry has been documented previously (Kalus et al., 2020), providing a plausible mechanistic link between improved systemic redox balance and enhanced intestinal architecture. Biochar may further support epithelial stability by reducing luminal irritants, binding ammonia, and minimizing exposure to microbial toxins (Kazemi, 2025). The coordinated action of antioxidant protection and detoxification therefore provides a coherent explanation for improvements in intestinal structure and function. Improvement in intestinal morphology (Fig. 4 and 5) along with favorable changes in intestinal microbiome (e.g., reduced *coliforms*, and *Salmonella* populations and higher *Lactobacillus* count) (Fig. 1) in birds receiving diets containing saffron petal extract without or with biochar may indicate that plant-derived additives can promote the integrity of intestinal epithelial cells, which further increase nutrient absorption and growth performance in quails. Comparative trials using other plant-derived materials could identify material-specific effects and optimal compositions.

The lowest NH₃ emission values in birds fed the combined biochar and saffron petal extract diet, indicating a greater reduction in nitrogen volatilization relative to other treatments. These findings are consistent with previous reports indicating that biochar, owing to its extensive surface area and highly porous structure, possesses a strong capacity to adsorb and retain ammonia (Prasai

et al., 2018). The reduction in ammonia emissions associated with biochar application appears to involve multiple mechanisms. Biochar may elevate manure pH, facilitating the shift between ammonium (NH_4^+) and ammonia (NH_3), after which gaseous ammonia can be adsorbed onto biochar surfaces (Agyarko-Mintah et al., 2017). Furthermore, the porous matrix of biochar enhances nitrogen retention by providing abundant adsorption sites, thereby limiting ammonia volatilization and associated odor emissions. Environmental sustainability is an increasingly critical consideration in poultry production. Ammonia emission from manure contributes to air quality deterioration and nitrogen loss. Biochar has been shown to adsorb ammonium ions and potentially inhibit microbial urease activity, thereby reducing nitrogen volatilization (Schmidt et al., 2017). Improved nitrogen retention efficiency may reflect enhanced protein utilization and reduced catabolic waste. The integration of pistachio biochar with saffron petal extract thus represents a strategy not only for improving bird physiology but also for mitigating environmental impact.

Taken together, these findings highlight that the integration of plant-derived antioxidants and agricultural byproduct based biochar offers a sustainable nutritional strategy to enhance intestinal morphology, oxidative resilience, and environmental performance in quail production systems.

CONCLUSION

The present study demonstrates that dietary inclusion of PB biochar and saffron petal extract exerts complementary physiological effects in Japanese quails. Their combined supplementation was associated with improved growth efficiency, enhanced antioxidant defense, modulation of humoral

immunity, favorable shifts in lipid metabolism, stabilization of cecal microbial populations, improved intestinal architecture, and reduced ammonia emission potential. The observed responses appear to be mediated through synergistic mechanisms involving the adsorptive and detoxifying properties of biochar together with the antioxidant and bioactive functions of saffron polyphenols. Improvement in oxidative balance and gut microenvironment likely contributed to enhanced nutrient utilization and immune competence, while reduced nitrogen volatilization highlights the environmental relevance of this strategy. Importantly, the use of saffron petals and PB biochar, both agricultural byproducts, offers a sustainable and residue-free nutritional approach aligned with current efforts to reduce reliance on antibiotic growth promoters. Although the present findings support their practical application in quail nutrition, further research is warranted to evaluate long term safety, dose optimization, and economic feasibility under commercial production conditions. Overall, integration of plant-derived antioxidants with carbon-based feed additives represents a promising multifunctional strategy for improving productive performance, intestinal health, and environmental sustainability in modern poultry systems.

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Authorship contribution statement

RV: Investigation, conducting experiments, laboratory analyses, project administration, supervision, conceptualization, methodology, writing—review. OZ and AM: Conceptualization, data analysis, Review and editing. VP: Writing—original draft and editing. AZM: Methodology,

data analysis, writing—original draft and editing. SE: Methodology. All the authors read and approved the manuscript.

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Data availability

Upon reasonable request, the corresponding author will make the data generated during and/or analyzed during the current study available.

Code availability

Not Applicable.

Conflict of interest

The authors declare no conflicts of interest.

Ethics Approval

This study protocol was approved by the Research Animal Ethics Committee at Saffron Institute, Torbat Heydaryeh University, and was performed in compliance with the guidelines of the Iranian Council of Animal Care (1995). Ethical approval was approved by the Research Animal Ethics Committee of the Saffron Institute (Approval code: 167788).

