Dose titration of plantain herb (*Plantago lanceolata* L.) supplementation on growth performance, serum antioxidants status, liver enzymatic activity and meat quality in broiler chickens

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**ABSTRACT**

This study was aimed to find out the suitable dose of fresh plantain (*Plantago lanceolata* L.) supplementation for optimum growth, serum antioxidants status, liver health, and meat quality in broilers. A total of 1152-days-old Cobb-500 broilers (average weight: 45 ± 0.7 g) were randomly assigned into four dietary treatments, including (i) control (CON): corn-soya based basal diet, and plantain (PL) supplemented groups (ii) PL40: CON + 40 g fresh PL/kg diet; (iii) PL80: CON + 80 g fresh PL/kg diet; and (iv) PL120: CON + 120 g fresh PL/kg diet. Improved growth efficiency (*p* < .05) was observed in PL supplemented groups compared to CON, where PL80 and PL120 groups had the highest value. Serum superoxide dismutase and glutathione peroxidase concentrations were comparable in the PL80 and PL120 groups, but higher (*p* < .05) than other groups. The lowest concentrations of aspartate aminotransferase and alanine aminotransferase were found in the PL80 group, while alkaline phosphatase was the highest in the PL40 group. Furthermore, the PL80 group exhibited the lowest (*p* = .001) abdominal fat content and the highest (*p* = .002) breast meat yield. Meat linoleic acid content was nevertheless improved linearly with PL supplement levels, and the highest value was found in the PL120 group. Furthermore, the maximum meat redness (*a*) was observed in PL80 and PL120 groups, which was approximately twice that of the CON. Overall, the growth and health responses of both PL80 and PL120 groups were similar, while the latter had improved the meat fatty acid profile.

**HIGHLIGHTS**

- Supplementation of 80 g plantain/kg diet showed optimum growth performance, health status, and plasma antioxidants level in broilers.
- 120 g plantain/kg diet might be supplemented with the purpose of producing value-added broiler meat.

**Introduction**

The use of natural herbs and their extracts in broiler diets has been gaining popularity not only to ensure human health concerns but also for safe and value-added livestock products (Zheng et al. 2019). The European Union has banned the use of synthetic anti-biotic growth promoters in food animals, which provokes scientists to find out suitable alternatives (Selaledi et al. 2020). Plantain (PL; *Plantago lanceolata* L.), a narrow leaf perennial herb belonging to the family *Plantaginaceae* has been widely used as a human tonic and forage herb for ruminants and poultry (Camy et al. 2020; Redoy et al. 2020). It contains a rich phytochemical profile, among them acteoside, aucubin and catalpol have a broad aspect of beneficial effects on animal health and productivity (Yap et al. 2019). Bioactive components in PL exert anti-microbial, anti-oxidative, anti-inflammatory, anti-parasitic effects in the broiler (Ferrazzano et al. 2015; Boamah et al. 2016; Peña-Espinoza et al. 2018; Hammami et al. 2020). Besides, PL has higher free radical scavenging activity, which might prevent the meat pigments from oxidation leading to improved meat colour and sensory properties (Redoy et al. 2020). Moreover, the rich fatty acid profile in PL might enhance broiler meat

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nutrition (Bajer et al. 2016). However, the overdose of PL supplementation in broiler might carry some negative consequences as it also contains tannin, saponin, and other polyphenolic compounds (Ebrahim et al. 2015). The higher level of these components leads to reduce feed intake, nutrients absorption, poisoning, bone formation, even death in the broiler (Ebrahim et al. 2015). Chacrabati et al. (2013) reported that a 50 g fresh PL/kg diet could be used in the broiler diet as a replacement for a commercial antioxidant to improve the growth performance. In contrast, Mazhari et al. (2016) found improved growth along with better immunity in broiler fed fresh PL at 100 g/kg diet compared to 50 g/kg diet and control diet.

So, there is a dearth of information pertaining to the safe and optimum level of fresh PL supplementation in the broiler to improve production efficiency and meat quality. Therefore, the objective of this research was to find out the optimum dose of fresh PL supplementation in broiler diet concerning growth, plasma lipid profiles, plasma antioxidants, liver health, and meat quality.

**Materials and methods**

**Experimental birds**

A total of 1152 one-day-old straight run broiler chicks (Cobb-500) were purchased from a local hatchery and placed in open-sided poultry shed in Manikganj, Dhaka for a period of 35 days. The chicks (initial body weight: 45 ± 0.7 g) were randomly allocated to four dietary

| Table 1. Ingredients and nutritional composition of experimental diets. |
|---------------------------------------------------------------|
| **Ingredients in basal diet** | **Amounts (%)** |
| Corn (7.9% CP) | 55.70 |
| Rice polish (13.7% CP) | 4.00 |
| Soybean meal (44.1% CP) | 30.79 |
| Pro-Pak® (60% CP) | 5.20 |
| Rice bran oil | 2.00 |
| Lime stone dust | 0.44 |
| DL-methionine-99% | 0.53 |
| L-Lysine-79% | 0.42 |
| Threonine-98.5% | 0.22 |
| Choline chloride 60% | 0.20 |
| Sodium bicarbonate | 0.20 |
| Sodium chloride | 0.22 |
| Vitamin-mineral premix | 0.08 |

