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Article

Feeding of mint leaf as an alternative to antibiotics on performance of broiler

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Abstract: The investigation was carried out through an experiment to examine how various quantities of mint leaves affect the production performance, carcass characteristics, immune parameters, and caecal microbial load of commercial broiler. A total of 225 one-day-old chicks of "Lohman Meat (Indian River)" strain having 43.00±0.66 g average body weight was divided into five investigational groups with three replications of 15 chicks each. The treatments were T_0= control (basal feed), T_1=basal feed + antibiotic, T_2=basal feed + 1.0% mint leaf, T_3=basal feed + 1.5% mint leaf and T_4=basal feed + 2.0% mint leaf. Weekly body weight, feed intake and FCR were recorded during the investigation stage and carcass characteristics, immune parameters and caecal microbial count were also studied after. The average live weight and body weight gain were notably increased in the 2.0% mint leaf treated group than the other groups. Significant improved FCR was perceived in birds fed with 2.0% mint leaf with basal diet. The results showed that, broilers feeding with 2.0% mint leaf have significant effects (P<0.05) on the thigh, wing, back, liver, neck, heart, gizzard intestine, spleen and bursa while it appeared insignificant (P>0.05) on dressing percentage, breast and drumstick. Significant (P<0.05) difference was observed for immune parameters (WBC, lymphocyte, granulocyte) in mint leaf treated groups than in the control which signifies better immunity in mint leaf treated groups. Addition of 2.0% mint leaf in the broiler diet results in a significant (P>0.05) lower amount of *Escherichia coli* and *Salmonella* counts than in the control group. This study concluded that, the addition of mint leaf resulted in improved growth performance, carcass yields, immunity and lower pathogenic microbes in broiler chickens. Therefore, the use of 2.0% of mint leaf in the diets of broilers has affirmative effects on their production performance and as an antibiotic alternative in broiler production can be recommended.

Keywords: mint leaf; feeding; antibiotics; performance; broiler

1. Introduction

For many years, antimicrobials have been added to poultry feed to promote growth and prevent diseases in the industry (Letlhogonolo *et al*., 2020; Paintsil *et al*., 2021). However, the use of antibiotics as a sub-therapeutic supplement in poultry feed has raised concerns as it has led to the emergence of multiple drug-resistant bacteria in recent years (Nhung *et al*., 2017; Oniciuc *et al*., 2018). Consequently, the prohibition of antibiotics as feed additives has prompted the exploration of alternative feed supplements for animal production (Begum *et al*., 2018; Islam *et al*., 2018; Amin *et al*., 2019; Arif *et al*., 2019). Herbal extracts have been used as a substitute for

antibiotics in feed supplements to boost growth performance in intensive management systems (Madhupriya *et al*., 2018). Due to the adverse effects of antibiotics, natural products such as phytogenics have been employed to improve feed utilization efficiency and enhance the growth performance of poultry (Adil *et al.*, 2015). Various studies have demonstrated the antimicrobial properties of certain medicinal plants in laboratory settings for human use (Asadi *et al.*, 2017).
The *Mentha* genus, which belongs to the Labiatae family, consists of 20 species that are found worldwide. One of these species is *Mentha pulegium* L., commonly referred to as pennyroyal. Pennyroyal is originally from Europe, North Africa, Minor Asia, and the Near East. The flowering aerial parts of pennyroyal have been traditionally used for their antimicrobial properties to treat a variety of conditions (Chalchat *et al*., 2000), such as colds, sinusitis, cholera, food poisoning, bronchitis, and tuberculosis (Ahmed *et al*., 2018). Additionally, pennyroyal has been utilized for its antiflatulent, carminative, expectorant, diuretic, antitussive, and menstruation properties (Qureshi *et al*., 2016). *Mentha pulegium* L. and other species within the *Mentha* genus have also been found to have antimicrobial, antioxidant, cytotoxic, and abortifacient properties (Shirazi *et al*., 2004; Soares *et al*., 2005; Kamkar *et al*., 2010; Ahmed *et al*., 2018). Mentha species, known as pudina locally (Khan and Khatoon, 2008), have been a significant source of therapeutic agents and are used in alternative systems of medicine (Naeem *et al.*, 2011). Furthermore, the mint plant is rich in active ingredients, including menthol (40-50%), carvone (67.3%), limonene (13.5%), 1,8-cineole (5.4%), linalool (2.8%), menthone (2.9%), and isomethone (1.2%) (Younis *et al*., 2004; Malik *et al*., 2012). These active ingredients have been shown to have positive effects on health and productivity (Ghazaghi *et al.*, 2014).
According to Ameri *et al*. (2016), mint contains bioactive compounds that exhibit antibacterial properties, suggesting its potential as a substitute for antibiotics. Additionally, supplementation of mint leaf powder was found to improve serum biochemical concentrations without any adverse effects on liver and kidney functions. Although Ghalamkari *et al*. (2012) found no significant improvement in broiler performance with the addition of 0.5% and 1.0% pennyroyal, Modiry *et al*. (2010) reported that the inclusion of 1.5% of a mixture of *Urtica dioica*, *M. pulegium*, and *T. vulgaris* in broiler diets improved performance and carcass quality. Similarly, the addition of 0.75% of these medicinal plant combinations during the growing period had positive effects on the growth performance and carcass traits of broilers (Nobakht *et al*., 2010; Khursheed *et al*., 2017). Therefore, given the limited use of mint leaf in broiler rations in Bangladesh, this study aimed to investigate the effects of different levels of mint leaf on broiler performance and carcass traits.