REFERENCES

- Abudabos, A. M., Alyemni, A. H., Dafalla, Y. M., and Khan, R. U. 2018. The effect of phytochemicals on growth traits, blood biochemical and intestinal histology in broiler chickens exposed to *Clostridium perfringens* challenge. *J. Appl. Anim. Res.* 46(1):691-695. <https://doi.org/10.1080/09712119.2017.1383258>
- Amprako, L., Alhassan, M., Buerkert, A., and Roessler, R. 2018. Influence of dietary wood charcoal on growth performance, nutrient efficiency and excreta quality of male broiler chickens. *Int. J. Livest. Prod.* 9(10):286-292. <https://doi.org/10.5897/IJLP2018.0486>
- Andrew Selaledi, L., Mohammed Hassan, Z., Manyelo, T. G., and Mabelebele, M. 2020. The Current Status of the Alternative Use to Antibiotics in Poultry Production: An African Perspective. *Antibiotics (Basel)*. 9(9):594. <https://doi.org/10.3390/antibiotics9090594>
- Arbestain, M. C., Jones, J. R., Condrón, L. M., and Clough, T. J. 2016. Research and application of biochar in New Zealand. *Agricultural and Environmental Applications of Biochar. Advances and Barriers*. 63:423-443. <https://doi.org/10.2136/sssaspecpub63.2014.0051>
- Association of Official Analytical Chemists (AOAC), 2005. *Official Methods of Analysis*. AOAC international, Gaithersburg, Maryland, USA.
- Bermúdez-Humarán, L.G., Chassaing, B. and Langella, P. 2024. Exploring the interaction and impact of probiotic and commensal bacteria on vitamins, minerals and short chain fatty acids metabolism. *Microb. Cell Fact.* 23(1):172. <https://doi.org/10.1186/s12934-024-02449-3>
- Cimrin, T., Tunca, R.I., Avsaroglu, M.D., Ayasan, T. and Küçükersan, S. 2020. Effects of an antibiotic and two phytochemical substances (cinnamaldehyde and 1, 8-cineole) on yolk fatty acid profile and storage period-associated egg lipid peroxidation level. *Revista Brasileira de Zootecnia*, 49:e20190270. <https://doi.org/10.37496/rbz4920190270>

- Cowieson, A. J., and Kluentner, A. M. 2019. Contribution of exogenous enzymes to potentiate the removal of antibiotic growth promoters in poultry production. *Anim. Feed Sci. Technol.* 250:81-92. <https://doi.org/10.1016/j.anifeedsci.2018.04.026>
- Cui, S., Ge, B. Zheng, J., and Meng, J. 2005. Prevalence and antimicrobial resistance of *Campylobacter* spp. and *Salmonella* serovars in organic chickens from Maryland retail stores. *Appl. Environ. Microbiol.* 71(7):4108-4111. <https://doi.org/10.1128/AEM.71.7.4108-4111.2005>
- Daneshmand, A., Sadeghi, G. H., and Karimi, A. 2012. The effects of a combination of garlic, oyster mushroom and propolis extract in comparison to antibiotic on growth performance, some blood parameters and nutrients digestibility of male broilers. *Brazilian J. Poult. Sci.* 14(2):141-147. <https://doi.org/10.1590/S1516-635X2012000200009>
- Dibner, J.J. and Ivey, F.J., 1990. Hepatic protein and amino-acid metabolism in poultry. *Poult Sci.* 69(7):1188-1194. <https://doi.org/10.3382/ps.0691188>
- El-Ghalid, O.A., Abdel-Hamid, A.E., Harfoush, A.S. and ELnagggar, A.S., 2022. Effect of graded levels of biochar supplementation as a growth promoter on productive and physiological performance of broiler chicks. *Egyptian. Poult. Sci. J.* 42(3).
- ELnagggar, A. S., and El-Kelawy, M. I. 2018. Effect of humic acid supplementation on productive performance, blood constituents, immune response and carcass characteristics of Sasso chicken. *Egypt J. Anim. Prod.* 55(1):75-84. <https://doi.org/10.21608/ejap.2018.93258>
- Elnagggar, A. S., Elghalid, O., El hamid Elsayed, A., and Harfoush, A. 2022a. Effect of graded levels of biochar supplementation as a growth promoter on productive and physiological performance of broiler chicks. *Egypt. Poult. Sci. J.* 42(3):243-63. <https://doi.org/10.21608/epsj.2022.263846>

- Elnaggar, A. S., Eltahawy, W., Ghalwash, A., and Ghazalah, A. 2022b. Productive and physiological response of broiler chicks to dietary humic acid. *Egypt. Poult. Sci. J.* 42(2):157-170. <https://doi.org/10.21608/epsj.2022.249542>
- Food and Agriculture Organization (FAO). 2019. The future of food and agriculture: Alternative pathways to 2050. Retrieved from <http://www.fao.org/3/ca6640en/ca6640en>
- Fouladi, P., Ebrahimnezhad, Y., Shahryar, H. A., Maheri, N., and Ahmadzadeh, A. 2018. Effects of organic acids supplement on performance, egg traits, blood serum biochemical parameters and gut microflora in female Japanese quail (*Coturnix coturnix japonica*). *Brazilian J. Poult. Sci.* 20:133-44. <https://doi.org/10.1590/1806-9061-2016-0375>
- Gahruie, H. H., Parastouei, K., Mokhtarian, M., Rostami, H., Niakousari, M. and Mohsenpour, Z. 2020. Application of innovative processing methods for the extraction of bioactive compounds from saffron (*Crocus sativus*) petals. *J. Appl. Res. Med. Aromat. Plants.* 19:100264. <https://doi.org/10.1016/j.jarmap.2020.100264>
- Giannenas, I., Tsalie, E., Chronis, E. f., Mavridis, S., Tontis, D., and Kyriazakis, I. 2011. Consumption of *Agaricus bisporus* mushroom affects the performance, intestinal microbiota composition and morphology, and antioxidant status of turkey poults. *Anim. Feed Sci. Technol.* 165(3–4):218-229. <https://doi.org/10.1016/j.anifeedsci.2011.03.002>
- Hien, N. N., Xuan Dung, N. N., Manh, L. H., and Le Minh, B. T. 2018: Effects of biochar inclusion in feed and chicken litter on growth performance, plasma lipids and fecal bacteria count of Noi lai chicken. *Livest. Res. Rural. Develop.* 30(7):Article #131. <http://www.lrrd.org/lrrd30/7/nnxdu30131.html>
- Hosseini-Vashan S. J, Mohammadian E, and Afzali N. 2018. Effect of hydroethanolic saffron petals' extract on performance, carcass characteristics and blood parameters of Japanese