| Plantain supplemented groups |
|-----------------------------|
| **Nutrient composition** | **CON** | **PL40** | **PL80** | **PL120** | **Plantain** |
| Analyzed chemical composition, g/100 g DM | |
| Dry matter | 88.72 | 89.22 | 89.72 | 90.22 | 12.53 |
| Crude protein | 23.30 | 23.38 | 23.46 | 23.54 | 15.82 |
| Crude fibre | 4.57 | 4.64 | 4.71 | 4.78 | 14.19 |
| Ether extract | 3.98 | 3.99 | 4.01 | 4.03 | 3.05 |
| Nitrogen free extract | 49.81 | 50.08 | 50.34 | 50.61 | 53.08 |
| Ash | 8.52 | 8.59 | 8.66 | 8.73 | 13.86 |
| ME, Kcal/kg | 3081 | 3087 | 3093 | 3103 | 2023 |
| Calcium | 0.91 | 0.92 | 0.93 | 0.94 | 1.81 |
| Available phosphorus | 0.54 | 0.54 | 0.54 | 0.55 | 0.41 |
| Iron | 0.0146 | 0.0150 | 0.0154 | 0.0158 | 0.08 |

Calculated fatty acid profile, g/100 g fat
- Saturated fatty acid: 19.97, 20.40
- Unsaturated fatty acid: 80.03, 79.61
- MUFA: 36.53, 92.88
- PUFA: 41.82, 7.12

Phytochemical profile, mg/g DM
- Acteoside: 1.48, 2.96, 4.44, 29.56
- Aucubin: 0.86, 1.73, 2.59, 17.26
- Catalpol: 0.24, 0.47, 0.71, 4.69

**CON:** corn-soya based basal diet; **PL40:** CON + 40 g fresh plantain/kg diet, **PL80:** CON + 80 g fresh plantain/kg diet; **PL120:** CON + 120 g fresh plantain/kg diet.

**Pro-Pak** is a protein concentrate manufactured by H. J. BAKER & BRO., LLC, USA which contained: DM, 94.80%; CP, 60%; CF, 8.1%; Phosphorus, 2.2%; Calcium, 4.0%; Ash, 12.3%; ME, 2865 Kcal/kg; Pepsin digestibility, 90.0%.

Each kg vitamin mineral premix contained: Vitamin A, 13.500 U; Vitamin D₃, 1.500 U; a-DL-Tocopherol acetate, 50 mg; Thiamine, 3 mg; Riboflavin, 5 mg; Pyridoxin, 4 mg; Cobalamin, 15 pg; Folsaure, 200 pg; Nicotenic acid, 60 pg; Ca-Pitothenate, 30 mg; Cholin, 750 mg; Ascorbic acid, 150 mg.

**MUFA:** monounsaturated fatty acids; **PUFA:** polyunsaturated fatty acids.

**GUILL-GUERRERO (2001).**

**Calculated value.**
treatments with six replications (48 birds/replication). This experiment was conducted using a completely randomised design.

**Experimental diets**

The experimental diets included (i) CON = corn-soya based basal diet having crude protein = 23.30% and metabolisable energy = 3081 kcal/kg diet, (ii) PL40 = CON + 40 g fresh plantain/kg diet, (iii) PL80 = CON + 80 g fresh plantain/kg diet, (iv) PL120 = CON + 120 g fresh plantain/kg diet. According to the suggested nutrient requirements for commercial Cobb 500 broiler starter feed, the experimental diet (Table 1) was formulated and maintained throughout the feeding trial (COBB 2018). Plantain herb (PL) cv. *Grasslands Lancelot* was cultivated nearby a poultry farm and harvested at the pre-flowering stage. After collection, the fresh PL was chopped properly (approximately 0.5–1.5 cm) by using a locally made manual chopper and mixed thoroughly. This herb was supplemented from the 4th day of the feeding trial at the rate of 5 g, 10 g and 15 g DM/kg of basal diet in PL40, PL80, PL120 groups, respectively.

**Housing and management**

Experimental birds were kept in 24-floor pens (4–5 cm sawdust bedding) having a living space of 1.08 ft² for each bird. In the first week, the brooding temperature was kept at 34 °C and thereafter weekly decreased 3 °C until it reached 21 °C. The standard lighting program was maintained according to COBB-500 commercial broiler management guidelines (COBB 2018). The feed was supplied at 0800 and 1600 h, and birds always had access to clean and fresh water throughout the experimental period. Feeders were cleaned every week, and drinkers were cleaned twice a day. On days 5 and 12, the experimental birds were vaccinated against new castle disease and infectious bursal disease, respectively. Strict bio-security measures were followed during the whole experimental period.

**Record keeping and sample collection**

Pen live weights were recorded weekly, and live weight gain (LWG) was calculated from the difference between the initial (ILW) and final live weight (FLW). Feed intake (FI) was calculated by subtracting refusal from the supplied feed. Feed conversion ratio (FCR) was determined from the ratio between FI and LWG. Birds were monitored twice a day; dead birds’ weight was used to adjust for feed consumption. The performance efficiency factor (PEF) was calculated using the equation described by Martins et al. (2016). On days 35, 18 birds from each group (three birds/replication) were slaughtered to collect blood and meat samples. Immediate after blood collection, it was centrifuged at 6000 × g for 15 min for plasma separation and stored at 4 °C (Camy et al. 2020). Both breast and thigh meat samples were collected for meat colour analysis, but only breast meat was used for proximate and fatty acid profile analysis.

