

Improving immune system and antioxidant status in Japanese quails through biochar supplementation

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Abstract

The study evaluated the effects of pistachio by-products biochar (PBB) on performance, blood metabolites, immune response, antioxidant status, and ammonia gas emissions in Japanese quails. A total of 500 one-day-old Japanese quails were assigned to a completely randomized design with five dietary treatments and five replicates for 35 days. The experimental diets included: (1) a basal feed without additives (control), (2) a basal feed with 0.05% flumequine 10% (positive control), (3) a basal feed with 0.35% PBB, (4) a basal feed with 0.65% PBB, and (5) a basal feed with 1% PBB. The results showed that weight gain significantly increased in birds fed 0.65% biochar compared to the control and flumequine groups, without any effect on feed intake. A trend towards a lower feed conversion ratio was observed in birds fed 0.65% biochar compared with the control. Quails fed 1% biochar had significantly lower cholesterol and LDL levels, while the control group exhibited the highest levels. The highest lymphocyte percentage was observed in quails fed 1% biochar, and increasing biochar levels in the diet significantly reduced the heterophil/lymphocyte ratio. However, biochar supplementation had no significant effect on immunoglobulin (IgG, IgM, IgY, and IgT) levels. Antioxidant markers, including total antioxidant capacity, glutathione peroxidase, and superoxide dismutase, were highest in birds receiving 1% PBB, with no significant difference between the 0.65% and 1% levels. Additionally, biochar supplementation significantly reduced ammonia gas emissions. Overall, incorporating at least 0.65% PBB in meat quail diets improved growth performance, blood parameters, antioxidant enzyme activity, and immune function, offering an eco-friendly alternative to antibiotics.

Key words: biochar, pistachio by-products, blood metabolites, immune response, ammonia emission

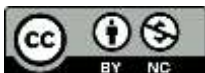
Introduction

Ensuring food security for a growing global population requires optimizing livestock production through sustainable resource utilization. Poultry production plays a crucial role in global food supply by providing meat and eggs. However, the industry faces challenges, including maintaining food safety, reducing environmental impact, and ensuring economic sustainability (Schmidt et al,

2019; Nair et al, 2023). Contaminated poultry products pose health risks, highlighting the need for safe and high-quality production systems (Vimal et al, 2022).

To achieve sustainability, utilizing locally available natural resources to enhance poultry performance and reduce production costs is essential (Man et al, 2021; Al-Khalaifah and Al-Nasser, 2023).

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Antibiotics have historically been used to control pathogens and improve productivity, but concerns over antimicrobial resistance led to their ban as growth promoters in the European Union in 2006 (Saleh et al, 2018). International organizations such as the FAO and WHO have since 2016 pushed for stricter regulations (FAO, 2016; WHO, 2019). Consequently, the poultry industry must identify alternative strategies to support animal health and maintain profitability while reducing antibiotic dependence (Abdel-Moneim et al, 2022).

Biochar, a carbon-rich material produced from the pyrolysis of plant residues and organic waste, has emerged as a promising natural alternative. Its properties vary based on feedstock type and pyrolysis conditions, with lignin-rich materials yielding higher biochar production (Ahmed and Hameed, 2020; Man et al, 2021). As a feed additive, biochar has demonstrated benefits in improving growth, feed efficiency, and nutrient utilization across various livestock species, including poultry, cattle, and fish (Vimal et al, 2022; Nair et al, 2023).

One of biochar's key advantages is its potential to mitigate environmental pollutants. While widely recognized for reducing methane emissions in ruminants, biochar may also decrease ammonia (NH₃) emissions in poultry production. Ammonia poses risks to farm workers, bird health, and air quality, making emission control a priority for farmers and regulatory agencies (Nowak et al, 2016; Kalus et al, 2019). Additionally, biochar supplementation has been associated with enhanced immune function, reduced mycotoxin toxicity, improved antioxidant status, and better liver enzyme activity in poultry (Jandosov et al, 2017; Rajput et al, 2017).

