



RESEARCH ARTICLE

Impact of Biochar and Organic Acid-Enriched Diet on Growth Performance and Intestinal Histomorphometry in Japanese Quail (*Coturnix coturnix japonica*)

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ABSTRACT

The current study aimed to assess the potential effects of biochar and propionic acid on production performance, and intestinal histomorphometry in Japanese quails. For this purpose, a completely randomized design randomly assigned 300, day-old birds to four treatment groups (T1, T2, T3, and T4). The T1 group received a basal diet and served as the control group while the T2 group received diet supplemented with corn cob biochar (@2%), T3 group diet was supplemented with corn cob biochar (@1.5%) and propionic acid (@0.5%), and the T4 group was offered propionic acid (@0.5%) only for six weeks. The weekly weight gain, feed intake, and FCR were recorded during the trial. At the completion of the trial, birds were euthanized humanely, and the intestine was collected and processed for the morphological parameters (weight, length, width). Statistical analysis revealed that significant ($P < 0.05$) variations were observed in body weight gain, feed intake, and FCR among the treatment groups supplemented with biochar and organic acids compared to the control. Histomorphometric analysis revealed significant ($P < 0.05$) improvements in villus height, villus width, crypt depth, villus height to crypt depth ratio, villus surface area, and thickness of tunica muscularis and externa in all experimental groups compared to the basal diet group. It has been concluded that biochar and propionic acid has the potential to be used as growth promoters, leading to improved growth performance and gut morphometry in Japanese quails.

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INTRODUCTION

Poultry feed contains biological and chemical growth promoters that facilitate maximum growth and production by improving feed utilization (Khalil *et al.*, 2020; Goodarzi *et al.*, 2024). Growth promoters in poultry include diverse substances including antibiotics, probiotics, prebiotics, enzymes, acidulants, antioxidants, and phytogens. These help to improve feed intake, enhance feed conversion rate, activate the body system, and promote energy by regulating gut microflora in poultry which is associated with improving the overall economic efficiency of production (Tous *et al.*, 2022; Gul and Alsayeqh, 2023; Raza *et al.*, 2024).

However, due to certain serious health concerns and the rise of antibiotic resistance, antibiotic growth promoters have been banned for use in poultry feed (Gul and Alsayeqh, 2023; Raza *et al.*, 2024).

Alternatively, acidulants, including propionic acid, formic acid, and citric acid could be considered a great choice since they lower the pH of the digestive tract, thereby inhibiting the growth of pathogenic bacteria and promoting the growth of beneficial bacteria in addition to their role in protein digestibility and gastric proteolysis (Haq *et al.*, 2017; Palamidi and Mountzouris, 2018; Raza *et al.*, 2024). Additionally, organic acids decrease amines and ammonia-like bacterial metabolites to improve feed proficiency (Abd El-Ghany, 2024) by enhancing the absorption of nutrients and ultimately their digestion. Biochar, a carbon-rich product obtained through the pyrolysis process (Willson *et al.*, 2019), has been associated with neutralizing the GIT toxins (Prasai *et al.*, 2016), improving feed efficiency and overall bird performance by acting as a good source of minerals (Gerlach and Schmidt, 2012). Additionally, it promotes bone

mineralization (Evans *et al.*, 2017), improves growth performance and litter quality (Kalus *et al.*, 2020) without harming broiler performance (Linhoss *et al.*, 2019).

However, the properties of biochar could be improved by various physical and chemical modifications (Yang *et al.*, 2019) which could result in significantly improving its physicochemical properties including specific pore size, surface area, molecular weight, type of functional groups, and cation exchange capacity (CEC) (Nazari *et al.*, 2019; Mihoub *et al.*, 2022). Among various modifiers of biochar, organic acids utilization with biochar as a chemical modifier can result in the enrichment of biochar improving its overall quality. This study aimed to analyze the impact of biochar enriched diet combined with propionic acid supplementation on the histomorphometric analysis of growth parameters and intestinal structure in Japanese quail (*Coturnix coturnix japonica*).

MATERIALS AND METHODS

Ethical approval: The following study was conducted at an experimentally controlled poultry shed of the University of Agriculture Faisalabad after ethical approval from the Institutional Biosafety and Bioethics Committee (IBBC), vide letter D.No:3476/ORIC dated: 15-07-2022.

Preparation of biochar: Biochar was prepared in a portable metallic kiln (CCM-7). It is a UK-designed carbon catcher model. The corn cob was burnt at 450–550°C in an oxygen-limited environment for 120–150 minutes, within a kiln. It was cooled down by an immediate sprinkling of water. Otherwise, they were converted into ash (Rajput *et al.*, 2024). Biochar was enriched further with propionic acid by mixing its solution with biochar at ambient temperature (25±2°C). The impregnated biochar was dehydrated for 24 hours at room temperature. Finally, the biochar was pulverized and used for supplementation.

