Evaluation of Herbal Extracts on Performance, Survivability, and Gut Microflora of Broiler Chickens: An Antibiotic-Free Alternative

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Abstract

Background: The use of herbal extracts as natural growth promoters has gained attention as an alternative to antibiotics in poultry farming. This study aimed to evaluate the effects of aloe vera, neem leaves, and ginger extracts, as well as their combination, on the production performance, carcass characteristics, survivability, and gut microflora of broiler chickens. Methods: A total of 150 day-old Lohman IR straight-run commercial broiler chicks were randomly assigned to five treatment groups: T1 (Control), T2 (Aloe vera extract, 3 ml/L), T3 (Ginger extract, 3 ml/L), T4 (Neem leaves extract, 2 ml/L), and T5 (Cocktail of herbal extracts, 4.5 ml/L). Each group had three replications, with 10 chicks per replication. Performance parameters including feed intake, live weight (LW), live weight gain (LWG), feed conversion ratio (FCR), water consumption, carcass yield, survivability, and gut microflora composition were assessed. Microbial analysis was conducted to identify pathogens in the gut samples. Results: Significant differences ($P \leq 0.05$) were observed in feed intake, LW, and LWG among the treatment groups. However, no significant differences were found in water consumption, FCR, and carcass yield. The survivability rate was highest in T3, T4, and T5 (100%), and lowest in the control group (99.5%). Microbial analysis revealed the presence of E. coli and Salmonella in the control group, Staphylococcus in T2 and T3, and Salmonella and Streptococcus in T4 and T5. Notably, herbal supplementation removed E. coli and Salmonella in T2, T3, and T5 samples. Conclusion: The supplementation of aloe vera, ginger, neem leaf extracts, and their combination improved the production performance of broiler chickens without negatively impacting carcass characteristics, survivability, or gut health. These herbal extracts can serve as effective, antibiotic-free alternatives to promote growth and gut health in poultry.

Keywords: Antibiotics-free, herbal extracts, survivability, production performance, supplementation, alternative

Significance | This study highlights herbal extracts as potential natural growth promoters to replace antibiotics in poultry without affecting performance.

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1. Introduction

Broiler farming is important in improving livelihood, food security, and poverty alleviation in rural and semi-urban communities in developing countries, including Bangladesh (Mamun *et al.*, 2021). In Bangladesh, poultry alone contributes 37% of total meat (Hamid, 2016). The average per capita availability of meat is 23.6 g/head/day in comparison to the standard human requirement of 120 g/head/day (BBS 2010). Therefore, a huge gap is observed between the requirements and availability of meat in Bangladesh (Ali, 2015). The demand for meat, egg, and their products has been expanding dramatically with income growth, population growth,

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urbanization, and dietary changes (Hamid, 2016). Broiler farming can play a vital role in supplying the meat requirement to a proper level. However, this sector is facing many challenges, and among them, infectious and non-infectious diseases are the most important. So, poultry farm owners are frequently using antibiotics to overcome these infectious and non-infectious diseases.

It is noteworthy that antibiotics used as antimicrobial growth promoters (AGPs) in broiler feed have been shown to increase meat yield and improve feed efficiency with a substantial reduction in pathogenic bacteria in the host gut (Gaskins *et al*., 2002). Nowadays, antibiotics are being used not only to control, prevent, and treat infectious diseases but also to enhance animal growth and feed efficiency (Tollefson *et al.*, 2000). Unfortunately, antibiotics are being used without following the successive withdrawal period, which tends to accumulate in tissues and organs to form residues at different concentrations (Haque et al., 2020). In Bangladesh, a recent study has shown that high levels of residues of major antibiotics like tetracycline, ciprofloxacin, enrofloxacin, and amoxicillin were found mostly in the liver, kidney, thigh meat, and breast meat of broilers (Sattar *et al.*, 2014). In many countries, the supply of antibiotics is controlled through prescription by healthcare professionals. Unfortunately, in developing countries like Bangladesh, sale and distribution are allowed without a prescription, which is often associated with unnecessary use, shorter/incomplete treatment courses, incorrect selection of antibiotics, and inappropriate dose (Auta *et al.*, 2019).