**Sample analysis**

All the samples were analysed in triplicate. Proximate components of fresh PL, basal feed, and meat samples were analysed according to AOAC (2005). Exactly 1 g of finely ground PL and basal feed samples were digested in 10 mL of a di-acid mixture ([HNO₃:HClO₄ = 2:1]) at 180–200 °C. For calcium determination, 1 mL of aliquot was mixed with dibromo-p-methylsulfonazo solution (2.5 mL), hydrochloric acid (1.5 mL), and distilled water (5 mL). For phosphorus determination, sulphomolybdc acid (4 mL) and stannous chloride solution (6 drops) were added to the aliquot (1 mL), followed by adding distilled water to make the volume 100 mL. Finally, the absorbance was taken at 624 nm wavelength for calcium and 600 nm wavelength for phosphorus in a UV spectrophotometer (T60; PG Instruments, UK) as a method followed by Li and Zhai (2020) and De Silva et al. (2015). In order to determine iron content, PL and basal feed samples (1 g) were digested using HNO₃ (10 mL) and H₂O₂ (2 mL) at 120 °C for 2 h. Then, the iron content was determined using a flame atomic absorption spectrophotometer (AA-7000; SHIMADZU, Japan) at 248.3 nm wavelength, where ferric nitrate solution was used as the standard solution (Marinova and Vladimirova 2010). For determination of acteoside, aucubin, and catalpol contents in PL, exactly 250 mg of powdered sample was dissolved into 25 mL of methanol (HPLC grade) followed by shaking and filtration. Thereafter, these components were quantified using ultra-high-performance liquid chromatography (UltiMate™ 3000; Thermo Fisher Scientific, USA). In this chromatography, the mobile phase used for acteoside determination was a water–methanol-acetic acid solution (14:6:1, v/v/v), whereas acetonitrile–water (2:98, v/v) solution was used for catalpol and aucubin determination. The flow rate was 0.8 mL/min. Finally, the acteoside was quantified at 330 nm wavelength, and catalpol and aucubin contents were determined at 204 nm wavelength in a diode array detector (DAD-3000; Thermo Fisher Scientific, USA) according to the method described by Al-Mamun et al. (2008). For serum triglyceride and
total cholesterol concentration determination, 10 μL serum sample was mixed with 1000 μL triglycerides reagent (Cat. No. 1155010, Linear Chemicals (LC), Spain) and 1000 μL cholesterol aqueous solution (Cat. No. 1118010; LC, Spain), respectively. After that, both the mixtures were incubated at 37°C for 5 min. Finally, the concentrations of both triglyceride and total cholesterol were measured at 505 nm wavelength in a bio-analyser (Urít-810; URIT Medical Electronic Group Co., Ltd., Guangxi, China). For high-density lipoprotein (HDL) content, 500 μL serum samples were mixed with 1000 μL precipitating reagent (Cat. No. 1133010, LC, Spain) followed by incubating the mixture at room temperature for 10 min, finally, the concentration was measured at 546 nm wavelength in bio-analysers. The low-density lipoprotein (LDL) and very-low-density lipoprotein (VLDL-C) were determined by using the following equations.

\[
LDL \text{ (mg/dL)} = \text{Total serum cholesterol} - \text{HDL} - \frac{\text{Triglyceride}}{5}
\]

(Lee and Siddiqui 2021)

\[
VLDL = C - \frac{\text{Triglyceride}}{5}
\]

(Lee and Siddiqui 2021)

For plasma superoxide dismutase (SOD) determination, 20 μL sample was mixed with 200 μL water-soluble tetrazolium salt solution and 20 μL enzyme working solution. The mixture was kept at 37°C for 20 min and after that took absorbance at 450 nm using a microplate reader (EL 10 A; BIOBASE, China) as instruction provided in Cat. No. 19160, Sigma-Aldrich, Germany. For glutathione peroxidase (GPx) determination specific ELISA kit (Cat. No. 354102, Sigma-Aldrich, Germany) was used, and exactly 20 μL sample was mixed with 50 μL assay buffer, 50 μL co-substrate mixture and, 50 μL NADPH according to manufacturer instruction. Finally, the absorbance was taken at 356 nm wavelength. Similar to SOD and GPx, catalase was determined using an assay kit according to the manufacturer’s protocol (Cat. No. 100, Sigma-Aldrich, Germany). Meat fatty acid profile was analysed using a gas chromatograph (14B; SHIMADZU, Japan) fitted with a flame ionisation detector as method followed by Redoy et al. (2020). Immediately after slaughter, meat samples were appropriately cleaned by rinsing the running tape water, and meat colour was determined by using a meat calorimeter (CR-410 colorimeter, Minolta, Japan) according to manufacturer guidelines. Meat colour was expressed by lightness (L*), redness (a*), and yellowness (b*).

### Table 2. Effects of different level of plantain herb (*Plantago lanceolata* L.) supplementation on performance index of broiler chickens at 35 days.

| Parameters          | CON   | PL40  | PL80  | PL120 | SEM  | Linear | Quadratic | Cubic |
|---------------------|-------|-------|-------|-------|------|--------|-----------|--------|
| Initial LW, g       | 45 ± 0.70 | 45 ± 0.50 | 45 ± 0.80 | 44 ± 1.00 | 0.23 | .348   | .141      | .977   |
| Final LW, g         | 1574 ± 7 | 1645 ± 10 | 1681 ± 9 | 1699 ± 11 | 14.69 | .000   | .001      | .389   |
| LWG, g              | 1529 ± 7 | 1600 ± 9 | 1635 ± 8 | 1655 ± 12 | 14.74 | .000   | .001      | .395   |
| Fl, g               | 2672 ± 16 | 2727 ± 24 | 2704 ± 20 | 2726 ± 29 | 8.72  | .042   | .255      | .066   |
| FCR (feed/gain)     | 1.74 ± 0.01 | 1.70 ± 0.01 | 1.65 ± 0.01 | 1.64 ± 0.02 | 0.01  | .000   | .082      | .265   |
| Mortality, %        | 2.33 ± 0.58 | 1.00 ± 0.00 | 1.00 ± 0.00 | 1.00 ± 0.00 | 0.24  | .003   | .050      | .620   |
| PEF, %              | 304.8 ± 4.45 | 331.9 ± 3.21 | 350.9 ± 6.32 | 356.5 ± 6.96 | 6.24  | .000   | .009      | .732   |