Experimental design: A total of 300-day-old Japanese quail (non-sexed) chicks were used in this study. The chicks were kept in formalin disinfected control house cages disinfected by formalin at 37°C and 75% relative humidity level with 16 hours of light period. The quail chicks were weighed individually and wing-banded for purposes of identification. They were randomly allocated into four equal groups, with 75 chicks per group. Each group was further divided into three replicates, consisting of 25 birds per replicate. The experimental birds were randomly divided as; Group (T1) served as a negative control and was supplemented with a commercially available basal diet. The Group (T2) received a basal diet with 2% corn cob biochar. The group (T3) was supplemented with 1.5% corn biochar blended with 0.5% propionic acid, while the group (T4) received 0.5% propionic acid only. The experiment was conducted over a period of six weeks. At the end of the trial, the birds were humanely euthanised. The feeding composition of commercially available basal diet of the birds has been mentioned in Table 1.

Table 1: Ingredients of the Basal Diet Formulated According to NRC (1994) Guidelines

Ingredients	Percentage (%)
Corn	35.12
Wheat Bran	1.00
Canola Meal	15.01
Fish Meal	2.00
Soybean Meal	15.64
Corn Gluten Meal	1.21
Poultry Byproduct meal	0.00
Rapeseed Meal	4.00
Marble Chips	0.79
DCP	0.80
Lysine sulphate	0.56
DL Methionine	0.08
Threonine	0.07
Molasses	0.63
Premix	0.24
Salt	0.18
Phazyme	0.05
Rice Broken	22.60
Percentage	100.00

* Crude protein (CP) = 23%.

Production parameters: The quail body weight was taken using an electrical balance with a precision of ±0.01g on weekly basis. The feed intake was computed in every replicate. The mortalities were recorded throughout the rearing period to adjust the bird's number and for accurate calculation of feed intake per quail. Feed intake (FI), was recorded using this equation

$$\text{Feed intake (g/bird)} = \frac{\text{feed intake in a replicate}}{\text{number of birds in a replicate}}$$

For body weight gain (BWG) the following equation was used.

$$\text{Body weight gain (BWG)(g)} = \text{Final weight} - \text{Initial weight}$$

While feed conversion ratio (FCR) was computed weekly using the following equation.

$$\text{Feed conversion ratio (FCR)} = \frac{\text{Feed intake (kg)}}{\text{Weight gain (kg)}}$$

Collection of samples: Samples of the small intestine and large intestine of each quail were collected for histomorphometric analysis. After the necropsy, gross examination of the intestines was completed. Subsequently, the organs were rinsed in 0.5% saline solution, followed by fixation in 10% neutral buffered formalin for microscopic analysis.

Macroscopic measurements: The intestine was studied carefully for macroscopic appearance and to measure the anatomical dimensions (width, length, weight). The measurements of length and width were taken with the help of a digital vernier calliper and measuring tape. The electrical weighing balance was used for the weight of the intestine. The relative weight (%) of the small and large intestine was calculated using following formula.

$$\text{Relative weight (\%)} = \frac{\text{organ weight (g)}}{\text{body weight (g)}} \times 100$$

Histomorphometric analysis: For histological analysis, fixed tissue samples were dehydrated, cleared, and embedded in paraffin wax using standard tissue processing

techniques. Thin sections (5 μ m) were cut using a microtome, mounted on slides, and stained with hematoxylin and eosin (H&E) manually for detailed microscopic examination of cellular and structural features as described by Suvarna et al. (2018). Following slide preparation, photomicrographs of each sample were taken with the help of a Nikon microscope at 200X. The Image J[®] Computer software was used for the measurement of the thickness of muscularis mucosa and muscularis externa layers of intestines). The villus width (vw)(μ m), villus height (vh)(μ m), crypt depth (μ m), and villus height/ crypt depth ratio were recorded. The height of the villi was recorded from the tip of the villi to the junction of the villi and crypt. The depth of the crypt was recorded from the base of the crypt to the crypt and villus junction (Awad *et al.*, 2009). The following equation was used to calculate the surface area of villi (μ m²), where VW represents villus width and VL represents villus length (μ m) (Sakamoto *et al.*, 2000).

Surface area of villi $= (2\pi)(VW/2)(VH)$

Statistical analysis: The standard method of one-way analysis of variance (ANOVA) with complete randomized design (CRD) was used to evaluate the data and Tukey's Test was used to compare treatment means, as suggested by Montgomery, (2019).