While most biochar research has focused on conventional biomass sources such as wood and bamboo, agricultural by-products like pistachio by-products (PB) offer a sustainable and cost-effective alternative. PB, generated during the de-hulling

process, include soft outer shells, twigs, leaves, hard shells, and green kernels (Mokhtarpour et al, 2014). The effectiveness of biochar varies based on feedstock type and production techniques (Al-Khalaifah and Al-Nasser, 2023), and while excessive biochar inclusion may interfere with nutrient availability, moderate supplementation levels (0.5–1%) have shown beneficial effects (Schmidt et al, 2019). However, the impact of PB-derived biochar on poultry performance, particularly in Japanese quail, remains largely unexplored. Given its unique structural properties and potential bioactive effects (Mirheidari et al, 2020), we hypothesize that PB-derived biochar may influence bird health. Therefore, this study aimed to evaluate the effects of PB-derived biochar on hematological parameters, immune response, antioxidant status, and ammonia emissions in Japanese quail. By identifying an effective biochar source from agricultural waste, this research contributes to sustainable poultry production by improving bird health, reducing environmental pollutants, and providing an eco-friendly alternative to antibiotic growth promoters.

Materials and Methods

Biochar Preparation and Experimental Design

Pistachio by-products were obtained from a pistachio de-hulling factory in Kashmar, Khorasan Razavi Province, Iran. The material was sun-dried and ground using a 2-mm mesh screen before undergoing pyrolysis according to the method described by Mirheidari et al. (2020). The total carbon (C), hydrogen (H), and nitrogen (N) content in PB biochar were 55.3%, 2.1%, and 1.6%, respectively (CHNS analyzer, Thermo Finnigan, Flash EA 1112 Series).

Five hundred day-old Japanese quails were assigned to five experimental diets with five replications (20 quails per replicate) in a completely randomized design for 35 days.

Temperature and management conditions were maintained according to standard breeding guidelines (Du Sert et al, 2020). The experimental diets (Table 1) formulated based on the nutritional requirements recommended by the NRC (1994) included: 1) basal diet without additives (control), 2) basal diet supplemented with 0.05% flumequine (10%) (positive control), 3) basal diet supplemented with 0.35% PBB, 4) basal diet supplemented with 0.65% BPP, 5) basal diet supplemented with 1% PBB. All birds had ad libitum access to feed and water.

Table 1: Ingredients and composition of basal diet

Ingredient	Amount (%)
Corn	49.25
Soybean meal (46%)	46.00
Soybean oil	1.00
Di-calcium phosphate	1.00
Limestone	0.80
L-Lysine	0.67
DL-Methionine	0.63
NaCl	0.32
Mineral premix*	0.15
Vitamin premix†	0.15
Nutrient composition	
AME (Kcal/kg)	2950
CP (%)	24.6
Total Lysine (%)	1.86
Total Methionine (%)	1.00
Total Methionine + Cysteine (%)	1.38
Total Tryptophan (%)	0.35
Calcium (%)	0.75
Available Phosphorus (%)	0.25

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

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

Measurements

Daily feed intake was determined by measuring the amount of feed offered and the refusals. Body weights were recorded weekly to track weight gain, and the feed conversion ratio (FCR) was calculated by

dividing the total feed consumed by the corresponding weight gain for each treatment group.

On day 35, two quails were randomly selected from each replicate (n = 10 per treatment), and blood samples were collected from the jugular vein. The samples were maintained at 4°C for 10 minutes and then centrifuged at 3000 rpm to separate serum. The serum samples were stored at -80°C for further analysis.

Biochemical parameters, including total cholesterol, triglycerides, low-density lipoprotein (LDL), high-density lipoprotein (HDL), glucose, and total protein, were determined using an autoanalyzer (BT3000, Roma, Italy). Oxidative stress status was assessed by measuring plasma malondialdehyde (MDA) levels (Pilz et al, 2000). Intracellular antioxidant markers were evaluated by determining the activities of superoxide dismutase (SOD) and glutathione peroxidase via spectrophotometry. All measurements were performed in duplicate.

To evaluate the humoral immune response, 0.5 mL of a 7% suspension of sheep red blood cells (SRBC), prepared in phosphate-buffered saline (PBS), was injected into the right subwing vein of birds at 28 days of age. Seven days post-injection (day 35), blood samples were collected from the left wing vein to assess antibody titers. After clotting, the serum was separated and incubated at 56°C for 30 minutes to inactivate complement. The total anti-SRBC antibody titer, as well as immunoglobulin M (IgM) and immunoglobulin Y (IgY) levels, were determined using serial dilution. The titers were expressed as log₂ values, with the highest dilution exhibiting complete agglutination. IgM levels were measured by treating serum samples with 0.01 M 2-mercaptoethanol, which selectively inactivates IgM. The difference between total anti-SRBC and IgY titers was used to estimate IgM concentrations.