LW: live weight; LWG: live weight gain; Fl: feed intake; FCR: feed conversion ratio; PEF: production efficiency factors; SEM: standard error mean.

CON: corn-soya based basal diet having CP = 23.30% and ME = 3081 Kcal/kg diet; PL40: CON + 40 g fresh plantain/kg diet; PL80: CON + 80 g fresh plantain/kg diet; PL120: CON + 120 g fresh plantain/kg diet.

**ab** Mean values with dissimilar superscripts differ significantly (p < .05).

**Statistical analysis**

Raw data were organised using the Microsoft Excel program and then analysed using IBM SPSS software (Version 23.0, IBM Corp., USA). All parameters were subjected to one-way analysis of variance (ANOVA), and data were presented as mean ± standard deviation. The orthogonal polynomial contrast test was performed to obtain linear, quadratic and cubic effects of increasing supplementation rate of PL in the basal diet on each tested parameter. Duncan’s multiple range test (DMRT) was conducted to find the difference among the treatment groups, and the differences at p < .05 were considered statistically significant. The following statistical model was considered:

\[
Y_{ij} = \mu + t_i + e_{ij}
\]

Where Y_{ij} was the overall response of broiler; μ was the overall mean; \( t_i \) was the effect of PL supplementation (treatment effect); \( e_{ij} \) was the error due to the \( j \)th replication of the \( i \)th treatments; data were normally distributed with zero mean and constant variance.

**Results**

**Growth performance**

Higher LWG (p = .000) and PEF (p = .000), and lower FCR (p = .000) were observed in the PL supplemented groups compared to the CON group (Table 2). With
the increasing doses of PL supplementation, LWG was improved but better influenced up to the PL80 group (Figure 1). Compared to CON, 2.30–5.75% lower FCR was found in PL supplemented groups where PL80 and PL120 groups exhibited the lowest value.

Plasma lipid profile, antioxidants and liver activity

Based on the result presented in Table 3, PL had a positive influence \((p < .05)\) on plasma lipid profiles, antioxidants concentration, and liver enzymatic activity. The PL80 group exhibited the lowest \((p < .05)\) triglyceride concentration, whereas HDL and LDL concentrations were similar to the PL120 group but lower \((p < .05)\) than others. Besides, the highest \((p < .05)\) concentrations of SOD and GPx were found in both PL80 and PL120 groups. However, catalase concentration was highest \((p < .05)\) in the PL80 group which was 5% higher than the PL120 group. The AST \((p = .002)\), ALT \((p = .000)\), ALP \((p = .005)\) and AST: ALT \((p = .001)\) were significantly improved in PL supplemented groups and the lowest \((p < .05)\) value was found in both PL80 and PL120 groups.

Carcase characteristics and meat quality

Compared to CON group, 3–8% higher dressing percentage, 12–21% higher breast meat, and 8–15% lower abdominal fat were found in PL supplemented groups (Table 4). Amongst the supplemented groups, both PL80 and PL120 groups exhibited the highest dressing percentage \((p = .004)\) and breast \((p = .002)\) yield, whereas the lowest abdominal fat \((p = .001)\) was recorded in the PL80 group. No difference was found in meat proximate components except ether extract

Table 3. Effect of different level of fresh plantain herb \((Plantago lanceolata\) L.) supplementation on plasma lipid profile, plasma antioxidants and liver enzymatic activity of broilers at 35 days.

| Parameters                        | CON          | PL40         | PL80         | PL120        | SEM         | Linear | Quadratic | Cubic |
|-----------------------------------|--------------|--------------|--------------|--------------|-------------|--------|-----------|--------|
| Triglyceride, mg/dL               | 60.67±0.28   | 58.50±1.0    | 55.08±0.52   | 57.50±1.00   | 0.63        | .000   | .001      | .007   |
| T-Cholesterol, mg/dL              | 84.75±3.03   | 84.73±0.68   | 82.50±1.00   | 82.00±1.32   | 0.58        | .051   | .818      | .410   |
| HDL, mg/dL                       | 49.50±1.00   | 51.50±0.87   | 53.17±0.76   | 52.00±0.50   | 0.45        | .002   | .009      | .263   |
| LDL, mg/dL                       | 23.12±2.03   | 21.53±0.25   | 18.32±1.50   | 18.50±1.99   | 0.73        | .003   | .372      | .262   |
| VLDL, mg/dL                      | 12.13±±0.06  | 11.70±0.20   | 11.02±0.10   | 11.50±0.20   | 0.13        | .000   | .001      | .007   |
| SOD, mg/dL                       | 1.83±0.03     | 1.85±0.02    | 1.91±0.03    | 1.90±0.03    | 0.01        | .005   | .363      | .152   |
| GPx, mg/dL                        | 33.45±1.79   | 36.04±1.28   | 38.83±0.91   | 38.49±1.96   | 0.76        | .002   | .139      | .427   |
| Catalase, U/L                     | 41.17±0.96   | 44.03±1.35   | 46.89±1.00   | 44.66±1.43   | 0.68        | .003   | .006      | .140   |
| AST, U/L                          | 182.26±4.91  | 174.16±2.11  | 167.18±3.00  | 170.69±1.05  | 1.85        | .001   | .012      | .276   |
| ALT, U/L                          | 15.60±0.98   | 12.22±0.72   | 10.27±0.54   | 11.72±0.65   | 0.62        | .000   | .000      | .332   |
| ALP, U/L                          | 183.79±2.09  | 192.96±3.81  | 191.79±1.49  | 188.52±0.54  | 1.21        | .061   | .002      | .204   |
| AST:ALT                           | 11.71±0.78   | 14.28±0.76   | 16.30±0.63   | 14.60±0.92   | 0.53        | .001   | .001      | .153   |