RESULTS

Production parameters: The production parameters involving weekly weight gain (g), feed intake (g), and FCR are presented in Table 2 (a, b, and c). The results of one-way ANOVA on weekly body weight gain revealed significant differences ($P < 0.05$) among the experimental groups throughout the trial (Table 2a). The multiple comparisons using Tukey's test indicated a significant difference ($P < 0.05$) in the mean values among all groups. Group T3, (supplemented with 1.5% corn biochar blended with 0.5% propionic acid), consistently exhibited the highest performance during the initial weeks, followed closely by Group T2, (which received a basal diet with 2% corn cob biochar). However, Group T2, outperformed as compared to other groups, including (T3), in the later weeks, particularly during Weeks 5 and 6. Group (T4), supplemented with 0.5% propionic acid only, demonstrated intermediate performance, generally below Groups (T2) and (T3) but superior to Group (T1). Group (T1), serving as the negative control with a commercially available basal diet, recorded the lowest performance throughout the study. The observed differences between groups were statistically significant ($P < 0.05$), underscoring the effectiveness of the dietary interventions, particularly the combination of biochar and propionic acid, to enhance performance metrics. These findings suggest a synergistic effect of biochar and propionic acid supplementation, warranting further investigation into their mechanisms of action.

Both Tables 2b and 2c show that Group (T1), serving as the negative control, consistently demonstrated the significantly highest feed intake throughout the trial, indicating that the commercially available diet supported greater consumption and hence lower FCR compared to

the experimental diets ($P < 0.05$) indicative of less efficient feed utilization. Group (T3), exhibited the lowest feed intake throughout the study and consistently demonstrated the lowest FCR, reflecting optimal feed conversion and enhanced feed efficiency. Groups (T2) and (T4), receiving 2% corn cob biochar and 0.5% propionic acid, respectively, showed intermediate feed intake, with Group (T4) exhibiting slightly higher values in the later weeks, displaying intermediate FCR values. These findings highlight distinct differences in feed intake patterns, likely influenced by dietary treatments.

Gross morphometry of intestine: The small and large intestines were weighed, and their relative weight was calculated after removing intestinal content in all treatment groups provided biochar, biochar + propionic acid, and propionic acid. The empty weight as well as the relative weight of small and large intestines showed a significant difference ($P < 0.05$) in mean value among the treated groups. In the small intestine, the highest weight (g) was recorded in Group T3, while the highest relative weight (%) was observed in Group T2. For the large intestine, both weight and relative weight were highest in Group T3.

Regarding morphometric parameters, in the small intestine, the greatest length (cm) was recorded in Group T2, while the highest width (mm) was observed in Group T3. In contrast, for the large intestine, both length and width were highest in Group T3. These findings indicate that dietary supplementation with corn biochar and propionic acid significantly influenced intestinal morphometry, with Group T3 showing the most pronounced effects overall. The results are graphically presented in Fig. 1.

Intestinal histomorphometry: The impact of biochar, biochar + propionic acid, and propionic acid alone on histomorphometry of the small intestine parts of duodenum, jejunum, and ileum, was recorded. Parameters including villus (width, height) (μ m), crypt depth (μ m), and villus height/ crypt depth ratio, and the thickness of tunica muscularis and muscularis mucosa were evaluated, revealing significant differences ($P < 0.05$) among the treatment groups.

The histological analysis revealed significant improvements ($P < 0.05$) in intestinal morphometry across the duodenum, jejunum, and ileum in response to dietary treatments. In the duodenum, Group T3 (1.5% corn biochar+0.5% propionic acid) exhibited the highest villus height (Fig. 2a), villus height-to-crypt depth ratio (Fig. 2e), muscularis externa thickness (Fig. 2f), and mucosa thickness (Fig. 2g), while Group T2 showed the highest villus width (Fig. 2b). Villus surface area (Fig. 2c) was significantly higher in Groups T2 and T3, whereas crypt depth (Fig. 2d) was greatest in Group T1, followed by Group T2.

In jejunum, group T3 consistently demonstrated superior results, with the highest villus height (Fig. 3a), villus width (Fig. 3b), villus surface area (Fig. 3c), villus height-to-crypt depth ratio (Fig. 3e), muscularis externa thickness (Fig. 3f), and crypt depth (Fig. 3d). However, mucosa thickness (Fig. 3g) was higher in Groups T1 and T2. In ileum, Group T3 again showed the highest villus

height (Fig. 4a), villus surface area (Fig. 4c), villus height-to-crypt depth ratio (Fig. 4e), muscularis externa thickness (Fig. 4f), and mucosa thickness (Fig. 4g). Interestingly, the control group (T1) had the highest villus width (Fig. 4b), while Group T2 showed the deepest crypt depth (Fig. 4d). These findings indicate that dietary

supplementation with 1.5% corn biochar blended with 0.5% propionic acid (Group T3) significantly enhanced the histological architecture of the intestinal segments, particularly in the duodenum, jejunum, and ileum, compared to the control group (T1).