Consumers have additional concerns about antibiotic residues/resistance, pesticide residues, additives, nutritional content, flavor, traceability, regional production, GMOs, social justice, etc. More interestingly, nowadays, consumers are demanding antibiotic-free safe foods (Islam *et al.*, 2024). As a result of the negative outcome of antibiotics, there is considerable research interest in the possible use of natural herbal plant extracts, such as Aloe vera, Ginger, and Neem leaves, for the replacement of antibiotics. The beneficial effects of natural products are greater than those observed with antibiotics, including a lower cost of production and reduced toxicity hazards (Manesh, 2012). Many types and forms of herbal feed supplements have been used to maintain and improve the health of human beings and chickens (Ahmed, 2015). Herbal extracts can also reduce the blood cholesterol level and improve immune system functions (Mathivanan, 2007). Herbal extracts activate immune responses, promote antimicrobial and anthelminthic actions, and stimulate endogenous enzymes that enhance the digestion process (Omar *et al.*, 2016). Thus, herbs could be expected to serve as safer alternatives as growth promoters due to their suitability and preference, lower cost of production, reduced risk of toxicity, minimum health hazards, and environmental friendliness.

Aloe vera (*Aloe barbedensis*) is an herbal plant that could be a possible alternative to chemical antibiotics, and its gel has been reported to possess anti-inflammatory activities, wound healing, and anticoccidial (Mwale *et al.,* 2005). Farmers in rural areas use *Aloe vera* for the prevention and treatment of disease (Mwale *et al*., 2005). Ginger (*Zingiber officinale)* is a perennial herbaceous plant that is a part of the Zingiberaceae family. It has carminative, diuretic, tonic, and disinfectant compounds that contain glucosinolate, sterols, and triterpenes. It is also a natural growth promoter and can be a potential alternative for common artificial growth promoters like antibiotics (Demir, 2003). The main compounds in ginger are gingerol and gingerdione, which can stimulate digestive enzymes, gastric secretion, and blood circulation and act as an enterokinetic (Incharoen, 2009).

Furthermore, neem leaves (NL) (*Azadirachta indica*) are one of the medicinal plants. NL and its constituents have been demonstrated to have immunomodulatory, anti-inflammatory, antihyperglycaemic, antiulcer, antimalarial, antifungal, antibacterial, antiviral, antioxidant, antimutagenic, and anticarcinogenic properties (Subapriya, 2005). Feeding different types of herbs as feed supplements has decreased oxidative stress and increased the productive performance of birds (Bashir *et al.,* 2014). While individual studies have examined the effects of aloe vera, ginger, or neem leaf extracts on broiler performance and gut health, comprehensive research evaluating their combined impact remains limited. Specifically, there is a need to investigate the synergistic effects of these herbal extracts on production performance, survivability, and gut microflora in antibiotic-free broilers (Khan et al., 2022). Additionally, standardized dosages and administration methods are lacking, leading to inconsistent results across studies. Addressing these gaps could provide valuable insights into the potential of these herbal combinations as effective alternatives to antibiotics in poultry production. Therefore, considering the health hazards of antibiotics and the advantages of several herbal extracts, as well as consumers' demand for antibiotic-free meat, this new approach was undertaken for antibiotic-free broiler production, aiming to determine the effect of aqueous extract of aloe vera, neem leaves, ginger, and their combination on antibiotics-free broilers' performance and gut microflora.

2. Materials and methods

2.1 Experimental design, duration, and preparation of the herbal extract

450 one-day-old Lohman IR straight-run commercial broiler chicks were subjected to a five-week (marketable age) experiment. Upon arrival, the chick's body weight was measured individually, and the average weight of the chicks was 46 g. The chicks were then allotted into five treatment groups each with three replicates each containing 30 chicks: T_1 (control), T_2 (Aloe vera extract, 3 ml/L), T_3

(Ginger extract 3 ml/L), T_4 (Neem extract 2 ml/L), and T_5 (cocktail, 4.5 ml/L) (**Supplementary Table 1**).

Fresh aloe vera leaves, ginger rhizomes, and neem leaves were collected for extract preparation. Aloe vera leaves were purchased from the local market, thoroughly washed under tap water, and the thorny margins along with the yellow sap-containing portions were removed. The cleaned leaves were chopped and processed using a Jaipan blender to extract the juice, which was then strained 2-3 times using a fine sieve, followed by muslin cloth filtration to obtain a clear extract. Similarly, ginger rhizomes were procured from the local vegetable market, cleaned, cut into small pieces using a metallic grinder, and blended into a paste with a Jaipan blender. The paste was then mixed with distilled water to form the extract. Mature neem leaves were freshly collected from trees within the campus, washed thoroughly to remove dirt, and ground using a grinder. All three plant materials, 250 g of aloe vera, ginger, and neem leaves, were each mixed with 750 ml of distilled water to prepare 25% aqueous extracts (**Figure. 1**). The prepared herbal extracts were stored at 4°C to preserve their active components. Each extract solution was uniformly mixed with drinking water on a daily routine basis. Furthermore, a cocktail was produced by uniformly mixing 1.5 ml of each extract solution.