quails challenged by aflatoxin. J. Saffron. Agron. Technol. 6(2):237-252.
<https://doi.org/10.22048/jsat.2017.70025.1203>

Hosseini-Vashan, S. J., and Piray, A. H. 2021. Effect of dietary saffron (*Crocus sativus*) petal extract on growth performance, blood biochemical indices, antioxidant balance, and immune responses of broiler chickens reared under heat stress conditions. Italian J. Anim. Sci. 20(1):1338-1347. <https://doi.org/10.1080/1828051X.2021.1921628>

Hussein, E.A., El-Kassas, N.E. and Alderey, A.A. 2023. Effect of dietary supplementation of clove, peppermint, cinnamon oils and their blends on growth performance, carcass characteristics, blood biochemical parameters and antioxidant status of broiler chicks. Egyptian J. Anim. Prod. 60(1):33-41.

Kalus, K., Konkol, D., Korczyński, M., Koziel, J. A., and Opaliński, S. 2020. Effect of biochar diet supplementation on chicken broilers performance, NH₃ and odor emissions and meat consumer acceptance. Animals (Basel). 10(9):1539. <https://doi.org/10.3390/ani10091539>

Kazemi, M., 2025. Biochar in Animal Agriculture: Enhancing Health, Efficiency and Environmental Sustainability. Vet. Med. Sci. 11(6):e70629.
<https://doi.org/10.1002/vms3.70629>

Liu, J., Xue, M., Morais, S., He, M., Wang, H., Wang, J., Pastor, J. J., Gonçalves, R. A., and Liang, X. 2022. Effects of a phytogenic supplement containing olive by-product and green tea extracts on growth performance, lipid metabolism, and hepatic antioxidant capacity in Largemouth bass (*Micropterus salmoides*) fed a high soybean meal diet. Antioxidants. 11(12):2415. <https://doi.org/10.3390/antiox11122415>

Mahfuz, S., Long, S., and Piao, X. 2020. Role of medicinal mushrooms in growth performance and physiological responses in broiler chickens. World's Poult. Sci. J. 76(1):1-18.
<https://doi.org/10.1080/00439339.2020.1729670>

- Man, K. Y., Chow, K. L., Man, Y. B., Mo, W. Y. and Wong, M. H. 2021. Use of biochar as feed supplements for animal farming. *Crit. Rev. Environ. Sci Technol.* 51(2):187-217. <https://doi.org/10.1080/10643389.2020.1721980>
- Masouri, L., Salari, S., Sari, M., Tabatabaei, S. and Masouri, B., 2017. Effect of feed supplementation with *Satureja khuzistanica* essential oil on performance and physiological parameters of broilers fed on wheat-or maize-based diets. *Br. Poult. Sci.* 58(4):425-434. <https://doi.org/10.1080/00071668.2017.1327701>
- Mousa, M.A., Asman, A.S., Ali, R.M., Sayed, R.K., Majrashi, K.A., Fakiha, K.G., Alhotan, R.A. and Selim, S. 2023. Impacts of dietary lysine and crude protein on performance, hepatic and renal functions, biochemical parameters, and histomorphology of small intestine, liver, and kidney in broiler chickens. *Vet Sci.* 10(2):98. <https://doi.org/10.3390/vetsci10020098>
- Neuvonen, P.J., Kuusisto, P., Vapaatalo, H. and Manninen, V. 1989. Activated charcoal in the treatment of hypercholesterolaemia: dose-response relationships and comparison with cholestyramine. *European J. Clinic. Pharmacol.* 37(3):225-230.
- Ochsenbein, A. F., and Zinkernagel, R. M. 2000. Natural antibodies and complement link innate and acquired immunity. *Immunol Today.* 21(12):624-30. [https://doi.org/10.1016/s0167-5699\(00\)01754-0](https://doi.org/10.1016/s0167-5699(00)01754-0)
- Percie du Sert, N., Ahluwalia, A., Alam, S., Avey, M. T., Baker, M., Browne, W. J., Clark, A., Cuthill, I. C., Dirnagl, U., Emerson, M., Garner, P., Holgate, S. T., Howells, D. W., Hurst, V., Karp, N. A., Lazic, S. E., Lidster, K., MacCallum, C. J., Macleod, M., Pearl, E. J., Petersen, O. H., Rawle, F., Reynolds, P., Rooney, K., Sena, E. S., Silberberg, S. D., Steckler, T., and Würbel, H. 2020. Reporting animal research: Explanation and elaboration for the ARRIVE guidelines 2.0. *PLoS Biol.* 18(7):e3000411. <https://doi.org/10.1371/journal.pbio.3000411>