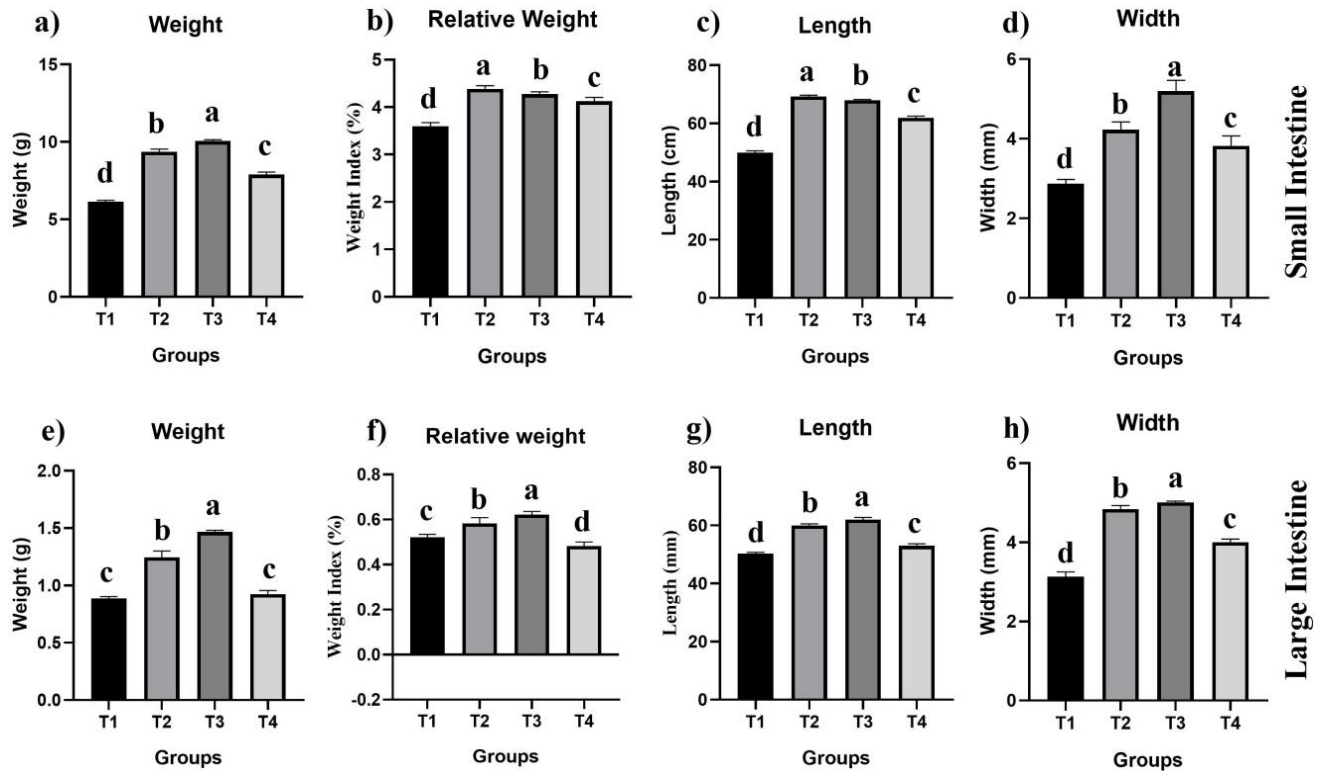


Fig. 1: Bar graph showing weight (g), relative weight (%), length (cm), and width (mm), of small and large intestines in Japanese quail. The bars with different superscripts show statistical differences ($P < 0.05$). T1 = negative control, T2 = experimental group supplemented with basal diet along with 2% corn cob biochar, T3 = experimental group supplemented with basal diet containing 1.5% corn biochar blended with 0.5% propionic acid, T4 = fed on diet mixed with 0.5% propionic acid only.

