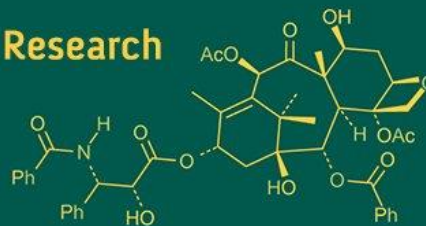


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## Effect of supplementation of herbal growth promoters (*Andrographis paniculata*, *Boerhaavia diffusa*, *Achyranthus aspera* and *Azadirachta indica*) on the nutrient digestibility and gut health parameter in broilers

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### Abstract

The present study was conducted to evaluate the effect of dietary supplementation of herbal growth promoters (HGP), namely *Andrographis paniculata*, *Boerhaavia diffusa*, *Achyranthus aspera*, and *Azadirachta indica*, at equal proportion on nutrient digestibility, gut health, and intestinal microbiota in broiler chickens. A total of 420 day-old Cobb-400 broiler chicks were randomly allotted to seven treatment groups and reared under standard management conditions for six weeks followed by 5 days of metabolic trial. T<sub>1</sub> served as the negative control (basal diet without antibiotic), T<sub>2</sub> received the antibiotic growth promoter (AGP) Enramycin 10% and T<sub>3</sub> through T<sub>7</sub> received the HGP at different doses of 200, 400, 600, 800, and 1000 g/ton of feed, respectively. Nutrient digestibility was assessed through a metabolism trial, while gut health was evaluated based on intestinal morphology and microbial load. Results revealed that organic matter, ether extract and nitrogen free extract were significantly ( $P \leq 0.05$ ) influenced by supplementation of different levels of herbal growth promoters in broilers. In the present study among the supplemented groups, higher organic matter ( $71.46 \pm 0.59$ ), ether extract ( $75.81 \pm 0.50$ ) and nitrogen free extract ( $76.28 \pm 0.49$ ) digestibility was observed in birds fed with T<sub>4</sub> diet containing herbal growth promoters @ 400 g/ton of feed, during the entire period (1-42 days of age). However, dry matter, crude protein and crude fibre digestibility show no significant ( $P \leq 0.05$ ) difference between different levels of herbal growth promoters and the control group. Intestinal morphology was significantly improved in herbal-supplemented birds, with T<sub>4</sub> exhibiting higher villus height in the duodenum ( $1406.70 \pm 11.15$ ), jejunum ( $1261.06 \pm 9.59$ ), and ileum ( $913.25 \pm 14.97$ ), and favorable crypt architecture indicating enhanced absorptive capacity. Microbial analysis showed reduced *Escherichia coli* counts in T<sub>4</sub> ( $4.56 \pm 0.04$ ) and increased *Lactobacillus* populations ( $8.24 \pm 0.05$ ), suggesting a favorable shift in gut microflora. Overall, the study concludes that inclusion of herbal growth promoters at @ 400 g/ton of feed, improves gut morphology, enhances nutrient digestibility, and promotes beneficial intestinal microbiota. These findings support the potential of phytochemical feed additives as effective natural alternatives to antibiotic growth promoters in broiler production systems.

**Keywords:** *Azadirachta indica* (Neem), Nutrient digestibility, *Escherichia coli* (E coli), *Lactobacillus* and Broiler

### Introduction

The poultry industry is one of the fastest-growing sectors of agriculture and plays a vital role in ensuring food security, nutritional security, and rural livelihoods. In India, the poultry population reached 851.81 million according to the 20th Livestock Census (2019), representing a growth of 16.8% over the previous census. The sector contributes approximately 51.14% of the country's total meat production and provides direct and indirect employment to nearly 6.5 million people. With annual growth rates of 5.57% in egg production and 11.44% in broiler production, poultry farming has become a major source of affordable animal protein and farm income. Modern broiler production focuses on maximizing growth performance and feed efficiency while minimizing production costs. Feed accounts for nearly 70–80% of the total production cost, making nutrient utilization and