Hematological Analysis

At the end of the experiment, blood samples were also collected to determine the heterophil-to-lymphocyte (H:L) ratio. Blood smears were prepared and stained with Wright's stain following the method of Lucas and Jamroz (1961). A total of 100 white blood cells were counted per sample, and the H:L ratio was calculated.

Ammonia Emission Measurements

Ammonia (NH₃) emissions from manure were assessed in the final week of the experiment following the method described by Kalus et al. (2020) with some modifications. Approximately 200 g of excreta was collected from each cage and stored in plastic zipper bags. These bags were then placed in a plastic container with a lid, which had two holes; one sealed with a membrane filter and the other used for ammonia measurement. The samples were allowed to ferment at room temperature, and ammonia levels were recorded throughout the process with time zero indicating the start of the ammonia emission monitoring. A gas-sampling pump (AP-20, Gastec Corp., Kitagawa, Japan) fitted with a detector tube (3LA, 3M) was used to measure ammonia emissions at 0 and 24

hours. The NH₃ concentration was reported as ppm per 100 mL.

Statistical Analysis

Data were analyzed using a completely randomized design. The GLM procedure in SAS (2001, version 9.1) was used for statistical analysis. Differences among treatment means were compared using Duncan's multiple range test at a significance level of P<0.05.

Results

Effect of pistachio by-products biochar and antibiotic on growth performance of Japanese quails are showed in Table 2. The effects of PBB and antibiotic supplementation on selected blood parameters in Japanese quails are presented in Table 3. Blood glucose, total protein, and HDL levels were not significantly affected by dietary treatments (P>0.05). However, cholesterol levels were significantly lower in quails fed 1% PBB (116 mg/dL) compared to the control group, which exhibited the highest cholesterol level (165 mg/dL, P<0.05). Similarly, LDL levels were significantly reduced in birds receiving 0.65% and 1% biochar, showing the lowest values among all experimental groups (P<0.05).

Table 2: Effect of pistachio by-products biochar and antibiotic on growth performance of Japanese quails

Item	Control	Treatment				SEM	P value
		Flumequine	0.35% Biochar	0.65% Biochar	1% Biochar		
Feed intake (g)	719	740	743	735	749	5.11	0.41
BW gain (g)	236 c	250 b	255 ab	263 a	256 ab	2.29	0.001
FCR	3.05	2.96	2.91	2.78	2.93	0.031	0.08

^{a, b, c} Different superscripts within the same row indicate significant differences (P<0.05).

Table 3: Effect of pistachio by-products biochar and antibiotic on blood biochemical parameters of Japanese quails (mg/dl)

Item	Control	Treatment				SEM	P value
		Flumequine	0.35% Biochar	0.65% Biochar	1% Biochar		
Glucose	184	189	191	206	210	3.61	0.06
Protein	3.45	3.48	3.52	3.55	3.62	0.033	0.61
Triglycerides	225	218	213	205	201	4.46	0.49
Cholesterol	165 a	137 ab	141 ab	129 b	116 b	5.51	0.04
HDL	63.8	59.2	69.9	67.6	71.5	1.79	0.17
LDL	80.1 a	64.5 ab	71.0 a	52.8 b	55.4 b	3.32	0.02

^{a, b} Different superscripts within the same row indicate significant differences (P<0.05).

Table 4 presents the differential leukocyte counts, heterophil-to-lymphocyte (H:L) ratio, and humoral immune response. The addition of PBB to the diet significantly influenced lymphocyte percentage and the H:L ratio ($P < 0.05$). The highest lymphocyte percentage was observed in quails receiving 1% biochar, whereas the control and antibiotic-treated groups exhibited the lowest levels. Moreover, increasing dietary biochar inclusion led to a significant reduction in the H:L ratio ($P < 0.05$), with the

lowest ratio recorded in the 1% biochar group, while the control group displayed the highest ratio. However, heterophil percentages did not differ significantly among treatments ($P > 0.05$). Assessment of humoral immunity, based on the levels of IgG, IgM, IgY, and IgT, revealed no significant effects of biochar supplementation ($P > 0.05$). Nonetheless, a numerical increase in IgG, IgY, and IgT levels was observed in biochar-fed groups.