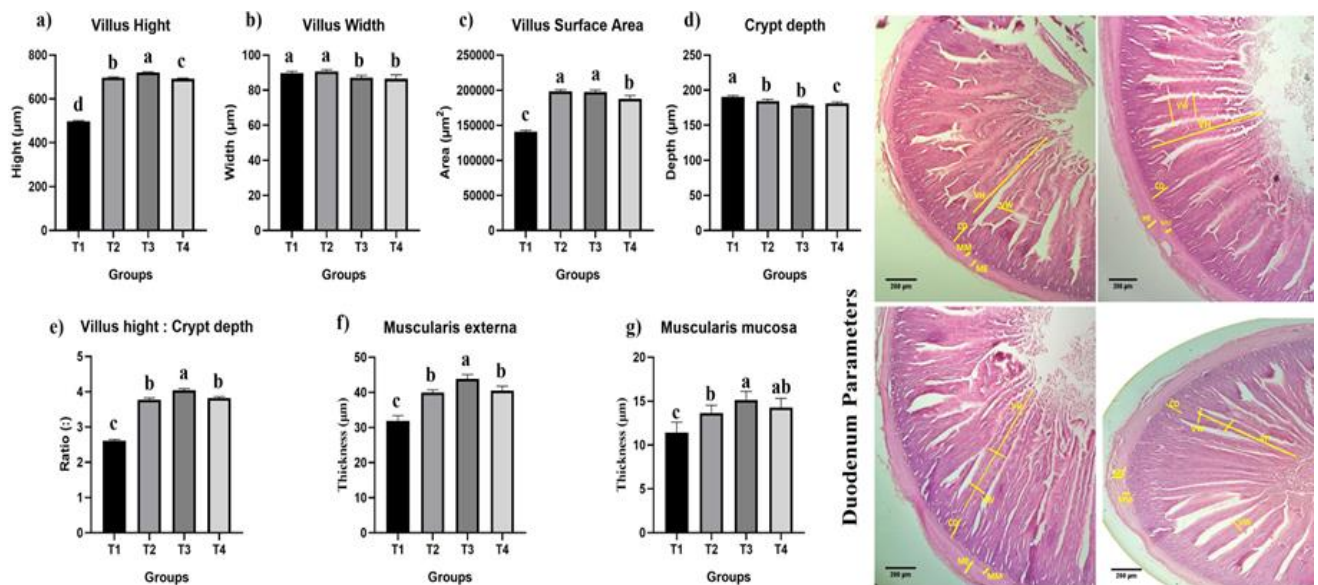


Fig. 2: (A) Graphical representation of the impact of biochar and propionic acid supplementation in basal diet on the histomorphometric characteristic of duodenum in Japanese quail. The bars with different superscripts show statistical differences ($P < 0.05$). a) villus height (μm) b) villus width (μm), c) villus surface (μm^2), d) crypt depth (μm), e) villus height: crypt depth, f) muscularis externa (μm), g) thickness of muscularis mucosa (μm). (B) H & E staining of duodenum for histomorphometric analysis illustrating mucosa, muscularis, and serosa. VH: Villus height, VW: Villus width, CD: Crypt depth, VSA: Villus surface area, MM: Muscularis mucosa thickness, ME: Muscularis externa thickness, VH:CD: Villus height/crypt depth ratio. T1 = negative control, T2 = experimental group supplemented with basal diet along with 2% corn cob biochar, T3 =

experimental group supplemented with basal diet containing 1.5% corn biochar blended with 0.5% propionic acid, T4= fed on diet mixed with 0.5% propionic acid only.