gut health critical determinants of profitability. Traditionally, antibiotic growth promoters (AGPs) have been used to enhance growth and feed conversion efficiency; however, concerns regarding antimicrobial resistance and drug residues in poultry products have led to restrictions on their use worldwide. Consequently, phytogenic feed additives have gained considerable attention as natural and sustainable alternatives to AGPs. Medicinal herbs possess antimicrobial, antioxidant, immunomodulatory, and digestive-stimulatory properties that can improve gut health, nutrient digestibility, and overall bird performance.

In the present study, a polyherbal phytobiotic comprising *Andrographis paniculata*, *Boerhaavia diffusa*, *Achyranthes aspera*, and *Azadirachta indica* was selected based on their complementary bioactive properties. *A. paniculata* is rich in andrographolide, which exhibits antimicrobial, anti-inflammatory, and digestive-stimulatory activities that can improve gut function and nutrient assimilation (Akbar, 2011). *B. diffusa* possesses potent antioxidant and hepatoprotective properties that support liver function and enhance nutrient metabolism (Mishra *et al.*, 2014). *A. aspera* contains saponins and other phytoconstituents known to improve digestive efficiency, nutrient utilization, and microbial balance in the gastrointestinal tract (Varadharajan *et al.*, 2012). *A. indica* (neem) is well recognized for its antimicrobial, antioxidant, and immunomodulatory effects, which contribute to maintaining intestinal health and reducing pathogen load (Subapriya and Nagini, 2005).

The combination of these medicinal plants extract in equal proportion was expected to provide synergistic effects by improving gut microbial ecology, enhancing digestive processes, promoting nutrient digestibility, and strengthening host health, thereby serving as a potential natural alternative to AGPs in broiler nutrition. Therefore, the present study was undertaken to evaluate the effects of dietary supplementation of this polyherbal phytobiotic on nutrient digestibility and gut health of broiler chickens.

## Material and methods

A feeding trial was conducted to evaluate the efficacy of a polyherbal growth promoter (HGP) as an alternative to antibiotic growth promoters in broiler chickens. The herbal formulation consisted of equal proportions of *Andrographis paniculata*, *Boerhaavia diffusa*, *Achyranthes aspera*, and *Azadirachta indica* plant extracts. A maize-soybean meal-based basal diets (Table 1) were formulated using locally available feed ingredients to meet the nutrient requirements of broiler chickens during the pre-starter, starter, and finisher phases, and subsequently seven experimental diets (Table 2) were prepared by supplementing the basal diet with either an antibiotic growth promoter or graded levels of a polyherbal growth promoter. A total of 420-day-old, Cobb-400 broiler chicks were obtained from a commercial hatchery. Upon arrival, the chicks were individually weighed, wing-banded for identification, and randomly segregated into seven dietary treatment groups (T<sub>1</sub>-T<sub>7</sub>). Each treatment consisted of four replicates with 15 birds per replicate, making a total of 60 birds per treatment. The experimental birds were maintained under a deep-litter housing system and managed according to standard broiler rearing practices throughout the six-week experimental period. Routine vaccination and health management protocols were followed uniformly across all treatment

groups. The experiment was conducted at the Department of Livestock Production and Management, Veterinary College, Bidar, under Karnataka Veterinary, Animal and Fisheries Sciences University (KVAFSU), Bidar. All experimental procedures were performed in compliance with the ethical standards approved by the Institutional Animal Ethics Committee (IAEC), Veterinary College, Bidar, Karnataka, under Project Proposal No. VCB/IAEC/LPM-09/2023-24.