- Placer, Z. A., L. L. Cushman, and B. C. Johnson. 1966. Estimation of product of lipid peroxidation (malonyl dialdehyde) in biochemical systems. *Anal. Biochem.* 16:359–364. [https://doi.org/10.1016/0003-2697\(66\)90167-9](https://doi.org/10.1016/0003-2697(66)90167-9)
- Pourmollaei, F., Ghazaghi, M., Rokouei, M., Bagherzadeh-Kasmani, F. and Mehri, M. 2025. Calcium requirements in growing Japanese quail from 21 to 35 days post-hatch. *Poult. Sci.* 104(2):p.104700. <https://doi.org/10.1016/j.psj.2024.104700>
- Rashidi, N., Khatibjoo, A., Taherpour, K., Akbari-Gharaei, M., and Shirzadi, H. 2020. Effects of licorice extract, probiotic, toxin binder and poultry litter biochar on performance, immune function, blood indices and liver histopathology of broilers exposed to aflatoxin-B1. *Poultry science.* 99(11):5896-5906. <https://doi.org/10.1016/j.psj.2020.08.034>
- Raz, M., Bagherzadeh-Kasmani, F., Karimi-Torshizi, M.A., Ghazaghi, M., Mokhtarpour, A. and Mehri, M. 2025. Boosting antioxidant defense and enhancing product quality by biochar and probiotics under chronic aflatoxicosis in quails. *Poult. Sci.* 104(7):105183. <https://doi.org/10.1016/j.psj.2025.105183>
- Schmidt, H.-P., Hagemann, N., Draper, K., and Kammann, C. 2019. The use of biochar in animal feeding. *PeerJ. Life. Environ.* 7:e7373. <https://doi.org/10.7717/peerj.7373>
- Schmidt, H-P., Pandit, B. H., Cornelissen, G., and Kammann, C. 2017. Biochar-based fertilization with liquid nutrient enrichment: 21 field trials covering 13 crop species in Nepal. *Land. Degrad. Develop.* 28(8):2324–2342. <https://doi.org/10.1002/ldr.2761>
- Sheikh, G. G., Malik, N. A., Sheikh, A. A., Ganai, A. M, Khan, A. A., Haq, Z., Farooq, J., and Rather, A. M. 2023. Saffron petals (*Crocus sativus L.*) enhance productive performance and carcass quality in broiler birds by improving their immunity, antioxidant status and biochemical profile. *Antioxidant Status and Biochemical Profile. J. Agric. Food. Res.* 12:100562. <https://doi.org/10.1016/j.jafr.2023.100562>

- Srivastava, R., Ahmed, H., Dixit, R. K., and Dharamveer Saraf, S. A. 2010. *Crocus sativus* L.: A comprehensive review. *Pharmacogn Rev.* 4(8):200-8. <https://doi.org/10.4103/0973-7847.70919>
- Tomsič, K., Seliškar, A., Lukanc, B. and Nemeč Svete, A. 2016. Plasma total antioxidant capacity and activities of blood glutathione peroxidase and superoxide dismutase determined in healthy dogs by using commercially available kits. *Acta Vet.* 66(4):534-48. <https://doi.org/10.1515/acve-2016-0046>
- Tufarelli, V., Ghavami, N., Nosrati, M., Rasouli, B., Kadim, I.T., Suarez Ramirez, L., Gorlov, I., Slozhenkina, M., Mosolov, A., Seidavi, A. and Ayasan, T. 2023. The effects of peppermint (*Mentha piperita* L.) and chicory (*Cichorium intybus* L.) in comparison with a prebiotic on productive performance, blood constituents, immunity and intestinal microflora in broiler chickens. *Anim. Biotech.* 34(7):3046-3052. <https://doi.org/10.1080/10495398.2022.2130798>
- Vakili, R., Mokhtarpour, A. and Hosseini Ghaffari, S.A. 2025. Functional feed for laying hens: application of saffron extract as eco-friendly supplement with cholesterol-lowering properties. *Vet Med. Sci.* 11(1):e70155. <https://doi.org/10.1002/vms3.70155>
- Vakili, R., Toroghian, M., and Torshizi, M. E. 2022. Saffron extract feed improves the antioxidant status of laying hens and the inhibitory effect on cancer cells (PC3 and MCF7) Growth. *Vet Med Sci.* 8(6):2494-2503. <https://doi.org/10.1002/vms3.910>
- Willson, N. L., Van, T. T. H., Bhattarai, S. P., Courtice, J. M., McIntyre, J. R., Prasai, T. P., Moore, R. J., Walsh, K., and Stanley, D. 2019. Feed supplementation with biochar may reduce poultry pathogens, including *Campylobacter hepaticus*, the causative agent of Spotty Liver Disease. *PLoS One.* 14(4):e0214471. <https://doi.org/10.1371/journal.pone.0214471>