2. Materials and Methods

2.1. Location of the experiment

From July to August 2021, the study was conducted at the poultry farm of Sher-e-Bangla Agricultural University in Dhaka, Bangladesh, and lasted for 28 days.

2.2. Collection of experimental broilers

The research comprised a total of 225 one-day-old chicks belonging to the "Lohman Meat (Indian River)" strain, with an average weight of 43.00±0.66 g. The chicks were procured from Kazi farm limited hatchery in Gazipur, Dhaka.

2.3. Preparation of experimental house

The broiler shed utilized in this study was an open-sided natural house with a concrete floor that was appropriately cleaned and disinfected before the commencement of the experiment. To maintain sanitation, a Benzalkonium chloride solution (Timsen TM, Eon Animal Health Products Ltd., Bangladesh) was applied to the house and divided into pens using polythene sheets to prevent air flow between them. Additionally, prior to the arrival of the chicks, the house was subjected to fumigation using a combination of formalin and potassium permanganate (500 ml formalin and 250 g potassium permanganate in a 2:1 ratio) in a 35 m^3 experimental area.

2.4. Experimental materials

Upon arrival, the chicks were placed in electric brooders and kept under standard brooding conditions for a period of 7 days. During this period, the chicks were provided with a basal diet. After 7 days, mint leaves were introduced to the diet as per the assigned dietary treatments. To help the chicks cope with stress from transportation, they were given glucose water with vitamin C for the first 3 hours and small feed particles on newspapers for the first 24 hours. Following the 7-day brooding period, the chicks were randomly assigned to different dietary treatment groups. Data on feed intake, live weight, body weight gain, feed conversion ratio, carcass characteristics, and total blood count were collected after 28 days.

2.5. Experimental treatments

Two hundred twenty-five (225) heads of one-day-old broiler chicks were divided into 5 experimental groups with 3 replications of 15 chicks each (Table 1).

Table 1. The experimental layout.

Treatments	Plan of treatments	Replications			Total number of birds
		1	2	3	
T_0	Basal feed	15 birds	15 birds	15 birds	45
T_1	Basal feed + Antibiotic	15 birds	15 birds	15 birds	45
T_2	Basal feed + 1.0% Mint leaf	15 birds	15 birds	15 birds	45
T_3	Basal feed + 1.5% Mint leaf	15 birds	15 birds	15 birds	45
T_4	Basal Feed + 2.0% Mint leaf	15 birds	15 birds	15 birds	45
	Grand Total	75	75	75	**225**

2.6. Collection of mint leaves

Mint leaf was purchased from the local market. Freshly purchased mint leaf was washed, cleaned with water, and drained. Mint leaves were then hand-picked from the stems and dried. Then, the dried mint leaves were ground by means of a blender and formerly filtered.

2.7. Experimental diets

The study utilized commercially available starter and grower broiler feed from the Kazi Farms group. The feed was given to the chickens four times per day following the management manual for Lohman meat (Indian River). Drinking water was provided *ad libitum* twice daily (Table 2).

Table 2. Chemical composition of the basal diet (starter and grower).