Table 4. Effect of pistachio by-products biochar and antibiotic on immune system parameters of Japanese quails

Item	Control	Treatment				SEM	P value
		Flumequine	0.35% Biochar	0.65% Biochar	1% Biochar		
Heterophils (%)	36.6	34.2	35.8	33.5	32.7	0.72	0.45
Lymphocytes (%)	56.9 c	57.2 c	60.7 bc	65.1 ab	67.8 a	1.31	0.003
Heterophil/ Lymphocyte Ratio	0.64 a	0.60 ab	0.59 ab	0.51 bc	0.48 c	0.019	0.009
IgG (mg/mL)	4.34	4.36	4.41	4.65	4.81	0.070	0.11
IgM (mg/mL)	1.27	1.31	1.30	1.42	1.36	0.034	0.81
IgY (mg/mL)	1.96	2.02	2.19	2.26	2.22	0.045	0.12
IgT (mg/mL)	7.56	7.69	7.90	8.31	8.38	0.122	0.10

^{a, b, c} Different superscripts within the same row indicate significant differences ($P < 0.05$).

The effects of biochar and antibiotics on antioxidant indices are summarized in Table 5. Serum malondialdehyde (MDA) levels were not significantly influenced by dietary treatments ($P > 0.05$). However, total antioxidant capacity and red blood cell antioxidant enzyme activities were significantly improved by dietary supplementation ($P < 0.05$). The highest

values for total antioxidant capacity, glutathione peroxidase, and superoxide dismutase were observed in the 1% biochar group ($P < 0.05$), though no significant difference was detected between the 0.65% and 1% biochar levels. The lowest antioxidant enzyme activities were recorded in the control group.

Table 5: Effect of pistachio by-products biochar and antibiotic on antioxidant parameters of Japanese quails

Item	Control	Treatment				SEM	P value
		Flumequine	0.35% Biochar	0.65% Biochar	1% Biochar		
Malondialdehyde (nmol/mL)	2.97	2.88	2.91	2.69	2.72	0.043	0.13
Total Antioxidant Capacity (mmol/L)	1.02 b	1.09 ab	1.18 ab	1.21 ab	1.28 a	0.032	0.07
Glutathione Peroxidase (mmol/L)	3.10 b	3.21 b	3.63 ab	4.09 a	4.28 a	0.016	0.02
Superoxide Dismutase (U/L)	178 c	182 bc	196 abc	228 ab	240 a	8.64	0.04

^{a, b, c} Different superscripts within the same row indicate significant differences ($P < 0.05$).

Table 6 presents the effects of dietary treatments on ammonia (NH₃) gas emissions from quail litter. Ammonia release at 0 and 24 hours post-excretion significantly decreased as biochar levels

increased (P<0.05). The most pronounced reduction in ammonia emissions was observed in quails fed 0.65% and 1% biochar, demonstrating its effectiveness in reducing environmental nitrogen losses.

Table 6. Effect of pistachio by-products biochar and antibiotic on ammonia gas emission from Japanese quail litter (ppm)

Item	Control	Treatment				SEM	P value
		Flumequine	0.35% Biochar	0.65% Biochar	1% Biochar		
Hour_0	98 a	100 a	65 ab	53 b	41 b	7.67	0.01
Hour_24	126 a	119 ab	84 bc	77 c	59 c	7.99	0.007

^{a, b, c} Different superscripts within the same row indicate significant differences (P<0.05).

Discussion

The PB biochar was included at 0.35%, 0.65%, and 1% to assess dose-dependent effects, following prior studies and preliminary data (Schmidt et al, 2017). The previous findings on biochar's effects in poultry diets have been inconsistent, even with similar inclusion levels. For example, Evans et al. (2016) reported that 2% poultry litter biochar (PLB) increased the feed conversion ratio (FCR) without affecting feed intake, while 4% PLB reduced weight gain. In contrast, Al-Jumaily et al. (2022) observed improved growth performance and feed efficiency at both 2% and 4% PLB, whereas other studies found no significant impact on feed intake, weight gain, or FCR at similar inclusion rates. These conflicting results highlight the influence of biochar's source and composition on its effectiveness. In the present study, biochar supplementation had no adverse effect on feed intake, indicating that palatability remained intact. However, the observed improvement in weight gain led to a more efficient FCR in biochar-fed groups, enhancing productivity without increasing feed costs. This aligns with Kana et al, (2011), who reported enhanced weight gain and FCR in broilers fed 0.2%–0.6% maize cob and Canarium charcoal, though higher levels reduced feed intake and growth. The optimal FCR in their study was achieved at 0.6% inclusion, similar to the 0.65% identified as optimal in the current research.