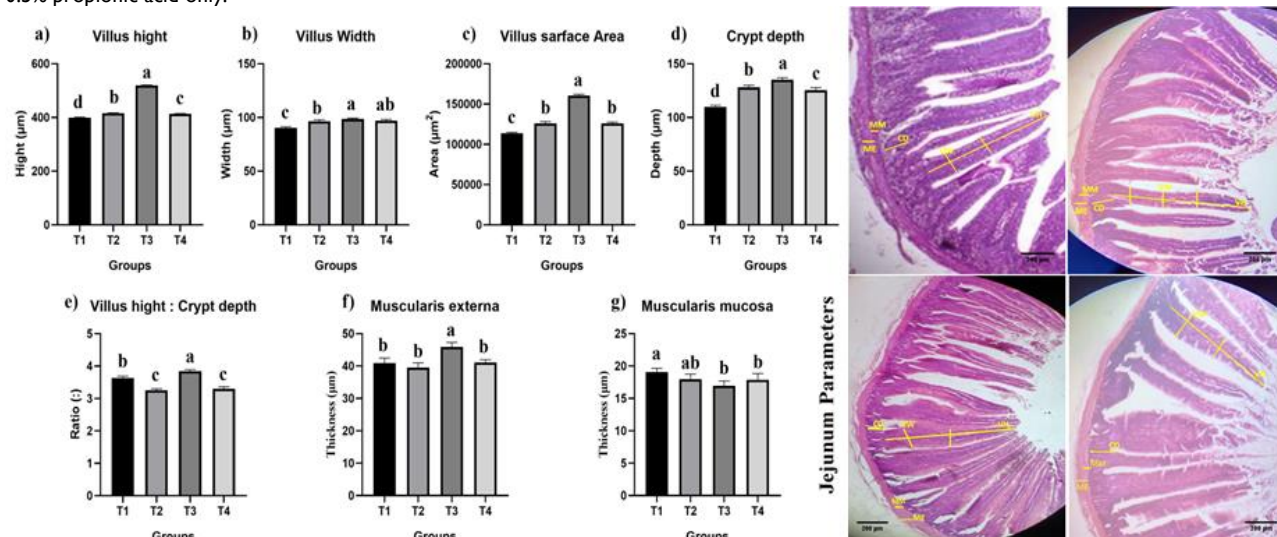


Fig. 3: (A) Graphical representation of the impact of biochar and propionic acid supplementation in basal diet on the histomorphometric characteristic of jejunum in Japanese quail. The bars with different superscripts show statistical differences ($P < 0.05$). a) villus height (μm) b) villus width (μm), c) villus surface (μm^2), d) crypt depth (μm), e) villus height: crypt depth, f) muscularis externa (μm), g) thickness of muscularis mucosa (μm). (B) H & E staining of jejunum for histomorphometric analysis illustrating mucosa, muscularis, and serosa. VH: Villus height, VW: Villus width, CD: Crypt depth, VSA: Villus surface area, MM: Muscularis mucosa thickness, ME: Muscularis externa thickness, VH:CD: Villus height/crypt depth ratio. T1= negative control, T2 = experimental group supplemented with basal diet along with 2% corn cob biochar, T3 = experimental group supplemented with basal diet containing 1.5% corn biochar blended with 0.5% propionic acid, T4= fed on diet mixed with 0.5% propionic acid only.

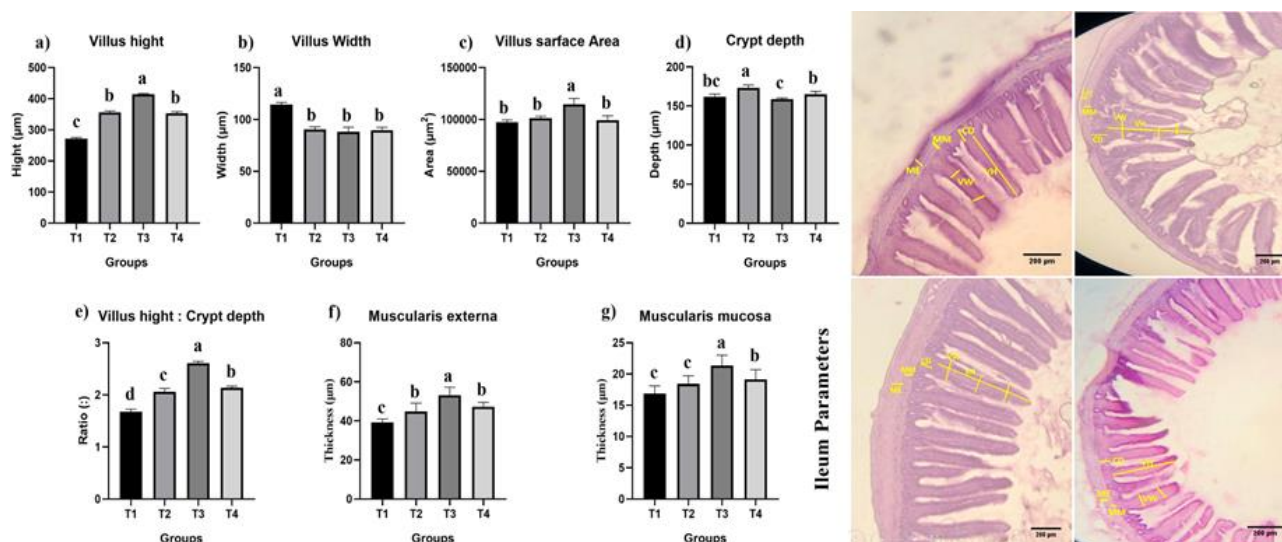


Fig. 4: (A) Graphical representation of the impact of biochar and propionic acid supplementation in basal diet on the histomorphometric characteristic of ileum in Japanese quail. The bars with different superscripts show statistical differences ($P < 0.05$). a) villus height (μm) b) villus width (μm), c) villus surface (μm^2), d) crypt depth (μm), e) villus height: crypt depth, f) muscularis externa (μm), g) thickness of muscularis mucosa (μm). (B) H & E staining of ileum for histomorphometric analysis illustrating mucosa, muscularis, and serosa. VH: Villus height, VW: Villus width, CD: Crypt depth, VSA: Villus surface area, MM: Muscularis mucosa thickness, ME: Muscularis externa thickness, VH:CD: Villus height/crypt depth ratio. T1= negative control, T2 = experimental group supplemented with basal diet along with 2% corn cob biochar, T3 = experimental group supplemented with basal diet containing 1.5% corn biochar blended with 0.5% propionic acid, T4= fed on diet mixed with 0.5% propionic acid only.