Parameter	Starter diet (0-14 days)	Grower diet (15-28 days)
Metabolizable energy (Kcal/Kg)	3000	3050
Protein (%)	21.0	19.0
Fat (%)	6.0	6.0
Fiber (%)	5.0	5.0
Ash (%)	8.0	8.0
Lysine (%)	1.20	1.10
Methionine (%)	0.49	0.47
Cysteine (%)	0.40	0.39
Tryptophan (%)	0.19	0.18
Threonine (%)	0.79	0.75
Arginine (%)	1.26	1.18

2.8. Management practices

In this study, rice husk was employed as a lightweight litter to absorb moisture from the fecal discharge of broiler chickens. Calcium oxide powder was added to the rice husk to serve as a disinfectant. The brooding temperature was set below 35°C and regularly checked every 2 hours using a digital thermometer to guarantee its consistency. Furthermore, the daily room temperature (in℃) and humidity were measured using a thermometer and a wet and dry bulb thermometer (Table 3).

Table 3. Average temperature and humidity.

Week	Date	Average temperature (ºC)	Average humidity (%)
1st	28.08.2021-03.09.2021	33.00	76.85
2nd	04.09.2021-10.09.2021	31.28	53.00
3rd	11.09.2021-17.09.2021	31.50	76.50
4th	21.11.2021-27.11.2021	29.87	78.27

2.9. Vaccination program

The experimental birds received vaccinations based on a predetermined schedule, utilizing vaccines obtained from Ceva Company in France (Table 4). Moreover, to prevent any potential deficiency-related illnesses, the birds were given a supplement of vitamin-B complex, vitamin-A, D3, and E.

Table 4. Vaccination schedule.

Age	Name of Disease	Name of Vaccine	Route of vaccination
Day 0	Infectious Bronchitis (IB) + Newcastle Disease (ND)	CEVAC BI L	One drop in eye
Day 09	Gumboro (IBD)	CEVAC IBDL	Drinking water
Day 17	Gumboro (IBD)	CEVAC IBDL	Drinking water

2.10. Recorded parameters

Throughout the study, the live weight and feed intake of the chicks were documented weekly, along with any incidences of mortality. Upon the slaughtering of the broiler chickens, the weight of the carcass, gizzard, liver, spleen, bursa, intestine, and heart were measured. The dressing percentage (DP) was then computed to assess the dressed carcass yield. The body weight gain, feed intake, feed conversion ratio (FCR), dressing percentage, and mortality of the birds were all evaluated using the following formula,

Body weight gain = Final weight – Initial weight

Feed consumption = Supplied amount of feed (g) – leftover of feed (g)

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

$$\text{Dressing percentage (DP)} = \frac{\text{Dressing yield (g)}}{\text{Live weight (g)}} \times 100$$

$$\text{Mortality rate (\%)} = \frac{\text{Total number of death}}{\text{Number of birds present in house}} \times 100$$

2.11. Determination of DP of broiler chicken

On the 28th day of the experiment, three broiler chickens were selected randomly from each replication, and their dressing percentage (DP) was determined. The birds were weighed before being humanely slaughtered using a halal method involving a knife. The external skin was carefully removed from the birds using scissors and hands, followed by a thorough washing to eliminate any loose feathers or other unwanted materials. The digestive system was detached from the carcass in line with the procedure outlined by Jones (1982), and the gall bladder, liver, and heart were separated from the viscera. To calculate the dressing yield, the weight of feathers, blood, head, shank, heart, liver, and digestive system was deducted from the weight of the bird.

2.12. Immunological study

Upon the completion of the study, blood samples were randomly collected from each replication. Using a syringe, approximately 2mL of blood was drawn from the wing vein and transferred to a vacutainer tube containing EDTA solution to prevent clotting. The blood samples were subsequently analyzed using an Auto Blood Analyzer in the laboratory.

2.13. Bacteriological study

Upon completion of the 28-day experiment, 15 birds from each treatment group were selected for slaughter, and their caecal contents were extracted for further analysis. EMB (Eosin Methylene Blue) and SS (*Salmonella-Shigella*) agar were purchased from the local market (HIMEDIA company) in Bangladesh. Following the extraction of caecal content from each sample, the content was diluted, and EMB and SS agar were employed to culture *E. coli* and *Salmonella* bacteria, respectively. The Petri dishes were subsequently placed in an incubator for 24 hours at a temperature of 37°C. The population of bacteria in each agar was determined as CFU g^{-1} (colony-forming unit).