The improved performance in biochar-fed quails may be attributed to the antimicrobial properties of PBB, which likely reduced *E. coli* counts (Reaggi et al, 2023) and increased *Lactobacillus* populations (Choi et al, 2009) leading to better digestion, nutrient absorption, and energy utilization. This supports the findings of Gerlach and Schmidt (2012), who noted biochar's role in enhancing digestion and feed efficiency. Nao Takeuchi-Storm et al, (2025) reported that both 2% biochar and the probiotic-like product (0.125% *Saccharomyces cerevisiae* fermentate) improved growth performance and gut health markers compared to the control group. Notably, biochar supplementation led to a significant reduction in *Campylobacter* in broilers highlighting its potential as an alternative or complementary strategy to probiotics in poultry nutrition. Similarly, Islam et al, (2014) concluded that 1% Sea tangle charcoal can be used as a potential alternative to antibiotic (0.01% Chlortetracycline) in duck production.

In this study, biochar supplementation at levels of 0.65% and 1% significantly reduced serum cholesterol and LDL levels compared to the control group. These findings are consistent with the previous research that demonstrated biochar's ability to modulate lipid metabolism, likely through its adsorption properties, which may alter lipid absorption in the

gastrointestinal tract (Boonanunatasarn et al, 2014). The reduction in cholesterol levels is particularly relevant, as elevated cholesterol is a risk factor for cardiovascular disease, even in poultry (Dim et al., 2018). Biochar may reduce serum cholesterol by binding bile acids, thereby disrupting the enterohepatic circulation and enhancing hepatic cholesterol catabolism (Neuvonen et al, 1989). This process reduces bile acid reabsorption, promoting increased conversion of cholesterol to bile acids and lowering blood cholesterol levels (Elghalid et al, 2022; Kramer and Glombic, 2006). Thus, the observed lipid-lowering effects of PB biochar highlight its potential as a functional feed additive for improving metabolic health in poultry.

Interestingly, while the antioxidant status of quails improved with biochar supplementation, the effects on glucose and protein levels were minimal. The slight increase in total protein levels in biochar-fed groups could be attributed to biochar's adsorption of mycotoxins, which may otherwise impair liver function and protein synthesis (Elghalid, 2022). While further research is warranted, this trend suggests that biochar may contribute to improved protein metabolism through its detoxifying action, promoting liver health and overall physiological stability.

Biochar supplementation significantly altered the differential leukocyte counts, particularly increasing the percentage of lymphocytes and reducing the heterophil-to-lymphocyte (H:L) ratio. These findings align with research indicating that biochar can enhance immune function by modulating the stress response (Salah et al, 2015). The reduction in the H:L ratio, in particular, may indicate a less stressful environment for the birds, as a lower H:L ratio is often associated with reduced stress and better immune competence (Minias, 2019). The elevated lymphocyte percentage suggests a stronger adaptive immune response, likely due to biochar's role in

improving overall health and possibly reducing subclinical infections. However, it is noteworthy that biochar supplementation did not significantly affect immunoglobulin levels (IgG, IgM, IgY, and IgT). This could be attributed to the relatively short experimental duration, and therefore future studies should explore the long-term effects of biochar on humoral immunity, particularly in terms of antibody production.

The numerical increase ($P=0.10$) in IgT levels in biochar-fed groups suggests that biochar may have immunomodulatory properties, although the lack of statistical significance warrants further investigation. Biochar's ability to reduce mycotoxin toxicity (through adsorption) could indirectly improve immune function, as mycotoxins are known to impair cellular immunity (Rajput et al, 2017). Therefore, the observed trends in immunoglobulin levels could reflect biochar's potential to alleviate the immunosuppressive effects of mycotoxins and other environmental stressors.