2.2 Feeding, watering, and management of birds

Chick starter was fed to the day-old chicks by dispersing it on newspaper during the first three days. Subsequently, feed was administered ad libitum in the linear feeders for one week during the study period. A measured amount of feed was provided to each feeder, which was then replenished upon depletion. After one week, almost two-thirds of the feeders remained unfilled to reduce feed waste. In the first 14 days of the experiment, the chicks were provided with a broiler starting diet (CP 22%, ME 3000 kCal/kg), followed by a grower diet (CP 21%, ME 3100 kCal/kg) from the 14th to the 32nd day, which was purchased from the local market. The aqueous extracts of aloe vera, ginger, neem leaves, and the cocktail were made as previously described and administered to the experimental birds by drinking water. All experimental chicks were vaccinated for Marek's disease on their first day of life at the hatchery, and the standard vaccination protocol was adhered to throughout the trial duration (Islam et al., 2024).

2.3 Parameters studied

From day one to the end of the experimental period (34 days), data were collected properly, including the production performance of the birds, as daily feed and water intake were recorded, and finally, the weekly feed intake was calculated. The body weight was also recorded at three-day intervals until the last day of the experiments. Feed conversion efficiency was calculated from the proportion of cumulative feed intake and final body weight gain. Carcass characteristics were also studied during the experimental period by randomly selecting two birds per replication to sacrifice, and the dressed weight, calculated dressing percentage, thigh, drumstick, and breast weight were recorded. Finally, the mortality of birds was recorded to calculate the survivability percentage of the broiler birds during the experimental period.

2.4 Gut microflora analysis of experimental birds

At the end of the experimental period, two broilers were randomly selected from each replicate. The birds were sacrificed, and cloacal swab samples were collected immediately from the mucosa of the cloacal opening of both broiler chickens. The collected samples were immediately placed in sterile vials, mixed with distilled water, and stored at -10°C. Different kinds of necessary culture media were prepared in our laboratory by following the standard protocols. However, **Figure. 2** represents the schematic flow chart of the microbial load assessment.

2.5 ***Statistical analysis***

The data were analysed using SPSS software analysis of variance (ANOVA) and a completely randomized design (CRD). Means were compared to determine the significance of differences by the LSD test. Statistical significance was set at the 1% level ($P \leq 0.01$) and the 5% level ($P \leq 0.05$).

3. Results

3.1 Effects of herbal extracts on feed and water intake of the experimental broilers

During the experimental period, the feed consumption per week was recorded and used for the calculation of the average total feed consumption per bird. The data for average total feed consumption (kg) are presented in **Table 1**. The average total feed consumption was 3.24±0.01, 3.20±0.02, 3.25±0.02, and 3.16±0.04 kg for T_1, T_2, T_3, T_4, and T_5, respectively. Mean water consumed for the treatment groups was 6.21±0.25, 6.47±0.09, 6.07±0.25, 6.34±0.10, and 6.23±0.13 L for T_1, T_2, T_3, T_4, and T_5, respectively (**Table 1**).

3.2 Effects of herbal extracts on the production performance of experimental broilers

The average live weight patterns of the experimental birds from 7 to 34 days of age under various treatments are presented graphically here in **Figure. 3.** The recorded average live weight for T_1, T_2, T_3, T_4, and T_5 was 2.05±0.05, 2.21±0.03, 2.19±0.07, 2.22±0.04, and 2.17±0.11kg, respectively (**Table 1**). The average final body weight gains and FCR at different treatments of birds in the current study are presented in **Table 1**. The recorded body weight gain for T_1, T_2, T_3, T_4, and T_5 was 1.89±0.08, 2.06±0.03, 2.04±0.07, 2.07±0.04, and 2.02±0.11kg, respectively. Furthermore, the obtained FCR of different groups of broilers after herbal supplementation through drinking water for T_1, T_2, T_3, T_4, and T_5 was 1.65, 1.57, 1.58, 1.56, and 1.57, respectively.