2.14. Statistical analysis

The information obtained from the investigation was arranged and examined through SPSS (Version 16.0). To meet the research objectives, the data were analyzed with a one-way ANOVA approach. The differences among the means were identified through the implementation of Duncan's multiple comparison test and LSD, and statistical significance was determined at a significance level of $P<0.05$.

3. Results and Discussion

3.1. Production performances of broiler chicken

3.1.1. Average live weight

At the conclusion of the fourth week, the average live weight of broiler chickens in dietary groups T_0, T_1, T_2, T_3, and T_4 were 2071.30±2.49, 2108.33±2.83, 2145.33±5.94, 2181.33±0.86, and 2234.70±1.15 g, respectively (Table 5). The difference between T_4 and the control group, as well as the other groups, was found to be significant ($P<0.05$). However, there was no noteworthy difference between the group administered antibiotics (T_1) and the other treatments, T_0, T_2, and T_3. The highest live weight was found in T_4 (2234.70±1.15 g) and the lowest result was in the T_0 (2071.30±2.49 g) group. Khurshid *et al.* (2016) reported that the birds fed diets accompanied with whichever fresh or enzyme-treated mint leaves reached significantly ($P<0.05$) higher live weight when related with the control (Table 5). The enhancement in the live body weight as found in the present study as a result of mint leaves supplementation might be due to the presence of antioxidant properties due to various phenolic and flavonoid compounds existing in it. Therefore, this study suggested that the addition of 2.0% mint leaves significantly increase the average live weight gain in the broiler.

Table 5. Effects of feeding mint leaf on production efficiency of broiler chicken.

Treatments	Average live weight (g/bird)	Average BWG (g/bird)	Average FI (g/bird)	Final FCR	Dressing (%)	Survivability (%)
T_0	2071.30^{b}±2.49	2028.30^{b}±2.52	2489.00±2.11	1.21^{a}±0.097	67.97±0.08	100.00±0.00
T_1	2108.33^{b}±2.83	2065.33^{b}±2.86	2480.33±2.72	1.18^{ab}±0.070	70.27±0.22	100.00±0.00
T_2	2145.33^{b}±5.94	2102.33^{b}±5.99	2480.00±2.49	1.16^{b}±0.101	68.70±0.13	100.00±0.00
T_3	2181.33^{b}±0.86	2138.33^{b}±0.87	2479.33±3.30	1.14^{ab}±0.087	69.57±0.16	97.78±0.40
T_4	2234.70^{a}±1.15	2191.70^{a}±1.14	2460.00±3.72	1.10^{ab}±0.055	70.03±0.24	100.00±0.00
Level of significance	*	*	NS	*	NS	NS

Here, all values presented are Mean±SE, T_0 = Control (Basal feed), T_1 = Basal feed + Antibiotic, T_2 = Basal feed + 1.0 % mint leaf, T_3 = Basal feed + 1.5 % mint leaf, and T_4 = Basal feed + 2.0 % mint leaf. Values are mean ± SE (n=45) one way ANOVA (SPSS, Duncan method). * Significant difference ($P<0.05$). NS: Non-significant. SE= Standard Error. ^{ab}: Values bearing different letters within each column differ significantly.

3.1.2. Average body weight gain

The body weight gain were 2028.30±2.52 g, 2065.33±2.86 g, 2102.33±5.99 g, 2138.33±0.87 g, and 2191.70±1.14 g for T_0, T_1, T_2, T_3, and T_4, respectively (Table 5). The highest outcome was obserbved in T_4 (2191.70±1.14) and the lowest result was in the T_0 (2028.30±2.52) group (Table 5). Dietary supplementation of Nobakht *et al.* (2011) showed that feeding 0.5% *Mentha Pulegium* resulted in a positive effect in broiler chicken. Medicinal plants i.e. tulsi, black pepper, black cumin, peppermint, papaya, garlic and cinnamon were considerably ($P<0.01$ and $P<0.05$) increased body weight during the experimental period (Amin *et al.*, 2019). The results highlighted that the feeding of 2.0% mint leaves improves the average body weight gain in commercial broilers.