HDL: high density lipoprotein; LDL: low density lipoprotein; VLDL: very low density lipoprotein; SOD: superoxide dismutase; GPx: glutathione peroxidase; AST: aspartate aminotransferase; ALT: alanine aminotransferase; ALP: alkaline phosphatase; SEM: standard error mean.

CON: corn-soya based basal diet having CP = 23.30% and ME = 3081 Kcal/kg diet; PL40: CON + 40 g fresh plantain/kg diet, PL80: CON + 80 g fresh plantain/kg diet; PL120: CON + 120 g fresh plantain/kg diet.

abcMean values with dissimilar superscripts differ significantly \((p < .05)\).

Figure 1. Effect of different level of fresh plantain herb (PL; Plantago lanceolata L.) supplementation on live weight gain of broilers at 35 days: (a) linear regression; (b) quadratic regression.
among the treatment groups (p > .05). The lowest meat ether extract was found in both PL80 and PL120 groups compared to PL40 and CON groups. No difference was found in meat saturated and mono-unsaturated fatty acid contents amongst the treatment groups (p > .05). The PL120 group exhibited the highest poly-unsaturated fatty acid, more specifically, higher linoleic acid contents compared to others.

Meat colour

The addition of PL at different levels in the broiler diet exhibited a positive influence on meat colour (Table 5). Antioxidant-rich PL significantly improved the breast and thigh meat colour. In the case of breast meat, both PL80 and PL120 groups exhibited similar L*, a*, and b* values which were better than CON and PL40 groups. The PL supplementation substantially improved (p < .05) the L* and a* values in thigh meat whereas similar (p > .05) L* value was found among the supplemented groups, and the highest (p = .001) a* value was found in both PL80 and PL120 groups compared to others.

Table 4. Effect of different level of fresh plantain herb (Plantago lanceolata L.) supplementation on carcase characteristics and meat quality of broilers at 35 days.

| Parameters | CON | PL40 | PL80 | PL120 | p-Value  |
|------------|-----|------|------|-------|----------|
| Carcase characteristics, % |     |      |      |       |          |
| DP | 58.54±1.09 | 61.33±1.09 | 62.51±1.40 | 63.15±0.89 | 0.60 .001 .140 .726 |
| Breast | 15.83±0.78 | 17.67±0.73 | 18.91±0.57 | 19.11±0.66 | 0.43 .000 .073 .813 |
| Thigh | 7.40±0.47 | 8.10±0.34 | 8.22±0.51 | 8.40±0.54 | 0.16 .034 .376 .615 |
| Abd. Fat | 1.36±0.03 | 1.26±0.05 | 1.16±0.02 | 1.23±0.03 | 0.02 .000 .002 .100 |
| Liver | 3.50±0.04 | 3.49±0.07 | 3.52±0.07 | 3.49±0.07 | 0.25 .907 .206 .541 |
| Heart | 0.63±0.02 | 0.63±0.02 | 0.64±0.03 | 0.64±0.04 | 0.01 .502 .919 .502 |
| Kidney | 0.21±0.02 | 0.21±0.03 | 0.23±0.02 | 0.20±0.03 | 0.01 .687 .404 .366 |
| Gizzard | 3.03±0.09 | 3.00±0.39 | 3.09±0.07 | 3.07±0.11 | 0.05 .687 .979 .704 |
| Meat proximate composition, % |     |      |      |       |          |
| Moisture | 73.50±1.00 | 71.67±0.76 | 71.66±1.61 | 73.33±1.25 | 0.39 .876 .035 .958 |
| Crude protein | 21.55±0.51 | 22.66±0.58 | 22.49±1.49 | 22.21±0.49 | 0.25 .446 .206 .619 |
| Ether extract | 1.92±0.05 | 1.83±0.03 | 1.57±0.06 | 1.58±0.04 | 0.06 .000 .115 .008 |
| NFE | 73.34±0.44 | 72.19±0.68 | 72.70±1.50 | 73.01±0.66 | 0.26 .841 .203 .460 |
| Ash | 1.17±0.06 | 1.25±0.09 | 1.30±0.05 | 1.25±0.06 | 0.02 .126 .125 .691 |
| Meat fatty acid profile, % |     |      |      |       |          |
| Saturated FA | 31.73±0.71 | 31.24±0.59 | 30.91±0.63 | 30.16±0.76 | 0.24 .020 .748 .748 |
| MUFA | 49.64±1.33 | 50.14±1.89 | 49.72±2.19 | 49.17±1.81 | 0.46 .709 .633 .871 |
| PUFA | 18.63±0.21 | 18.62±0.27 | 19.33±0.33 | 20.67±0.41 | 0.26 .000 .007 .802 |
| Linoleic acid | 12.61±0.08 | 13.43±0.16 | 14.91±0.07 | 15.46±0.11 | 0.34 .000 .060 .009 |
| Linolenic acid | 0.27±0.11 | 0.29±0.02 | 0.28±0.04 | 0.31±0.18 | 0.03 .703 .938 .808 |
| L/C3 | 49.12±2.06 | 46.39±1.25 | 45.33±1.12 | 44.71±1.89 | 0.65 .009 .295 .778 |
| a/C3 | 1.42±0.08 | 2.00±0.04 | 4.01±0.11 | 4.21±0.13 | 0.37 .000 .010 .000 |
| b/C3 | 2.49±0.40 | 2.75±0.16 | 4.29±0.02 | 4.43±0.03 | 0.26 .000 .582 .001 |