3.3 Effects of herbal extracts on carcass characteristics of the experimental broilers

Table 1. Variation in production performances of broiler chicken supplemented with herbal extracts in drinking water

Variables	Treatments (mean ± SD)					
	T_1	T_2	T_3	T_4	T_5	*p*-value
Water intake (L)	6.21± 0.25	6.47±0.09	6.07±0.25	6.34±0.10	6.23±0.13	0.5
Feed intake (Kg)	3.11^{e} ±0.07	3.23_{b} ±0.01	3.21^{c} ±0.04	3.24_{a} ±0.04	3.16^{d}±0.08	0.05
Body weight (kg)	2.05^{e} ± 0.05	2.21^{b} ±0.03	2.19^{c} ± 0.07	2.22^{a} ±0.04	2.17^{d} ±0.11	0.05
Body weight gain (kg)	1.89^{e} ± 0.08	2.06^{b} ± 0.03	2.04^{c} ± 0.07	2.07^{a} ±0.04	2.02^{d} ±0.11	0.05
FCR	1.65± 0.05	1.57±0.02	1.58± 0.04	1.56±0.05	1.57±0.07	0.23

Table 2. Variation in carcass yield of broiler chicken supplemented with herbal extracts in drinking water

Variables	Treatments (mean ± SD)					
	T_1	T_2	T_3	T_4	T_5	*p*-value
Dressing weight (kg)	1.70±0.13	1.85±0.14	1.89±0.07	1.95±0.17	1.86±0.23	0.40066
Dressing %	68.03±4.28	69.88±2.92	70.33±1.80	72.71±4.74	69.83±0.93	0.55681
Thigh meat (g)	276.67± 18.58	292.00± 46.70	300.67± 23.25	322.00±10.58	303.33±65.74	0.70416
Drumstick meat (g)	221.67± 24.44	240.00±20.30	249.33± 8.50	256.67±36.67	232.67±45.54	0.6529
Breast meat (g)	707.00± 10.44	687.67± 52.00	768.00± 84.50	761.33±152.73	681.67±59.52	0.62061

Table 3. Gut microflora detected in various of broiler chicken supplemented with herbal extracts.

Variables	**Detected bacteria**	**Media**
T_1	*E. coli., Salmonella*	EMB, SS Agar
T_2	*Staphylococcus*	N. broth
T_3	*Staphylococcus*	N. broth
T_4	*Salmonella*	SS Agar
T_5	*Streptococcus*	N. broth

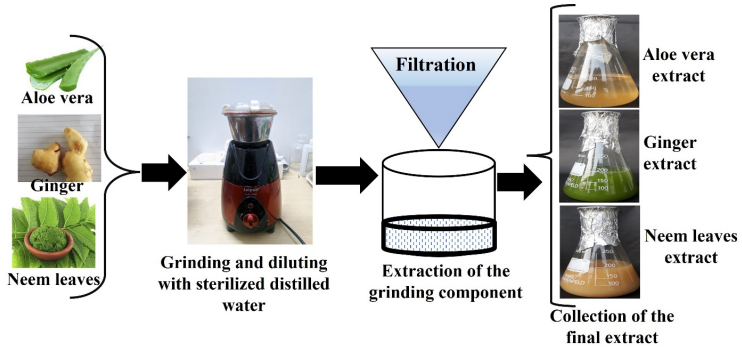


Figure 1. Preparation of aqueous herbal extracts.

The mean values for dressing weight of the experimental broilers under various treatment groups are displayed here in **Table 2**. The obtained dressing weight was 1.7, 1.85, 1.89, 1.95, and 1.86 kg for T_1, T_2, T_3, T_4, and T_5, respectively. Further, the observed dressing percentage relative to the body weight of broilers is presented in **Table 2**. The dressing percentage of the experimental broiler was 68.03, 69.88, 70.33, 72.71, and 69.83 % in T_1, T_2, T_3, T_4, and T_5, respectively.

The mean values for thigh meat, drumstick meat, and breast meat of the experimental broilers under various treatment groups are shown in **Table 2**. The obtained thigh meat weight was 276.67, 292.00, 300.67, 322.00, and 303.33 g for T_1, T_2, T_3, T_4, and T_5, respectively. The result revealed that the mean weight of drumstick meat was 221.67, 240.00, 249.33, 256.67, and 232.67 in T_1, T_2, T_3, T_4, and T_5, respectively. Furthermore, the result revealed that the mean breast meat weight was 707.00, 687.67, 768.00, 761.33, and 681.67 g in T_1, T_2, T_3, T_4, and T_5, respectively.

3.4 Effects of herbal extracts on the survivability of the experimental broilers

The observed survivability percentage of the experimental broilers supplemented with three different herbal extracts is presented in **Fig. 4**. The estimated survivability of the experimental broilers was 99.55, 99.77, 100, 100, and 100% in T_1, T_2, T_3, T_4, and T_5, respectively.