The beneficial effects of biochar supplementation were also demonstrated in broilers fed 0.5% biochar derived from rice husk, which resulted in lower mortality rates, enhanced growth performance, and improved immune function (Nair et al, 2023). These improvements were linked to the mitigation of aflatoxin-related immunosuppressive effects, as aflatoxin-contaminated feed is known to impair cellular immunity and reduce antibody production in response to sheep red blood cells (Bagherzadeh Kasmani et al, 2012). The immunosuppressive effects of aflatoxins are primarily attributed to the inhibition of protein synthesis, including key immunoglobulins such as IgG and IgA (Rajput et al, 2017), along with a reduction in complement hemolytic activity (Chen et al, 2014) and a decline in lymphocyte counts.

Biochar exhibits significant electron transfer capabilities that may help mitigate

oxidative stress in animals. It can activate hydrogen peroxide (H_2O_2), generating hydroxyl radicals ($\bullet\text{OH}$) through interactions with persistent free radicals in its structure (Fang, 2014). This process facilitates electron transfer, promoting the degradation of harmful compounds. The graphitic structure of biochar enhances this function by lowering the energy barrier for electron movement, increasing its efficiency (Dou, 2023). Additionally, biochar can act as an electron mediator between bacteria and minerals, further supporting its role in biological electron transfer processes (Kappler, 2014).

Moreover, biochar's potential to enhance the antioxidant defense system may involve modulation of key endogenous enzymes such as glutathione peroxidase (GSH-Px), catalase, and superoxide dismutase. Glutathione peroxidase (GSH-Px), in particular, eliminates hydrogen peroxide produced during lipid oxidation (Almeina et al., 2012). Biochar is proposed to exert antioxidant effects by stabilizing lipid membranes and inhibiting free radical-induced lipid peroxidation. Malondialdehyde (MDA), a marker of polyunsaturated fatty acid oxidation, reflects oxidative stress, with elevated levels indicating lipid damage (Gawel et al, 2004). These properties suggest that biochar may help mitigate oxidative stress by neutralizing free radicals and enhancing electron transfer.

One of the notable environmental benefits of biochar supplementation in poultry diets is its potential to reduce ammonia (NH_3) emissions from manure (Sha et al, 2019). In the present study, biochar inclusion at 0.65% and 1% significantly reduced ammonia emissions at both 0 and 24 hours post-excretion. This result aligns with the previous studies showing that biochar, with its high surface area and porosity, can adsorb and sequester ammonia (Prasai et al, 2018). The reduction in ammonia emissions not only mitigates air pollution in poultry houses but also

improves animal welfare by decreasing exposure to high concentrations of ammonia, which can lead to respiratory issues and stress in birds (Kalus et al, 2019). The mechanisms behind biochar's ability to reduce ammonia emissions are multifaceted. Biochar may increase the pH of manure, which could lead to the conversion of ammonium (NH_4^+) to ammonia gas (NH_3), which is then adsorbed by biochar particles (Agyarko-Mintah et al, 2017). Moreover, biochar's porous structure provides a vast surface area for nitrogen retention, further decreasing ammonia volatility and odor. The results from this study highlight the dual benefit of PBB in poultry diets: improving bird health while simultaneously reducing environmental pollutants.

While moderate levels of biochar inclusion—such as the 0.65% used in this study—have demonstrated positive outcomes, it is essential to recognize that higher dosages may introduce safety risks. At elevated concentrations, the inherently adsorptive properties of biochar may interfere with nutrient availability, potentially binding vital components like amino acids and vitamins (Al-Khalafah and Al-Nasser, 2023). Although the analysis of 112 scientific papers on biochar feed supplements has shown that no significant negative effects on animal health were found in any of the reviewed publications (Schmidt et al, 2019), comprehensive toxicological assessments, encompassing extended feeding trials and residue analyses, remain crucial to validating the safety of biochar as a feed additive.