- Zahed, O., Vakili, R. and Mokhtarpour, A., 2025. Pistachio by-product biochar as an antibiotic alternative: promoting growth and gut health in Japanese quails (*Coturnix japonica*). *Trop. Anim. Health. Prod.* 57(5):249. <https://doi.org/10.1007/s11250-025-04504-6>
- Zhao, B., Xu, X., Xu, S., Chen, X., Li, H., and Zeng, F. 2017. Surface characteristics and potential ecological risk evaluation of heavy metals in the bio-char produced by co-pyrolysis from municipal sewage sludge and hazelnut shell with zinc chloride. *Bioresour. Technol.* 243:375-383. <https://doi.org/10.1016/j.biortech.2017.06.032>

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Table 1

Major bioactive compounds (secondary metabolites) of Iranian *saffron* petal extract (Vakili et al., 2022)

Constituent	Content
Total phenolic compounds (mg)	3.42±0.11
Total flavonoids (mg/g)	2.75±0.07
Kaempferol (% w/w)	12.6±0.12
Crocin (% w/w)	0.6±0.03
Anthocyanin (mg/l extract)	1712±0.24

Table 2

Chemical composition of the PB biochar.

Dry matter (%)	93.20
C (%)	55.34
H (%)	2.11
N (%)	1.56
Yield (%) *	65.83

*Calculated as (mass of biochar (g)/oven dry mass of feedstock (g)) × 100 (Zhao et al., 2017).

Table 3

Ingredients and composition of the basal diet.

Ingredient	Amount (%)
Corn	51.80
Soybean meal (44%)	36.70
Corn gluten meal (62%)	5.21
Soybean oil	2.90
Di-calcium phosphate	1.65
Limestone	0.70
L-Lysine	0.13
DL-Methionine	0.11
NaCl	1.30
Mineral premix*	0.15
Vitamin premix [†]	0.15
Choline Chloride (50%)	0.20
Nutrient composition	
AME (Kcal/kg)	2995
CP (%)	24.00
Total Lysine (%)	1.30
Total Methionine (%)	0.80
Total Methionine + Cysteine (%)	0.93
Total Tryptophan (%)	0.35

Calcium (%)	0.80
Available Phosphorus (%)	0.45
DEB (mEq/kg) [§]	250

^{*}Mineral premix provided per kilogram of diet: 65 mg Mn (from MnSO₄·H₂O), 55 mg Zn (from ZnO), 50 mg Fe (from FeSO₄·7H₂O), 8 mg Cu (from CuSO₄·5H₂O), 1.8 mg I (from Ca(IO₃)₂·H₂O), 0.30 mg Se, 0.20 mg Co (from Co₂O₃), 0.16 mg Mo.

[†]Vitamin premix provided per kilogram of diet: 11,500 IU vitamin A (from vitamin A acetate), 2100 IU cholecalciferol, 22 IU vitamin E (from DL- α -tocopherylacetate), 0.60 mg vitamin B₁₂, 4.4 mg riboflavin, 40 mg nicotinamide, 35 mg calcium pantothenate, 1.50 mg menadione (from menadione dimethyl-pyrimidinol), 0.80 mg folic acid, 3 mg thiamine, 10 mg pyridoxine, 1 mg biotin, 560 mg choline chloride, 125 mg ethoxyquin.

[§]Dietary electrolyte balance: represents dietary Na + K – Cl in mEq/kg of diet.

Table 4

Growth performance in Japanese quails fed different feed additives.

Item	Treatment [†]					SEM	<i>P</i> value
	CON	PRO	PBB	SPE	PBB + SPE		
1-21 days							
Feed intake(g)	277	272	274	288	294	0.178	0.11
BW gain (g)	138b	142b	143b	154a	158a	2.09	0.003
FCR	2.00	1.91	1.92	1.87	1.86	0.029	0.13
21-35 days							
Feed intake(g)	435	432	434	449	457	4.90	0.11
BW gain (g)	99	104	110	112	114	1.77	0.12
FCR	4.39	4.15	3.95	4.01	3.97	0.087	0.73
1-35 days							
Feed intake(g)	712	704	708	737	751	5.55	0.41
BW gain (g)	237c	246b	253ab	266ab	273a	2.17	<0.001
FCR	3.01c	2.86b	2.80ab	2.77ab	2.75a	0.035	0.08

CON: Control diet without additives, PRO: basal diet + 1% probiotic protexin, PBB: basal diet + 1% pistachio byproduct biochar, SPE: basal diet + 500 mg/kg saffron petal extract and, PBB + SPE: basal diet + 1% pistachio byproduct biochar + 500 mg/kg saffron petal extract.

BW gain: body weight gain; FCR: feed conversion ratio.

SEM: standard error of the means.

Table 5

Carcass and relative organ weights in Japanese quails fed different feed additives.

Parameter	CON	PRO	PBB	SPE	PBB + SPE	SEM	P Value
Cecal digesta	6.94a	6.84a	6.26c	6.47bc	6.71ab	0.085	0.002
pH							
Carcass (%)	74.04	72.11	76.87	78.88	75.76	1.599	0.3779
Liver (%)	3.13	2.35	2.98	2.23	2.27	0.390	0.5037
Gizzard (%)	1.91	1.77	1.88	1.78	1.80	0.117	0.9175

CON: Control diet without additives, PRO: basal diet + 1% probiotic protexin, PBB: basal diet + 1% pistachio

byproduct biochar, SPE: basal diet + 500 mg/kg saffron petal extract and, PBB + SPE: basal diet + 1% pistachio

byproduct biochar + 500 mg/kg saffron petal extract.