3.5 Effects of aloe vera, ginger, and neem leaf extract on intestinal microbes

On day 34 of the experimental period, two broilers were randomly selected from each replication and were sacrificed. After slaughter, the intestinal content was collected immediately by swab. The samples were immediately mixed with distilled water and stored at -10°C. Samples were serially diluted from 10^{-1} to 10^{-7}. The E. coli count was performed in MacConkey agar after aerobic incubation at 37°C for 24 h. The numbers of colony-forming units (CFU) were expressed as Log10 CFU per gram. Microbiological analysis of intestinal content revealed the presence of several types of bacteria in different treatments (**Table 3**). The microscopic image of the observed bacteria in various groups of broilers after herbal supplementation is presented in **Figure 5.** The microbial analysis revealed that the non-supplemental control group (T_1) contained *E. coli.* and *Salmonella,* supplemental group T_2 and T_3 contained *Staphylococcus* whereas T_4 and T_5 contained *Salmonella,* and *Streptococcus*, respectively.

4. Discussion

4.1 Effects of herbal extracts on feed and water intake of experimental broilers

The results revealed a significant ($P \leq 0.05$) difference in feed intake among the treatments at 34 days. The highest feed intake was observed in T_4, followed by T_2, T_3, and T_5, while the lowest intake was recorded in the non-supplemented control group (T1). Significant differences ($P \leq 0.05$) were found between T_1 (control) and T_2, T_3, and T_4, whereas the difference between T_1 and T_5 was non-significant. The findings align with several studies supporting the positive effects of phytogenic supplements on feed intake. For instance, Hosseini (2010) reported that *Aloe vera* supplementation increased feed consumption in broilers. Similarly, Mitsch et al., (2014) noted that *Aloe vera* improves gut microflora, enhancing digestive health. Javed et al. (2009) and Owen and Amakiri (2011) highlighted ginger's role in improving palatability, nutrient utilization, and appetite stimulation due to its bioactive compounds (e.g., gingerol, shogaols). However, contrasting results exist. Amerah et al., (2013) found no effect of *Aloe vera* on feed consumption, while Fakhim et al., (2013) and Rahman et al., (2015) reported no significant differences with ginger or neem-ginger mixtures. Landy et al. (2011a) also observed no impact of neem leaf powder on feed intake.

In the current study, increased feed intake in supplemented groups (T_2-T_5) compared to the control (T_1) may be attributed to enhanced palatability, nutrient utilization, and the antimicrobial/antioxidant properties of *Aloe vera*, neem, and ginger, either alone or in combination. For water intake, no significant ($P > 0.05$) differences were observed among treatments at 34 days. The highest intake was in T_2, followed by T_4, T_5, and T_1, while T_3 had the lowest. This aligns with Odo et al. (2010) and Islam et al. (2017), who reported no significant effects of *Aloe vera* on water intake. Conversely, Durrani et al. (2008) noted reduced water intake with neem powder supplementation, whereas Nileshkumar et al. (2018) found no improvement with neem extract.

4.2 Effects of herbal extracts on the production performance of experimental broilers

The highest body weight was observed in T_4 (2.22 ± 0.04 kg), followed by T_2, T_3, and T_5, while the lowest was in the control group T_1 (2.05 ± 0.05 kg). Supplemental groups (T_2-T_5) showed significantly ($P \leq 0.05$) higher body weights than T_1, but no significant differences ($P > 0.05$) were found among supplemented groups. Similarly, T_4 had the highest body weight gain (2.07 ± 0.04 kg), followed by T_2, T_3, and T_5, with the lowest gain in T_1 (2.02 ± 0.11 kg). These findings align with Shokraneh et al., (2016), who reported that *Aloe vera* supplementation significantly increased broiler body weight. Khan et al., (2014) also observed greater weight gain with *Aloe vera* leaf supplementation (1–2%). Conversely, Fakhim et al., (2013) found no significant effect of ginger extract on body weight, while Ubua et al., (2019) reported no improvement with neem extract during the starter phase. The superior performance in T_4 (neem extract) may be attributed to improved gut health, antimicrobial effects, and enhanced nutrient absorption. Notably, antibiotic-free production was achieved, suggesting herbal extracts as viable alternatives.