Table 5. Effect of different level of fresh plantain herb (Plantago lanceolata L.) supplementation on meat colour of broilers at 35 days.

| Parameters | CON | PL40 | PL80 | PL120 | p-Value  |
|------------|-----|------|------|-------|----------|
| Breast meat colour |     |      |      |       |          |
| L* | 49.12±2.06 | 46.39±1.12 | 45.33±1.12 | 44.71±1.89 | 0.65 .009 .295 .778 |
| a* | 1.42±0.08 | 2.00±0.04 | 4.01±0.11 | 4.21±0.13 | 0.37 .000 .010 .000 |
| b* | 2.49±0.40 | 2.75±0.16 | 4.29±0.02 | 4.43±0.03 | 0.26 .000 .582 .001 |
| Thigh meat colour |     |      |      |       |          |
| L* | 47.46±0.49 | 44.28±0.89 | 44.77±1.44 | 43.74±1.84 | 0.53 .012 .183 .153 |
| a* | 2.87±0.19 | 3.92±0.07 | 4.69±0.09 | 4.79±0.17 | 0.23 .000 .000 .311 |
| b* | 3.76±1.25 | 3.86±0.19 | 4.40±0.49 | 4.80±0.35 | 0.21 .078 .183 .153 |

L*: lightness; a*: redness; b*: yellowness; SEM: standard error mean.

CON: corn-soya based basal diet having CP = 23.30% and ME = 3081 Kcal/kg diet; PL40: CON + 40 g fresh plantain/kg diet, PL80: CON + 80 g fresh plantain/kg diet, PL120: CON + 120 g fresh plantain/kg diet.

abcMean values with dissimilar superscripts differ significantly (p < .05).

Discussion

Growth performance

Previous research has demonstrated that supplementation of PL in fresh (Mazhari et al. 2016), aqueous extract (Camy et al. 2020), or dried powder (Saleheen among the treatment groups (p > .05). The lowest meat ether extract was found in both PL80 and PL120 groups compared to PL40 and CON groups. No difference was found in meat saturated and mono-unsaturated fatty acid contents amongst the treatment groups (p > .05). The PL120 group exhibited the highest poly-unsaturated fatty acid, more specifically, higher linoleic acid contents compared to others.

Meat colour

The addition of PL at different levels in the broiler diet exhibited a positive influence on meat colour (Table 5). Antioxidant-rich PL significantly improved the breast and thigh meat colour. In the case of breast meat, both PL80 and PL120 groups exhibited similar L*, a*, and b* values which were better than CON and PL40 groups. The PL supplementation substantially improved (p < .05) the L* and a* values in thigh meat whereas similar (p > .05) L* value was found among the supplemented groups, and the highest (p = .001) a* value was found in both PL80 and PL120 groups compared to others.
Lipia citridora leaves powder (which is high in verbascoside, a compound similar to acteoside in PL) did not influence broiler growth up to 5.0 mg/kg feed. Despite these inconsistent and controversial findings, the improved growth in PL supplemented groups could be attributed to the existence of bioactive components (acteoside, aucubin, and catalpol) that enhanced the broiler’s free radical scavenging activity and thus acted as a natural antioxidant (Chacrabati et al. 2013; Boamah et al. 2016; Mazhari et al. 2016). In addition, flavonoid components in PL induce a lethal chemical interaction with parasite molecules, resulting in a lower parasitic count in the broiler and thus might improve nutrients turnover (Camy et al. 2020). However, the dose-related response in growth performance was optimised at the PL80 group (Figure 1) and afterward did not carry any significant improvement. One of the reasons that might be responsible for getting optimised growth performance in the PL80 group instead of the PL120 group includes – dietary inclusion of different levels of bioactive components and their interaction. Acteoside in PL is phenylpropanoid glycoside which is effective to alleviate oxidative stress (Marco et al. 2015), and aucubin (iridoid glycosides) has a proven effect on immunomodulation by increasing lymphocyte production and interferon-gamma secretion (Venkatalakshmi et al. 2016). While the PL120 group obtained the highest concentrations of acteoside and aucubin (4.4 and 2.6 mg/kg DM, respectively), the PL80 group (2.9 and 1.7 mg/kg DM, respectively) also exhibited a similar response in terms of growth efficiency, which might be due to the interaction with other bioactive phytochemicals such as oxalate, tannin, and others. Broilers fed the PL120 diet ingested approximately 1.5-times the amount of oxalate as those fed the PL80 diet (106 vs 71 mg), which could have a detrimental effect on growth efficiency, explaining why the inclusion of higher acteoside and aucubin in the PL120 group failed to improve additional response over the PL80 group (Guil-Guerrero 2001; Zhang et al. 2020). Further study is needed to elucidate the antagonistic interaction among the phytochemicals in the broiler.