SEM: standard error of the mean

Table 6

Immunological parameters in Japanese quails fed different feed additives.

Parameter	CON	PRO	PBB	SPE	PBB + SPE	SEM	P Value
IgG (mg/dl)	0.24d	0.44bc	0.39c	0.51ab	0.56a	0.029	0.0004
IgA (mg/dl)	0.64a	0.40bc	0.43b	0.35bc	0.33c	0.027	<0.0001
IgM (mg/dl)	0.22c	0.42b	0.38b	0.49ab	0.58a	0.034	0.0009

CON: Control diet without additives, PRO: basal diet + 1% probiotic protexin, PBB: basal diet + 1% pistachio byproduct biochar, SPE: basal diet + 500 mg/kg saffron petal extract and, PBB + SPE: basal diet + 1% pistachio byproduct biochar + 500 mg/kg saffron petal extract.

SEM: standard error of the mean.

Table 7

Serum biochemical indices in Japanese quails fed different feed additives.

Parameter	CON	PRO	PBB	SPE	PBB + SPE	SEM	P Value
Total protein (g/dL)	2.57c	3.05b	3.49ab	3.28ab	3.58a	0.108	0.0034
Albumin (g/dL)	1.54b	1.86a	1.99a	1.88a	1.86a	0.069	0.0180
Globulin (g/dL)	1.03d	1.19cd	1.50ab	1.40bc	1.72a	0.063	0.0017
AST (IU/L)	174.43c	269.93a	250.13a	199.17bc	231.47ab	12.002	0.0039
ALT (IU/L)	10.10cd	12.84b	18.29a	10.79c	82.29d	0.562	<0.0001

CON: Control diet without additives, PRO: basal diet + 1% probiotic protexin, PBB: basal diet + 1% pistachio byproduct biochar, SPE: basal diet + 500 mg/kg saffron petal extract and, PBB + SPE: basal diet + 1% pistachio byproduct biochar + 500 mg/kg saffron petal extract.

SEM: standard error of the mean.

Table 8

Serum lipid profile in Japanese quails fed different feed additives (log CFU/g).

Parameter	CON	PRO	PBB	SPE	PBB + SPE	SEM	P Value
Total cholesterol (mg/dl)	580.81a	281.42c	372.43b	196.19d	234.11cd	13.861	<0.0001
Triglycerides (mg/dl)	349.53a	92.39c	225.25b	196.29b	101.73c	10.362	<0.0001
HDL (mg/dl)	29.91b	45.81a	48.22a	44.58a	52.87a	3.316	0.0085
LDL (mg/dl)	480.99a	216.96c	279.16b	112.35e	160.89d	11.878	<0.0001
VLDL (mg/dl)	69.91a	18.48c	45.05b	39.26b	20.35c	2.072	<0.0001

CON: Control diet without additives, PRO: basal diet + 1% probiotic protexin, PBB: basal diet + 1% pistachio byproduct biochar, SPE: basal diet + 500 mg/kg saffron petal extract and, PBB + SPE: basal diet + 1% pistachio byproduct biochar + 500 mg/kg saffron petal extract.

SEM: standard error of the mean.