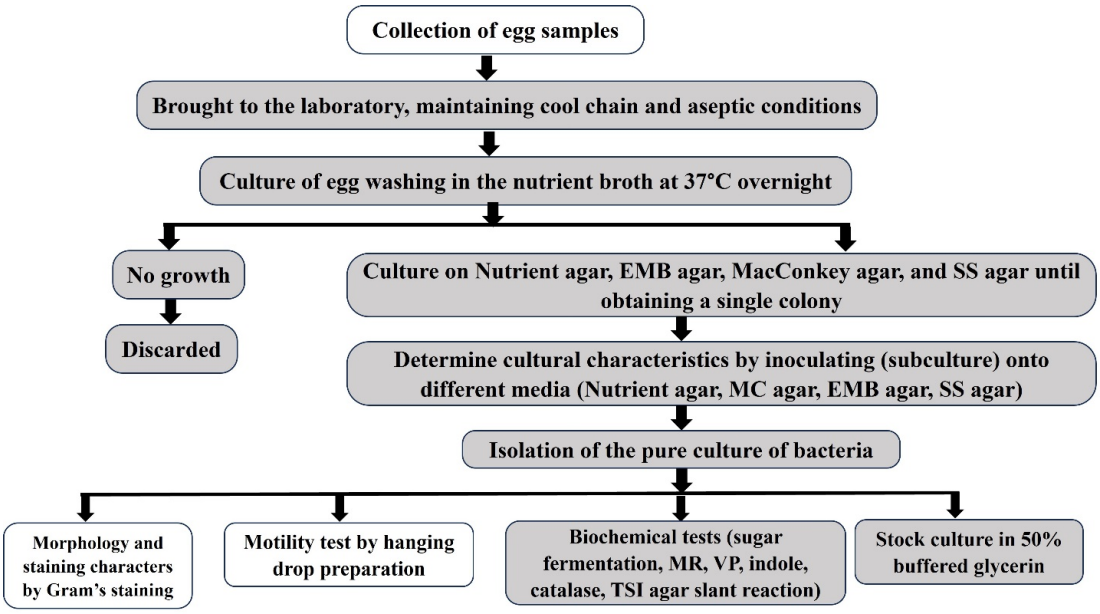


Figure 2. Flow chart of assessing the bacterial load in the gut of broilers under various groups. Here, n = 30.

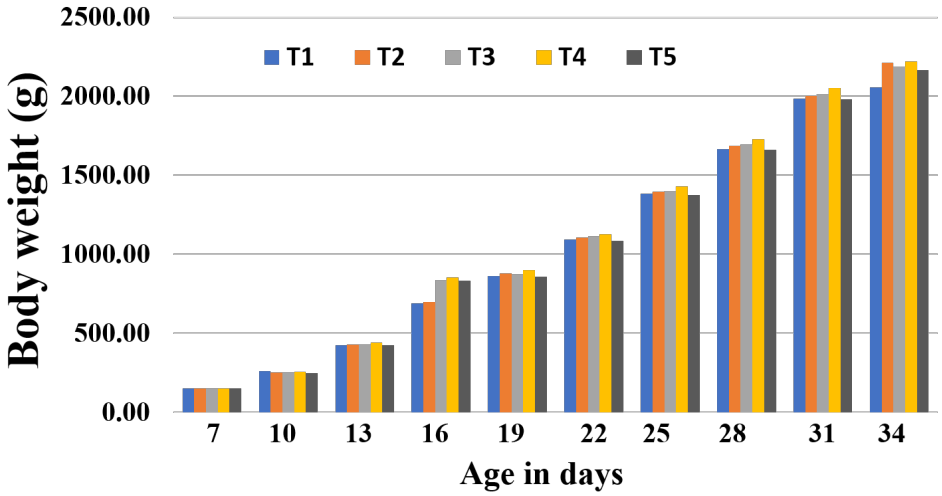


Figure 3. Live weight patterns of the experimental birds from 7 to 34 days of age under various treatments.

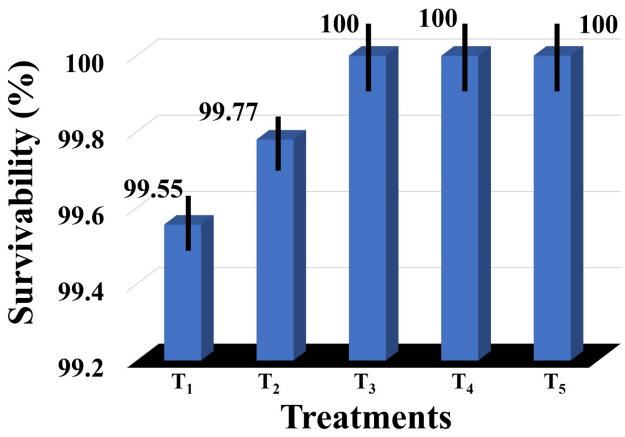


Figure 4. Survivability percentage of broilers supplemented with the herbal extract.