**Plasma metabolites, antioxidants and liver activity**

In this experiment, different levels of PL supplementation significantly reduced serum triglycerides in broiler but failed to reduce T-cholesterol content, which was consistent with the previous findings (Mazhari et al. 2016; Camy et al. 2020). However, some researchers reported that pomegranate pulp (Hosseini-Vashan and Raei-Moghadam 2019); garlic powder (Karim et al. 2018); turmeric (Mondal et al. 2015); thyme and curcumin (Fallah and Mirzaei 2016) supplementation in broiler diet reduced plasma T-cholesterol content. On the contrary, Akbari et al. (2016) and Mehrparvar et al. (2016) reported that peppermint and Lipia citridora leaves powder did not affect serum HDL and glucose content in laying hen and broiler, respectively. However, the essential oil extracted from PL successfully exerted the hypcholesterolaemia effect in mice by reducing the activity of HMG-CoA reductase which is responsible for cholesterol biosynthesis (Najafian et al. 2018). But we failed to exert this effect under the current study and the dose of PL supplementation might be a factor. The increased doses of PL oil extract application in mice drastically reduced plasma T-cholesterol levels (Najafian et al. 2018), and we found a similar trend but not statistically significant. However, the reduction of triglycerides concentration in PL supplemented groups might be due to the enhanced activity of lipoprotein lipase enzyme which promoted the breakdown of triglycerides into free fatty acids and glycerol (Adiputro et al. 2015) and the lowest triglycerides value in the PL80 group might be due to higher serum antioxidant capacity compared to others. As SOD has a negative correlation with triglyceride levels, it aids in the clearance of reactive oxygen species, thus reducing oxidative damage and peroxidation. Triglyceride, a result of lipid peroxidation, decreases as the activity of GPX, SOD and CAT increases (Ma et al. 2020). Besides, lower triglycerides concentration in the PL80 group might assist to reduce the VLDL and chylomicron concentration, as both lipoproteins are the transporters of glycerides (Fallah and Mirzaei 2016).

The presence of bioactive components in herbs improved serum antioxidants level in the broiler (Cherian et al. 2013; Camy et al. 2020). Elevated SOD and GPx concentration in both PL80 and PL120 groups might be due to higher consumption of
bioactive components, specially acteoside, though it is not clear why the PL120 group exhibited similar results to the PL80 group. Grossly, the acteoside, a polyphenolic component in PL, imparts free radical scavenging activity to the animal body, thereby reducing oxidative stress by increasing serum antioxidant levels (Mazhari et al. 2016). Furthermore, PL has a greater capacity for scavenging superoxide anion radicals, which might reduce superoxide anion concentration in broilers, resulting in elevated SOD levels in the supplemented group (Al-Mamun et al. 2007). This hypothesis may be supported by the findings of Lien et al. (2008), who reported a similar response in laying hens by dietary flavonoid supplementation. Additionally, the polyphenolic components in ginger powder (Habibi et al. 2014), grape pomace (Ebrahimzadeh et al. 2018), or Artemisia annua (Wan et al. 2016) improved serum antioxidant levels in the broiler. Simultaneously, the uronic acid content of PL is linked to arabinoxylans through an acid group that could be hydrolysed by certain intestinal microbes that possess arabinoxylases, resulting in the formation of arabinoxylan oligosaccharides that act as a prebiotic component (Divani et al. 2018). Findings from previous research suggested that prebiotic components exerted antioxidant activity both in vitro and in vivo conditions (Kogan et al. 2008; Álauwong et al. 2013), supporting the increased serum antioxidant levels in the PL supplemented groups.

Liver enzymatic activity was significantly improved in PL supplemented groups, which was in accordance with Sahoo et al. (2019), who reported similar findings with turmeric and ginger powder in broiler. Previous research illustrated that antioxidant-rich herbs like rosemary leaves (Ahmed et al. 2015), neem leaves (Ansari et al. 2012), aloe vera (Khan et al. 2014) and ajwain seed (Kolbadinejad and Rezaeipour 2020) were effective for improving liver enzymatic activity in the broiler. Besides, infusion of PL extract directly to rat blood significantly reduced the serum AST and ALT levels, and the lowest value was found at the infusion rate of 25 mg/kg body weight (Turel et al. 2009). In the current study, the lowest ALT and AST concentrations were found in both PL80 and PL120 groups, which indicated that both groups might induce a similar rate of inhibition of the degeneration and necrosis in the liver (Hussan et al. 2015). This phenomenon reduces liver enzyme activity by suppressing the reactive oxygen species and cytokine production (Moradi-Ozarlou et al. 2020).