**Table 1:** Per cent ingredient and nutrient composition of basal experimental diet Ingredient composition

| Ingredients         | Prestarter (0-7 d) | Starter (8-21 d) | Finisher (22-42 d) |
|---------------------|--------------------|------------------|--------------------|
| Yellow maize        | 51                 | 55               | 58.5               |
| Soyabean meal       | 41.92              | 37.5             | 32.5               |
| Vegetable oil       | 3.1                | 4                | 5.5                |
| Dicalcium phosphate | 1.0                | 1.0              | 1.0                |
| Common salt         | 0.3                | 0.3              | 0.3                |
| Mineral mixture*    | 2.0                | 2.0              | 2.0                |
| Vitamin premix **   | 0.1                | 0.1              | 0.1                |
| DL-Methionine       | 0.1                | 0.1              | 0.1                |
| Liver tonic         | 0.13               | 0.13             | 0.13               |
| Mycotoxin Binder    | 0.1                | 0.1              | 0.1                |
| Coccidiostat        | 0.09               | 0.09             | 0.09               |
| Total               | 100.0              | 100.0            | 100.0              |

## Chemical composition of different broiler diets (%DMB)

| Experimental diet | DM%   | CP%   | CF%  | EE%  | TA%  | NFE%  |
|-------------------|-------|-------|------|------|------|-------|
| Prestarter        | 89.35 | 22.53 | 4.32 | 4.32 | 7.35 | 61.48 |
| starter           | 89.74 | 20.81 | 4.85 | 4.17 | 7.86 | 62.31 |
| Finisher          | 89.17 | 19.72 | 5.21 | 5.31 | 7.12 | 62.64 |

<sup>1</sup> Provides per kg: Calcium – 230 g and Phosphorus – 180 g

<sup>2</sup> Provides per kg: Mn – 80 mg, Zn – 80 mg, I – 3.29 mg, Fe – 32 mg and Se – 0.1 mg

<sup>3</sup> Provides per kg: A – 12,375 IU, D<sub>3</sub> – 1,800 IU, B<sub>2</sub> – 7.5 mg, K – 1.5 mg, B<sub>1</sub> – 4 mg, B<sub>6</sub> – 8 mg, B<sub>12</sub> – 40 µg, E – 20 mg, Niacin – 60 mg and Calcium pantothenate – 12.5 mg

**Table 2:** Description of experimental design from 0 - 42 days period

| T <sub>1</sub> | Basal diet (Without Antibiotic)                        |
|----------------|--------------------------------------------------------|
| T <sub>2</sub> | Basal diet + AGP (Enramycin 10%) at 100g/ton of feed   |
| T <sub>3</sub> | Basal diet+ Herbal Growth Promoter (200g/ton of feed)  |
| T <sub>4</sub> | Basal diet+ Herbal Growth Promoter (400 g/ton of feed) |
| T <sub>5</sub> | Basal diet+ Herbal Growth Promoter (600g/ton of feed)  |
| T <sub>6</sub> | Basal diet+ Herbal Growth Promoter (800g/ton of feed)  |
| T <sub>7</sub> | Basal diet+ Herbal Growth Promoter (1000g/ton of feed) |

## Nutrient Digestibility

The feeding trial was conducted for six weeks, followed by a 5-day metabolic trial. The experimental diet (Table 1) and water were fed ad libitum during the trial. One bird from each replicate was randomly selected and housed individually in metabolic cages. The first two days served as an adaptation period, followed by a 3-day collection period during which daily feed intake and excreta output were recorded accurately to assess nutrient digestibility. Proximate composition of feed and excreta samples, including dry matter (DM), crude protein (CP), ether extract (EE), crude fibre (CF), and total ash, was determined according to standard procedures described by the Association of Official Analytical Chemists (AOAC, 2019). Nitrogen-free extract (NFE) was calculated by difference. Organic matter (OM) was calculated as the difference between dry matter and total ash.