DISCUSSION

The feed additives are one of the most essential factors for cost-effective poultry production. Many studies have reported the rational use of feed additives which ultimately leads to improved growth performance and health (Khalil *et al.*, 2020; Masood *et al.*, 2020). The organic and safe feed additives are replacing synthetic growth promoters including antibiotics due to growing concerns regarding resistance and food safety (Gul and Alsayeqh, 2023; Raza *et al.*, 2024). Alternatively, both

biochar and organic acid have emerged as alternative additives, offering potential benefits for enhancing overall production parameters in the poultry industry (Kalus *et al.*, 2020).

The current study demonstrated significant improvement in the production parameters of quail including body weight gain and feed efficiency, which was improved in all experimental groups particularly Group (T3), supplemented with 1.5% corn biochar blended with 0.5% propionic acid. These results are consistent with the findings of Dim *et al.* (2018) who

revealed that the final body weight, average daily weight gain, and FCR were improved in birds who were fed 6% biochar, propionic acid, and their combination on weekly weight gain (g/bird), feed intake in (g), and feed conversion ratio (FCR) of Japanese quail

Weeks	T1	T2	T3	T4	P-value
a). Weekly weight gain (g/bird)					
Week 1	34.89±0.19 ^{bc}	37.49±0.27 ^b	39.15±0.25 ^a	37.04±0.24 ^b	0.001
Week 2	62.54±0.14 ^d	70.27±0.15 ^b	71.99±0.15 ^a	66.29±0.42 ^c	0.001
Week 3	101.45±0.16 ^d	105.93±0.23 ^b	109.38±0.25 ^a	103.98±0.21 ^c	0.000
Week 4	137.45±0.17 ^d	148.15±0.39 ^b	150.56±0.16 ^a	140.50±0.18 ^c	0.007
Week 5	173.58±0.19 ^c	218.29±0.25 ^a	217.09±0.29 ^a	191.11±0.50 ^b	0.000
Week 6	173.01±0.28 ^d	219.74±0.12 ^a	217.66±0.15 ^b	192.06±0.52 ^c	0.001
b). Feed intake (g)					
Week 1	9.119±0.123 ^a	7.777±0.145 ^b	7.030±0.167 ^c	8.035±0.135 ^b	0.005
Week 2	13.470±0.142 ^a	12.093±0.152 ^b	11.349±0.191 ^c	12.487±0.086 ^b	0.001
Week 3	19.307±0.158 ^a	17.638±0.145 ^b	16.478±0.101 ^c	18.067±0.223 ^b	0.000
Week 4	22.709±0.164 ^a	21.272±0.149 ^b	20.919±0.073 ^b	22.520±0.116 ^a	0.001
Week 5	23.956±0.135 ^a	22.473±0.134 ^c	21.880±0.159 ^d	23.176±0.159 ^b	0.000
Week 6	24.171±0.152 ^a	23.249±0.139 ^b	22.062±0.109 ^c	23.503±0.151 ^b	0.001
c). Feed conversion ratio (FCR)					
Week 1	2.40±0.017 ^a	2.38±0.01 ^a	2.35±0.017 ^a	2.39±0.005 ^a	0.001
Week 2	3.10±0.058 ^a	2.97±0.02 ^{ab}	2.80±0.006 ^c	2.85±0.029 ^{bc}	0.001
Week 3	3.49±0.033 ^a	3.32±0.03 ^b	3.13±0.071 ^c	3.34±0.024 ^{ab}	0.004
Week 4	3.69±0.018 ^a	3.37±0.09 ^b	3.33±0.074 ^b	3.45±0.015 ^{ab}	0.001
Week 5	3.81±0.028 ^a	3.39±0.00 ^b	3.18±0.057 ^c	3.42±0.004 ^b	0.000
Week 6	4.06±0.035 ^a	3.32±0.01 ^b	3.23±0.047 ^{bc}	3.36±0.012 ^c	0.001

*The results are presented as mean± standard error of the mean (SEM). Within each row, values with different superscripts (a, b, c, d) differ significantly at P<0.05. T1= negative control, T2 = experimental group supplemented with basal diet along with 2% corn cob biochar, T3 = experimental group supplemented with basal diet containing 1.5% corn biochar blended with 0.5% propionic acid, T4= fed on diet mixed with 0.5% propionic acid only.

charcoal inclusion. Similarly, Flores *et al.* (2021), reported that biochar supplementation in turkey diet resulted in improved growth performance. Studies by Mohammed *et al.* (2018) and Beier *et al.* (2019) also reported the beneficial effects of the supplementation of propionic acid on the production parameters and the health status of birds.