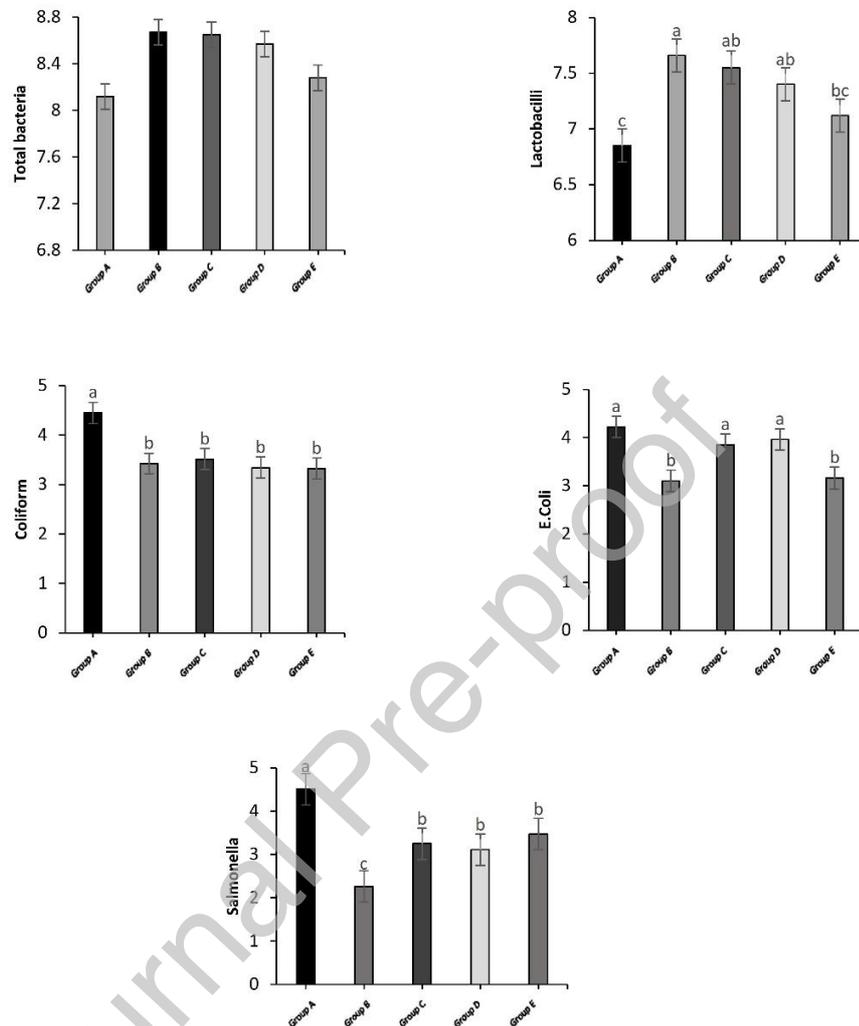


Fig. 1. Cecal microbial populations in Japanese quails fed different feed additives (log CFU/g).

(A) Control diet without additives, (B) basal diet + 1% probiotic protexin, (C) basal diet + 1% pistachio byproduct biochar (D), basal diet + 500 mg/kg saffron petal extract (E), and basal diet + 1% pistachio byproduct biochar + 500 mg/kg saffron petal extract.

Data are presented as means \pm SD.

a, b, c Significant differences for the different groups ($p < 0.05$).

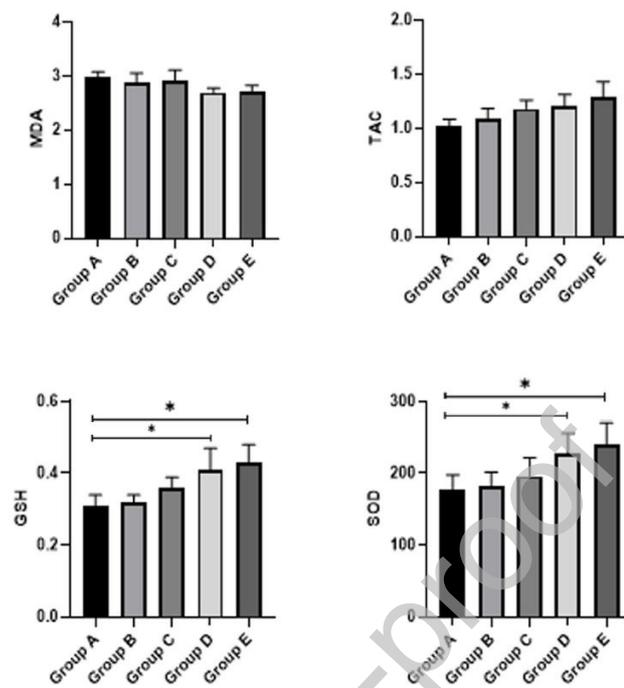


Fig. 2. Antioxidant status in Japanese quails fed different feed additives.

(A) Control diet without additives, (B) basal diet + 1% probiotic protexin, (C) basal diet + 1% pistachio byproduct biochar (D), basal diet + 500 mg/kg saffron petal extract (E), and basal diet + 1% pistachio byproduct biochar + 500 mg/kg saffron petal extract. MDA (malondialdehyde), TAC (total antioxidant capacity), GSH (reduced glutathione), and SOD (superoxide dismutase)

Data are presented as means \pm SD.

*Significant differences for the different groups ($p < 0.05$).