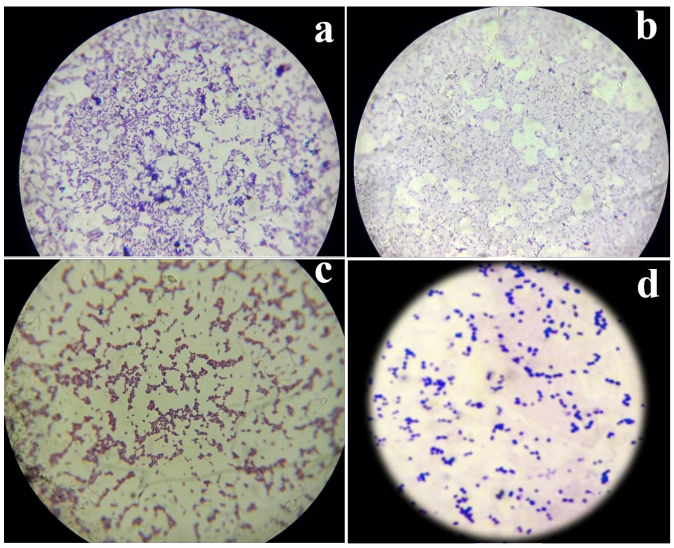


Figure 5. Microscopic image of bacteria obtained in the intestinal samples after supplementation of herbal extracts. Here *E. coli.*, *Salmonella, Staphylococcus*, and *Streptococcus* are indicated by plate (a), (b), (c), and (d), respectively.

No significant ($P > 0.05$) differences in FCR were observed among groups, though T_4 (1.56) had the best FCR, followed by T_5 (1.57), T_2 (1.57), and T_3 (1.58), while T_1 (1.65) performed worst. This indicates that neem extract (T_4) slightly improved feed efficiency. Zidan et al. (2016) and Moorthy et al. (2009) reported enhanced FCR with ginger powder supplementation, while Fakhim et al. (2013) attributed improvements to ginger's digestive-stimulating properties. In contrast, Ubua et al. (2019) found no FCR improvement with neem extract. Nileshkumar et al. (2018) noted that 4% neem infusion improved FCR, likely due to its antimicrobial effects.

4.3 Effects of herbal extracts on carcass characteristics of the experimental broilers

Statistical analysis showed that herbal extract supplementation had no significant ($P > 0.05$) effect on dressing weight or dressing percentage. However, the highest dressing percentage was observed in T^4 (neem extract, 72.71%), followed by T_3 (ginger extract, 70.33%), while the control group (T_1) had the lowest (68.04%). Groups supplemented with Aloe vera (T_2) or a mixture of all extracts (T_5) showed intermediate values (~69.8%). These findings align with Shokraneh et al. (2016) and Sinurat et al. (2002), who reported no significant impact of Aloe vera on carcass traits. Conversely, Talukder et al. (2017) found that ginger supplementation improved dressing percentage significantly ($P < 0.05$).

No significant differences ($P > 0.05$) were observed in thigh or drumstick weights among treatments. The highest thigh weight was recorded in T_4 (neem extract), followed by T_5 (mixture), T_3 (ginger), and T_2 (Aloe vera), with the lowest in T_1 (control).

Similarly, drumstick weights were highest in T_4 (256.7 g) and lowest in T_1 (221.7 g). These results are supported by Moorthy et al. (2009) and Iheanetu et al. (2014), who reported no significant effects of herbal supplements on cut parts. However, Fallah (2015) noted improved thigh and breast weights with Aloe vera + garlic supplementation, likely due to enhanced metabolism. Breast meat weights were statistically similar ($P > 0.05$) across groups, though T3 (ginger, 768.0 g) and T4 (neem, 761.3 g) showed numerically higher values than the control (T1, 707.0 g). Discrepancies exist in the literature: Ubua et al. (2019) and Obun et al. (2013) reported significant improvements with neem supplementation, while Doley et al. (2014) attributed positive effects to Aloe vera + yeast combinations. Most studies, including Islam et al. (2017) and Iheanetu et al. (2014), found no significant effects of herbal extracts on internal organs or abdominal fat. However, Ademola et al. (2009) observed that ginger (2%) + garlic (1.5%) significantly improved carcass part development ($P < 0.01$).

4.4 Effects of herbal extracts on the survivability of the experimental broilers

No significant difference ($P>0.05$) in survivability was observed among treatment groups. All supplemented groups (T_3-T_5) showed 100% survivability, while the control group (T_1) had 99.5% survivability. These findings demonstrate the safety and potential benefits of herbal supplementation in broiler production. Recent studies support these observations: Gaikwad et al. (2019) reported 100% survivability in ginger-supplemented broilers. Deka et al. (2020) found 100% livability with neem extract compared to 97.22% in controls. Dipin et al. (2017) confirmed no adverse effects of aloe vera on broiler health. The excellent survivability in supplemented groups (particularly T_3-T_5) may be attributed to: a) antimicrobial properties of herbal extracts suppressing pathogenic bacteria, b) stress-reducing effects of phytogenic compounds, and c) improved overall health status without antibiotic use. Notably, these results

were achieved under antibiotic-free conditions, suggesting that herbal extracts can effectively maintain flock health. While some studies like Durrani et al. (2008) reported significantly improved survivability with neem extracts, our findings align more closely with Ansari et al. (2012), who observed no significant mortality differences. The 100% survivability in T_3 (ginger), T_4 (neem) and T_5 (mixed extracts) groups indicates these supplements may offer particular benefits for broiler health management, though the differences from control were not statistically significant in this study.