It has been reported that the growth-enhancing properties of biochar and propionic acid are afflicted by their strong antibacterial actions and pathogen absorption capabilities (Schmidt *et al.*, 2019; Abbas *et al.*, 2021). Further, biochar reduces antibiotic residues, increases nutritional intake, and detoxifies contaminants leading to enhanced nutrient absorption in the gut (Khoa *et al.*, 2018), hence overall improvements in weight gain, immune response, feed intake, feed conversion rates, carcass features, and overall quality of animal products are observed (Hien *et al.*, 2018). Similar growth-promoting impacts of propionic acid were observed in quails (Khan *et al.*, 2016) and broilers (Rimbawanto *et al.*, 2019; Palupi *et al.*, 2020). Contrary to our findings, some researchers also reported inconsistent effects of biochar on poultry performance (Odunsi *et al.*, 2007; Bagal *et al.*, 2016; Hajati, 2018). We assume that these contradictions in results could be associated with improper dosage supplementation, and short experimental duration for using biochar and propionic acid.

It has been reported that the shorter villi and long crypts depth is an indication of poor absorptive activity but high villi, villi height/crypt depth ratio are directly correlated with improved cellular activity of intestinal epithelium (Khan and Iqbal, 2016). The results of our study demonstrated significant improvements in the intestinal histomorphometry of quails supplemented with biochar, biochar combined with propionic acid, and propionic acid alone. The observed improvements in villus height, villus height-to-crypt depth ratio, crypt depth, and thickness of the muscularis externa across the

duodenum, jejunum, and ileum suggest a positive impact on intestinal architecture and function. The combination of biochar and propionic acid (Group T3) consistently exhibited the most pronounced effects on intestinal morphology which are indicative of enhanced nutrient absorption and an efficient epithelial turnover rate, as taller villi provide a larger surface area for nutrient uptake. These findings are consistent with the results of Rodjan *et al.* (2018) and Yang *et al.* (2018), who observed improved villus width, height, crypt depth, and area of the ileum, duodenum, and jejunum in broilers fed on a diet supplemented with organic acids at different concentrations.

We assume that a significant increase in crypt depth observed in the experimental groups could be associated with a heightened proliferative activity in the intestinal mucosa, reflecting an active repair and renewal process. This is particularly relevant for maintaining intestinal integrity and optimal digestive function under intensive production systems. According to our results, the significant thickening of the muscularis externa and muscularis mucosa in the supplemented groups further underscores the role of dietary additives in supporting gut motility and overall digestive health. Similar to our findings, Majewska *et al.* (2011) reported improved gut morphology in broilers supplemented on a 1% bamboo charcoal diet. Consistent with our findings, Yamauchi *et al.* (2010) reported that the mixture of bamboo vinegar and bamboo charcoal enhances intestinal function in layers during the early laying stages. Likewise, Samanya and Yamauchi (2002) indicated charcoal powder, wood vinegar, or a combination of both improved intestinal villus function, increasing villus height, epithelial cell area, and mitotic activity. In another study, Rattanawut *et al.* (2017) reported that 1-1.5% dietary bamboo charcoal powder, including vinegar, enhanced villus size in the duodenum and jejunum, reduced ileal bacterial infection, and enhanced eggshell quality. The present study supports

these findings, showing that biochar supplementation in Japanese quails improved intestinal histomorphology, likely due to biochar's detoxifying and adsorption properties. Similarly, the antimicrobial activity of organic acids, which alter intestinal pH, further contributed to enhanced intestinal function.

Conclusions: The present study suggests that biochar and a combination of biochar and propionic acid have the potential to promote growth in poultry, specifically in quails. It was established that the biochar along with propionic acid are potential growth promoters. There was an overall improvement in gut and immune health with corn cob biochar and propionic acid that led to better growth performance in Japanese quail.

Authors contribution: MU, ASQ, RS and FD all were actively involved for conceiving the idea, research trail, data collection, write up and proof reading of the manuscript.

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