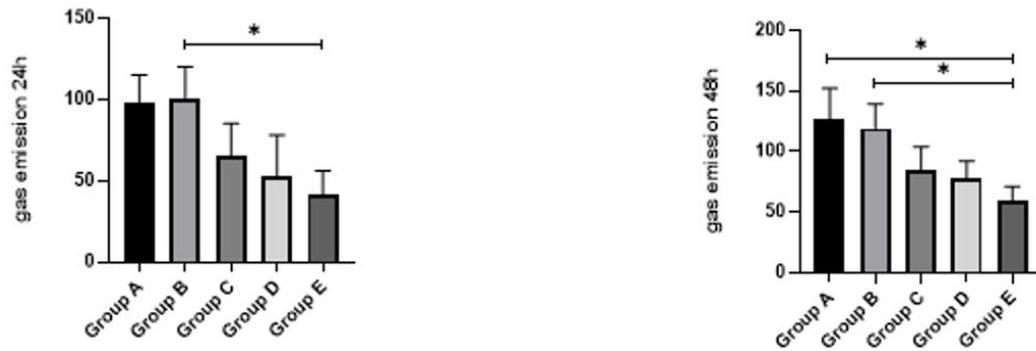


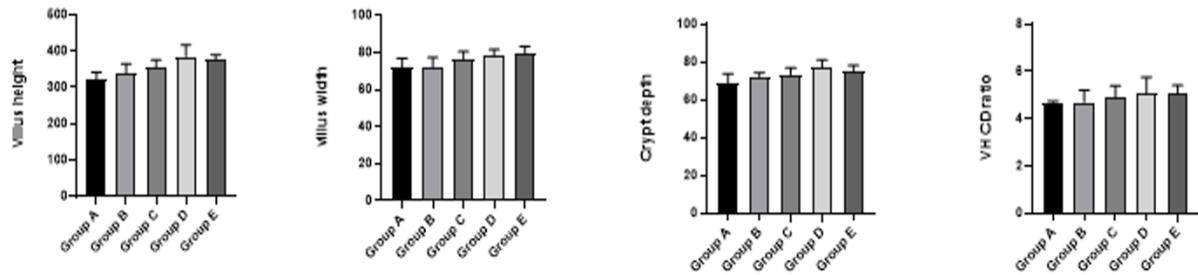
Fig. 3. Manure gas emission in Japanese quails fed different feed additives after 24 h and 48 h.

(A) Control diet without additives, (B) basal diet + 1% probiotic protexin, (C) basal diet + 1% pistachio byproduct biochar (D), basal diet + 500 mg/kg saffron petal extract (E), and basal diet + 1% pistachio byproduct biochar + 500 mg/kg saffron petal extract.

Data are presented as means \pm SD.

*Significant differences for the different groups ($p < 0.05$).

A) Ileum



B) Cecum

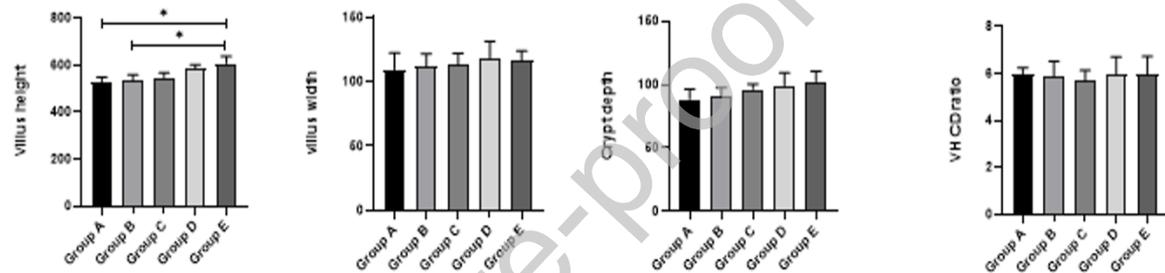


Fig. 4. Intestinal morphology in Japanese quails fed different feed additives; A) Ileum and B) Cecum.

(A) Control diet without additives, (B) basal diet + 1% probiotic protexin, (C) basal diet + 1% pistachio byproduct *biochar* (D), basal diet + 500 mg/kg saffron petal extract (E), and basal diet + 1% pistachio byproduct *biochar* + 500 mg/kg saffron petal extract.

Data are presented as means \pm SD.

*Significant differences for the different groups ($p < 0.05$).

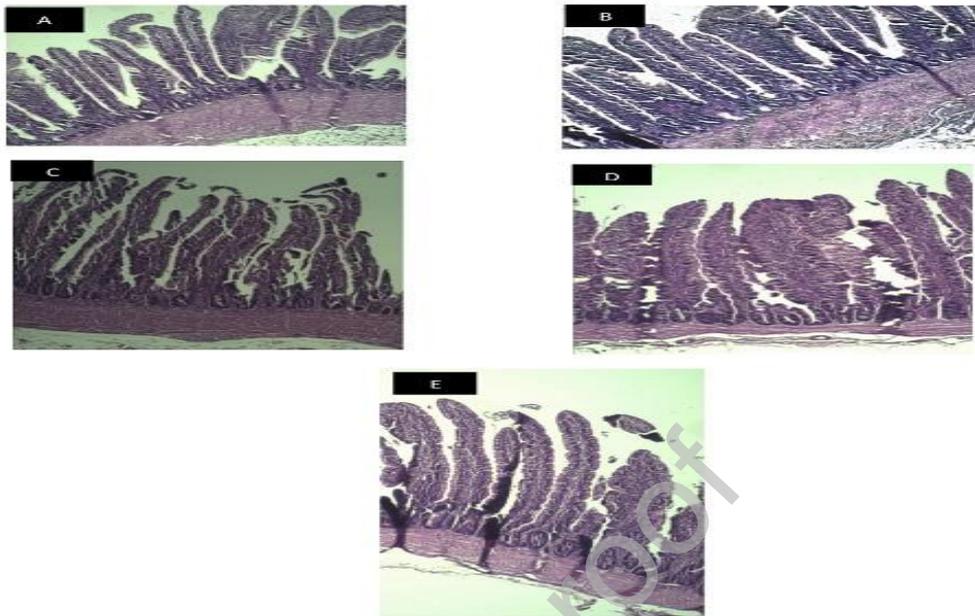


Fig. 5. Representative photomicrograph of the ileum in Japanese quails fed different feed additives.

(A) Control diet without additives, (B) basal diet + 1% probiotic protexin, (C) basal diet + 1% pistachio byproduct biochar (D), basal diet + 500 mg/kg saffron petal extract (E), and basal diet + 1% pistachio byproduct biochar + 500 mg/kg saffron petal extract.

Conflict of interest

No potential conflicts of interest were reported by the authors.