4.5 Effects of aloe vera, ginger, and neem leaves extract on intestinal microbes

Aloe vera plays a significant role in balancing and improving intestinal microflora in chickens due to its antibacterial properties. The microbial analysis of broiler chickens supplemented with aloe vera extract (T_2) revealed the presence of only Staphylococcus, while E. coli and Salmonella were detected in the control (T_1). Studies have shown that aloe vera can improve intestinal microflora by increasing Lactobacillus and decreasing harmful bacteria like E. coli (Darabighane et al., 2012; Shokraneh et al., 2016). Aloe vera's antimicrobial activity is attributed to acemannan, a polysaccharide that has immunomodulatory, antimicrobial, and antitumor effects (Chois & Chang, 2003).

Ginger is another natural growth promoter and an alternative to antibiotics in green broiler production. Microbial analysis revealed that broilers supplemented with ginger extract (T_3) also showed only Staphylococcus in their intestinal content, while E. coli and Salmonella were found in the control group. Studies (Amaduruonye et al., 2018; Adeyemo et al., 2016) have demonstrated that ginger supplementation can reduce microbial load in the caeca and limit the growth of harmful bacteria like E. coli.

Neem leaf extract has long been recognized for its medicinal properties, including antibacterial and antifungal effects. The microbial analysis of broilers supplemented with neem leaf extract (T_4) showed only Salmonella, in contrast to E. coli and Salmonella detected in the control group. Neem has been shown to boost immune function and exhibit antibacterial activity against a wide range of pathogens (Landy et al., 2011b; Subapriya & Nagini, 2005). Previous studies have also reported that neem leaf extracts effectively reduce bacterial counts in poultry (Odoh & Bratte, 2015). The microbial analysis indicated that E. coli and Salmonella were present in the control group (T_1), while only Staphylococcus was found in the aloe vera (T_2) and ginger (T_3) supplemented groups. The neem (T_4) and other herbal extract groups showed improvement in microbial safety, with T5 being the safest in terms of microbial contamination, as it contained Streptococcus but no harmful E. coli or Salmonella.

The use of herbal extracts such as aloe vera, ginger, and neem leaf as growth promoters offers a promising alternative to antibiotics in poultry production, particularly in countries like Bangladesh, where the ban on antibiotics in livestock is becoming more common. These natural additives not only improve growth performance but also enhance microbial safety, offering a sustainable approach to antibiotic-free poultry production.

5. Conclusion

The present study demonstrated that supplementation of selected herbal extracts—aloe vera, neem leaves, ginger, and their combination—through drinking water positively influenced the production performance and carcass traits of broiler chickens without causing significant adverse effects. Among the treatments, neem leaf extract (T_4) consistently showed superior results in terms of feed intake, body weight gain, feed conversion ratio (FCR), and dressing percentage. Although most differences in carcass characteristics were statistically non-significant, improvements in key parameters such as drumstick and breast meat weights were observed in the herbal-supplemented groups compared to the control. Additionally, survivability rates were slightly higher in the treated groups, with the highest (100%) observed in T_3, T_4, and T_5. Importantly, microbial analysis revealed the absence of *E. coli* and *Salmonella* in the samples from T_2, T_3, and T_5, indicating a potential antimicrobial benefit of the herbal extracts. Based on these findings, it can be concluded that herbal supplementation offers a promising, sustainable, and safe alternative to antibiotics in broiler production, particularly in developing countries like Bangladesh, where antibiotic-free poultry practices are increasingly essential for public health.

Author contributions

M.S.I. supervised the research and conceptualized the theme of the manuscript. A.H.H. conducted the research. M.Z.I., J.R., M.S.A., and M.M.H. prepared the herbal extracts and the images of the manuscript. performed most of the experiments in the manuscript. A.H.H. and M.Z.I. wrote the original manuscript. S.S.J. and M.S.I. edited and reviewed the manuscript. All authors reviewed, revised, and approved the final draft for publication.

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Competing financial interests

The authors have no conflict of interest.

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