3.1.3. Feed Intake (FI)

There was no significant effect ($P>0.05$) of different treatments on feed intake (grams per broiler chicken). The average total feed intake of broiler chickens at the end of the 4th week in the T_0, T_1, T_2, T_3, and T_4 dietary groups were 2489.00±2.11, 2480.33±2.72, 2480.00±2.49, 2479.33±3.30, and 2460.00±3.72 g, respectively (Table 5). The highest average feed intake was found in T_0 (2489.00±2.11) and the lowest result was in T_2 (2460.00±3.72) group (Table 5). The results of this study are consistent with previous studies conducted by Durrani *et al*. (2008) and Amasaib *et al*. (2013) which reported that the inclusion of spearmint in broiler diets did not have a significant effect ($P<0.05$) on feed intake. It is likely that the lack of effect on feed intake observed in this study is due to the fact that the diets were isocaloric. Thus, it can be concluded that the addition of mint leaf to broiler diets does not have a significant effect on feed intake.

3.1.4. Feed conversion ratio (FCR)

The FCR was significantly (P<0.05) different among the treatments and in the dietary groups of T_0, T_1, T_2, T_3 and T_4 they were 1.21, 1.18, 1.16, 1.14 and 1.10 respectively. The use of different levels of mint leaves resulted in enlightening the FCR (Table 5). Mohanad and Ulsagheer (2020) reported the significant enhancement in FCR with the feeding of combination of *Mentha pulegium* and *Thymus vulgaris* powder treated group in broiler. Use of peppermint (*Mentha piperita)* in broiler ration were significantly (P<0.05) improved FCR (Amin *et al.*, 2019). The presence of antimicrobial, antioxidant and phenolic substances may be the main cause for improvement in FCR as a result of dietary supplementation of mint (Souri *et al.*, 2004; Jazani *et al.*, 2009). Hence, the addition of different levels of mint leaves to the broiler diet showed better FCR than the control and antibiotic group.

3.1.5. Dressing percentage (DP)

In this study, the DP in T_4 (70.03±0.24) and T_1 (70.27±0.22) groups were better compared with the other treatment groups T_0 (67.97±0.08), T_2 (68.70±0.13) and T_3 (69.57±0.16) (Table 5). While the treatment groups did not show any substantial variation, it is worth highlighting that comparable outcomes have been documented in past research, indicating a distinctiveness to the findings. For instance, Gurbuz and Ismael (2016) found no significant difference in carcass yield among broilers fed with peppermint and basil dietary treatments. Similarly, Khursheed *et al*. (2017) reported that there was no significant difference in dressing percentage (P>0.05) between the mint leaf added group and the control group. Therefore, addition of different level of mint leaves can improve the DP of the broiler.

3.1.6. Survivability

The survivability percentage was not substantially different (P>0.05) among different treatments, with the control, antibiotic, and 1.0% and 2.0% mint leaves groups having a higher rate of 100±0.00 compared to the 1.5% mint leaves group (97.78±0.40) (Table 5). Previous studies by Ocak *et al*. (2008) and Asadi *et al*. (2017) have shown that the adding peppermint to broiler diets can lead to decreased mortality rates, possibly due to the antiseptic properties of the plant and its role as an immune-stimulating factor. It is possible that the immune-stimulating properties of the mint leaves played a role in the survivability rates observed in this study.

3.2. Carcass characteristics of broiler chicken

3.2.1. Weight of different parts of carcass

The weight of different carcass parts was found to be higher in the dietary treatment groups compared to the control cluster. Specifically, there was a substantial (P<0.05) increase in the weight of dressed thigh, wing, and back in the T_4 treatment group compared to the other treatments. However, there was no significant difference observed in breast and drumstick weights among the treatment groups (Table 6). These findings are consistent with a study by Khurshed *et al*. (2017) who reported no substantial variance (P>0.05) in carcass characteristics between control and groups fed with 1.0% and 2.0% mint leaves. Comparable outcomes were also conveyed by Goodarzi and Nanekarani (2014), who found that adding 2.0% pennyroyal medicinal plants in broiler feed led to improvements in growth performance and carcass traits.

Table 6. Effects of feeding mint leaf on carcass features of chicken.

Treatments	Breast (g)	Thigh (g)	Wing (g)	Drumstick (g)	Back (g)
T_0	546.00±2.89	185.00^{c}±0.88	99.67^{b}±1.06	178.30±0.69	245.70^{ac}±0.83
T_1	583.30±3.34	206.00^{ab}±0.35	118.30^{ac}±0.23	184.70±0.96	234.00^{bc}±0.26
T_2	596.70±5.11	215.70^{abc}±3.16	111.00^{ab}±1.57	182.00±2.15	254.30^{ab}±2.35
T_3	589.30±0.66	196.30^{bc}±0.47	114.70^{ac}±0.24	188.00±0.18	248.70^{a}±3.51
T_4	631.70±2.38	227.00^{a}±1.68	115.70^{ab}±0.63	193.30±0.92	258.70^{a}±15.4
Level of significance	NS	*	*	NS	*

Here, all values presented are Mean±SE; Coding and other information refer to footnote of Table 5. ^{abc} Values bearing different letters within each column differ significantly.