Dietary inclusion of PB biochar at 0.65% significantly improved weight gain, feed efficiency, and antioxidant enzyme activity in Japanese quails. Additionally, PB biochar enhanced immune function by increasing lymphocyte counts and decreasing the heterophil-to-lymphocyte ratio, without negatively affecting feed intake or immunoglobulin levels. Both

0.65% and 1% PBB effectively reduced ammonia emissions from manure, indicating environmental benefits. The findings suggest that PB biochar can be incorporated into quail diets at levels as low as 0.65%, providing comparable or even superior benefits to antibiotic supplementation, while simultaneously

reducing reliance on chemical additives. Despite the promising effects of biochar on performance, immunity, and environmental parameters, further research is needed to fully elucidate its long-term safety, optimal inclusion levels, and comparative efficacy against other non-antibiotic feed additives under commercial production conditions.

Ethics approval

This study protocol was approved by the Research Animal Ethics Committee at the Islamic Azad University (IR.IAU.AEC.) with the reference number of 162773541 (1402/02/25).

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Conflict of interest

No conflicts of interest are declared by the authors.

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بهبود سیستم ایمنی و وضعیت آنتی اکسیدانی در بلدرچین ژاپنی با استفاده از بیوچار

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چکیده

این مطالعه به ارزیابی تأثیرات بیوچار حاصل از محصولات جانبی پسته بر عملکرد، متابولیت‌های خونی، پاسخ ایمنی، وضعیت آنتی‌اکسیدانی و انتشار گاز آمونیاک در بلدرچین‌های ژاپنی پرداخت. در مجموع، ۵۰۰ قطعه بلدرچین یک روزه ژاپنی به طراحی کاملاً تصادفی با پنج تیمار غذایی و پنج تکرار (۲۰ قطعه بلدرچین در هر تکرار) به مدت ۲۵ روز تخصیص داده شدند. جیره‌های غذایی آزمایشی شامل موارد زیر بودند: (۱) جیره پایه بدون افزودنی (شاهد)، (۲) جیره پایه با ۰/۰۵ درصد فلوکوئین ۱۰ درصد (شاهد مثبت)، (۳) جیره پایه با ۰/۳۵ درصد بیوچار، (۴) جیره پایه با ۰/۶۵ درصد بیوچار و (۵) جیره پایه با ۱ درصد بیوچار. نتایج نشان داد که وزن در بلدرچین‌های تغذیه شده با ۰/۶۵ درصد بیوچار نسبت به گروه شاهد و مکمل شده با فلوکوئین به طور معنی‌داری بیشتر بود، بدون آن که مصرف خوراک تحت تأثیر قرار بگیرد. ضریب تبدیل نیز در پرندگان تغذیه شده با ۰/۶۵ درصد بیوچار نسبت به شاهد تمایل به معنی‌داری داشت. بلدرچین‌های تغذیه شده با ۱ درصد بیوچار به طور معنی‌داری سطح کلسترول و LDL کمتری داشتند، در حالی که گروه شاهد بالاترین مقدار را نشان دادند. بالاترین درصد لنفوسیت‌ها در بلدرچین‌های تغذیه شده با ۱ درصد بیوچار مشاهده شد و افزایش سطح بیوچار در جیره غذایی نسبت لنفوسیت/هتروفیل را به طور معنی‌داری کاهش داد. با این حال، مکمل کردن بیوچار تأثیر معنی‌داری بر سطوح ایمونوگلوبولین‌ها (IgY, IgM, IgG) و (IgT) نداشت. نشان‌گرهای آنتی‌اکسیدانی، از جمله ظرفیت آنتی‌اکسیدانی کل، گلوکاتیون پراکسیداز و سوپراکسید دیسموتاز، در پرندگان تغذیه شده با ۱ درصد بیوچار پوست پسته بالاترین مقادیر را داشتند ولی تفاوت معنی‌داری بین سطوح ۰/۶۵ درصد و ۱ درصد مشاهده نشد. علاوه بر این، مکمل کردن بیوچار انتشار گاز آمونیاک را به طور معنی‌داری کاهش داد. به طور کلی، افزودن حداقل ۰/۶۵ درصد بیوچار پوست پسته به جیره غذایی بلدرچین‌های گوشتی، عملکرد رشد، پارامترهای خونی، فعالیت آنزیم‌های آنتی‌اکسیدانی و پاسخ ایمنی را بهبود بخشید و جایگزینی دوستدار محیط زیست برای آنتی‌بیوتیک‌ها ارائه داد.

کلمات کلیدی: بیوچار، پوست پسته، متابولیت‌های خونی، پاسخ ایمنی، انتشار گاز آمونیاک

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