Carcass composition and meat quality

Improved dressed and breast meat yield in PL supplemented groups were consistent with the previous findings (Mazhari et al. 2016; Camy et al. 2020). Besides, supplementation of individual herb or mixture of herbs dry matter at the rate of 0.5–1.0% in poultry diet significantly reduced abdominal fat and meat fat contents (Narimani-Rad et al. 2011; Kusmayadi et al. 2019). In broiler, excessive fat is mostly deposited in the abdominal region, and several factors regulate this negative trait, like (i) genetics, (ii) nutrient concentrations in diet, (iii) plasma metabolites level, (iv) dietary inclusion of phytochemicals, etc. Genetics, obviously the prime factor for fat deposition (Fouad and El-Senousey 2014) but in this study all the experimental birds were a similar strain, so we can exclude this possibility. Lower energy and protein levels in broiler lead to reduce abdominal fat deposition (Fouad and El-Senousey 2014), which contradicts with our findings of reduced abdominal fat contents in PL supplemented groups (Table 1). Due to PL supplementation, both energy and protein contents in the diets were increased (<1%), which might not be sufficient to carry to any changes. However, lower plasma LDL and chylomicrons concentrations in PL supplemented groups might reduce the transportation of fatty acid from the liver to adipose tissue (Dubikovskaya et al. 2014), which resulted in lower abdominal fat content in the treatment groups. Besides, the lowest plasma triglycerides and LDL contents in the PL80 group might be responsible for getting the lowest abdominal fat content among the treatments. Additionally, polyphenolic compounds have been shown to inhibit dietary fat absorption (Fouad and El-Senousey 2014), lipase activity (Kang et al. 2012), and/or hepatic lipogenesis (Saha et al. 2019) in mouse models, which might be a fact for PL supplemented groups. Along with this, the highest serum antioxidants level in the PL80 group might additionally attribute to get the lowest fat deposition in the abdominal pad.

Fatty acids profiles

Fatty acid composition of broiler meat depends exclusively on dietary manipulation, where (i) fatty acid profile, (ii) antioxidants level, (iii) selenium content of supplied feed contributes significantly. The fresh PL is a good source of poly unsaturated fatty acids (PUFAs), which might help to increase these levels in PL supplemented groups (Mukemre et al. 2020). Previous research also illustrated that dietary intervention with PUFAs led to improve these concentrations in broiler meat (Saeed et al. 2018; Alagawany et al. 2019). The sources of these PUFAs and their dietary levels have a strong influence on lipid metabolism; however, excess
inclusion can lead to negative impacts by accelerating lipid oxidation (Wu et al. 2019). Besides, phenolic compounds in PL help to exert the anti-oxidative effect, which might stabilise the PUFAs in the intestine and muscle tissues (Brenes et al. 2016). Sohaib et al. (2015) reported that PUFAs content in broiler meat changed +4.2% when supplemented by 1% red ginseng (rich in saponin) compared to control. Furthermore, higher serum antioxidant levels improve PUFAs levels in broiler meat either by inhibiting the desaturase enzyme activity or improving the enzyme activity, which converts SFA into PUFA (Chung and Choi 2016). Moreover, PL contains selenium, an essential mineral for the anti-oxidative process, ranging from 40–50 μg/kg DM, which improved PUFAs content in broiler meat (Puerto et al. 2017; Sotek et al. 2019). In this experiment, the PL80 group had higher serum antioxidant levels, and the PUFAs content was highest in the PL120 group, which conflicts with the previous argument. The dietary inclusion of PUFAs might be more influential for improving meat PUFAs than other factors (Lee et al. 2012). That’s why the PL120 group had a higher value than the PL80 and other groups.

Meat colour

The broiler cannot synthesise meat colour pigments, and the key pigment extracted from the diet sometimes fails to exert the desirable meat colour (Díaz-Gómez et al. 2017). Therefore, both synthetic and natural pigments are often used in broiler diets, but natural pigments are mostly safe and eco-friendly (Wang et al. 2016). In this experiment, the lower L* value and higher a* and b* values in PL supplemented groups were consistent with the findings of Wang et al. (2016) and Kanani et al. (2017), who reported similar results with marigold extract and cinnamon, respectively. PL contains α-tocopherol and lutein, which might be responsible for increased redness and yellowness of breast and thigh meat in supplemented groups (Elgersma et al. 2013). The PL80 and PL120 groups showed similar results, while meat colouration was predicted to improve linearly with the increasing rate of PL supplementation. This phenomenon could be justified in a way that the correlation between the serum antioxidants level and broiler meat colouration (Karadas et al. 2016). In the current study, serum antioxidants level was improved consistently up to the PL80 group (Table 4) and then eventually did not carry any extra significance for additional supplementation in the PL120 group, which might be reflected in meat colouration. Owing to identical levels of serum antioxidants in both PL80 and PL120 groups, the defense against lipid oxidation, responsible for the formation of brown metmyoglobin from bright red oxygenated myoglobin, might be similar (Salami et al. 2015). Besides, elevated serum antioxidant concentrations in these groups might improve mitochondrial stability by (i) reducing the phospholipase A2 activity and/or (ii) by minimising calcium ions leakage, which is directly related to meat discolouration (Pedrão et al. 2015; Carvalho et al. 2017).

Conclusions

It seems that fresh PL supplementation at the rate of 40 g–120 g/kg basal diet significantly improved the growth performance, plasma antioxidant concentration, liver enzymatic activity and meat fatty acid profile in the broiler. Both 80 g and 120 g fresh PL supplementation executed similar responses in the aforementioned variables except meat fatty acid profile. The linoleic acid content in meat which is a major interest of consumers was highest at 120 g supplementation. So, it could be concluded that 80 g fresh PL/kg diet could be used for optimum growth performance and health status of broiler provided that poultry entrepreneurs who are interested to produce linoleic acid-enriched meat could supplement up to 120 g PL/kg diet.

Ethical approval

The experimental protocols, bird management and sample collection were reviewed and approved by the Animal Care Committee of Bangladesh Agricultural University Research System, Mymensingh 2202, Bangladesh (BAURES/ESRC/2019/AH/16).

Disclosure statement

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