3.2.2. Relative weight of internal organs

The weight of liver, heart, neck, gizzard, intestine, spleen, and bursa in broilers that were fed diets containing 1.0%, 1.5%, or 2.0% mint leaf, as well as a control group and an antibiotic-added group were found significantly difference (P<0.05) among the various treatment groups (Table 7). The T_4 (2.0% mint leaf), T_3 (1.5% mint leaf) and T_2 (1.0% mint leaf) treated group showed better results in the relative weight of internal organs than the control and antibiotic added group (Table 7). However, the highest result was found in T_4 (2.0% mint leaf). This result is dissimilar to the findings of Ismail *et al.* (2004), who stated no influence of treatment on the weight of the liver, in broilers, fed herbal plant extracts. However, the present research findings well approve with the result Goodarzi and Nanekarani (2014) who establish significant relative organ weight in the 2.0% mint leaf treated groups. The observed significant difference in the weight of internal organs (liver, heart, neck, gizzard, intestine, spleen and bursa) among the dietary treatment groups may be attributed to the positive effect of spices and herbs, which are the source of essential oils, on food digestion. Essential oils have been reported to increase the absorption of amino acids, improve body weight, and enhance the function of other organs. Mellor (2000) suggested that dietary essential oils could improve digestion. The higher weight of internal organs in the mint leaf treated groups compared to the control group might be due to the positive effect of feeding mint leaves on the overall carcass traits of broilers.

Table 7. Effects of feeding mint leaf on internal organs of broiler chicken.

Treatments	Liver (g/bird)	Heart (g/bird)	Neck (g/bird)	Gizzard (g/bird)	Giblet (g/bird)	Intestine (g/bird)	Spleen (g/bird)	Bursa (g/bird)
T_0	49.33^{bcd}±0.30	7.5^{bc}±0.19	46.33^{ab}±0.62	20.00^{b}±0.23	123.17^{b}± 0.71	132.00^{bcd}±0.26	4.67^{ab}±1.12	2.47^{a}±0.70
T_1	49.33^{bcd}±0.22	8.17^{ac}±0.10	41.00^{b}±0.15	22.00^{b}±0.22	120.50^{b}±0.24	118.00^{bc}±0.77	3.00^{b}±0.50	2.16^{a}±0.30
T_2	52.67^{ad}±0.72	8.8^{ac}±0.25	53.00^{ab}±1.57	24.67^{b}±0.95	139.13^{b}±1.74	138.00^{abd}±1.25	6.33^{ab}±0.79	2.17^{a}±0.46
T_3	49.33^{bcd}±0.43	9.07^{ac}±0.14	46.00^{a}±0.44	21.67^{b}±0.32	126.07^{b}±0.65	121.67^{bcd}±1.10	5.0^{ab}±0.41	2.74^{a}±0.70
T_4	60.00^{a}±0.33	9.5^{a}±0.16	47.33^{a}±0.22	32.67^{a}±0.35	149.50^{a}±0.52	154.00^{a}±0.08	6.67^{a}±0.93	3.05^{a}±0.44
Level of significance	*	*	*	*	*	*	*	*

Here, all values presented are Mean±SE; Coding and other information refer to footnote of Table 5. ^{abcd}: Values bearing different letters within each column differ significantly.

3.3. Immunity parameters

The immune parameters including WBC, Lymphocyte, and Granulocyte were analyzed and the results showed a significant difference (P>0.05) among the various treatments. The control group had the highest granulocyte count (11.73±0.06) which suggests low immunity, while the antibiotic treated group (T_1) and all the mint leaves treated groups (T_2, T_3 and T_4) had significantly lower granulocyte counts, indicating higher immunity. The lowest counts of WBC (5.83±0.05), Lymphocyte (2.22±0.01) and Granulocyte (3.55±0.11) were observed in T_1, T_3, and T_1 respectively (Table 8). Mint has been extensively used in herbal medication and has been shown to enhance immunity (Nanekarani *et al*. 2012). Al Ankari *et al*. (2004) reported that the use of herbal mint (*Menthal ongifolia*) in broiler chicken diets increased antibody titre. This may be due to the various essential oils present in mint leaf meal such as menthol, mentone, cavone, methyl acetate and piperitone.

Table 8. Effects of feeding mint leaf on immune parameters of broiler chicken.

Treatment	WBC (×10^9/L)	Lymphocyte(×10^9/L)	Granulocyte(×10^9/L)
T_0	14.60^{a}±0.07	2.69^{cd}±0.07	11.73^{a}±0.06
T_1	5.83^{ab}±0.05	2.54^{cd}±0.13	3.55^{b}±0.11
T_2	12.23^{bc}±0.09	2.68^{c}±0.11	9.69^{c}±0.06
T_3	6.43^{bd}±0.06	2.22^{b}±0.01	4.10^{d}±0.05
T_4	10.20^{be}±0.03	3.23^{a}±0.06	6.78^{e}±0.004
Level of significance	*	*	*

Here, all values presented are Mean±SE; Coding and other information refer to footnote of Table 5. ^{abcde}: Values bearing different letters within each column differ significantly.

3.4. Intestinal bacterial status

The number of *E. coli* and *Salmonella* in the caecum of birds was statistically significant (P<0.05) among the treatments. The highest *E. coli* colony was discovered in the control (T_0) group (16.30±0.02), indicating low immunity in the control group. The lowest *E. coli* colony was found in T_4 (1.50±0.08), T_3 (4.30±0.05), T_1

(8.10±0.03) and T_2 (8.70±0.03). The highest *Salmonella* colony was found in the control (T_0) group (7.47±0.02). The lowest *Salmonella* colony was found in T_4 (2.10±0.07), T_3 (2.50±0.06), T_2 (3.43±0.11) and T_1 (5.17±0.07) which indicates the addition of different level of mint leaves with feed reduces the number of pathogenic bacteria in the intestine and improves the gut health (Table 9).

Table 9. Effects of feeding mint leaf on microflora (log 10 CFU/g) in the caecum of broiler.

Treatments	No. of *E. coli* colony (CFU/g)	No. of *Salmonella* colony (CFU/g)
T_0	16.30^{a}±0.02	7.47^{a}±0.02
T_1	8.10^{b}±0.03	5.17^{ab}±0.07
T_2	8.70^{c}±0.03	3.43^{ac}±0.11
T_3	4.30^{d}±0.05	2.50^{ad}±0.06
T_4	1.50^{e}±0.08	2.10^{ae}±0.07
Level of significance	*	*

Here, all values presented are Mean±SE; Coding and other information refer to footnote of Table 5. ^{abcde}: Values bearing different letters within each column differ significantly.

In addition, the study found that the 2.0% mint leaves treated group had the lowest levels of *E. coli* and *Salmonella*. Cabuk *et al*. (2003) demonstrated the effectiveness of functional substances such as cinnamate and eugenol found in medicinal and aromatic plants as active substances and digestive stimulators, resulting in antimicrobial effects, particularly against intestinal microbes in the digestive system. Furthermore, the addition of phytogenic feed additives to broiler diets has been found to enhance intestinal functions and microbial activities (Wati *et al*., 2015). The antibacterial properties of mint leaves are primarily attributed to the presence of high levels of menthol, the principal phenolic constituent (Dorman *et al*., 2003). Thus, the current study suggests that the addition of 2.0% mint leaves to broiler diets can significantly reduce the number of *E. coli* and *Salmonella* in the caecum and improve the overall health status of broilers.

4. Conclusions

The findings of this study directed that nurturing commercial broilers with varying levels of mint leaves resulted in a significant improvement in their production in terms of body weight, carcass traits, feed conversion ratio, and immunity. The highest improvement was observed at the 2.0% level. Therefore, addition of 2.0% mint leaf with broiler feed as antibiotic alternative in broiler production can be recommended to avoid the human health hazard.

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Data availability

The data presented in this study are contained in this manuscript.

Conflict of interest

None to declare.

Authors' contributions

Conceptualization: Mofassara Akter, Methodology: Mofassara Akter and Md. Asaduzzaman, Laboratory analysis: Md. Asaduzzaman, Data collection and Statistical analysis: Mofassara Akter, Writing-original draft preparation: Mofassara Akter, Writing- review and editing: Mofassara Akter. All authors have read and approved the final manuscript.

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