The apparent dry matter digestibility (DMD) was calculated using the following equation:

$$\text{DMD (\%)} = \frac{\text{Weight of the dry feed consumed (g)} - \text{Weight of the dried excreta voided (g)}}{\text{Weight of the dry feed consumed (g)}} \times 100$$

Similarly, the apparent digestibility coefficients of organic matter, crude protein, ether extract, crude fibre, and nitrogen-free extract were calculated as:

$$\text{Digestibility of Nutrient (\%)} = \frac{\text{Unit nutrient intake (g)} - \text{Unit nutrient out go (g)}}{\text{Unit nutrient intake (g)}} \times 100$$

## Gut Health Parameter

### Gross Morphology

The intestinal sample collection for microbial analysis was done according to the procedure outlined by (Song *et al.*) [27]. On 42<sup>nd</sup> day eight birds from each treatment (two birds per replicate) were slaughtered. After evisceration the entire intestine was aseptically removed. The contents of the intestine from the distal end of the duodenum to the ileo-cecal junction were collected by squeezing the intestine gently and rapidly into a sterile sample container placed in ice box. The samples of intestinal contents were transported to the laboratory for microbial enumeration. On 42<sup>nd</sup> day during slaughter, 2 cm long segment of duodenum, jejunum and ileum were collected and then washed with physiological saline solution and fixed in 10% neutral buffered formalin solution. Samples collected from duodenum, jejunum and ileum of birds were processed for histomorphological examination in terms of measurement of parameters like villus length (VL), crypt depth (CD). Histological technique involves processes like fixation of tissue, dehydration, clearing, embedding, cutting and staining.

Fixation in 10% formalin with approximately 10-20 times the volume of the specimen was done. Tissues were dehydrated by using increasing the strength of alcohol like 50%, 70%, 90% and 100%. Clearing was done by replacing alcohol by xylene for 30min – 1h. Impregnation of tissue with wax was done at the melting point temperature of paraffin wax and the volume of wax was about 25-30 times the volume of tissues for a total duration of 4 h. Impregnated tissues were placed in a mould with their labels and then fresh melted wax was poured in it and allowed to settle and solidify. These paraffin embedded tissues were sectioned at 5µm thickness and stained routinely with Hematoxylin-Eosin stain (H&E). Histological sections were examined under 2X of light microscopy with micrometry and photographic attachment. The images were analyzed using image analyzing software (OLYMPUS cell Sens Standard, version 1.13). A total of 20 intact well oriented crypt-villus units per bird were selected randomly, measured and the mean length was calculated for each sample. Villus length was measured from the tip of the villi to the base between individual villi, and crypt depth measurements were taken from the valley between individual villi to the basal membrane.

## Microbial Load of Gastro Intestinal Tract

The intestinal contents collected were subjected to the total viable counts of *Escherichia coli* and *Lactobacilli spp.* eight birds from each dietary treatment were slaughtered on 42<sup>nd</sup> day intestines were dissected at Meckel's diverticulum. Eosin methylene blue agar (EMB) for *E. coli* growth, and MRS agar (De Man, Rogosa and Sharpe agar) for *Lactobacillus spp* growth was used. Then, 9 sterile test tubes with lids containing 9mL of phosphate buffer solution (PBS, pH-7.4) as diluent was prepared. Approximately 1g of the intestinal contents taken by sterile swab and homogenized for 3 min, aseptically mixed, added to the tubes, and diluted up to 10<sup>9</sup>. Later, 1ml of the contents of each test tube was transferred to one of three selective agar media on petri plates, respectively. Aerobic bacterial plates (*E. coli.*) were placed in an incubator at 37° C for 24 h. Anaerobic (*Lactobacilli spp.*) medium plates were placed in an anaerobic jar with an anaerobic gas pack system at 37°C for 24 h. Finally, the intestinal bacterial colony populations formed in each plate was counted by a colony counter and the number of colonies was expressed as log10 value.

## Statistical Analysis:

The data obtained from the present study was analyzed using Two-way ANOVA with Bonferroni Post Test utilizing Graph Pad Prism version 5.0 software to compare the groups at P<0.05 level of significance.

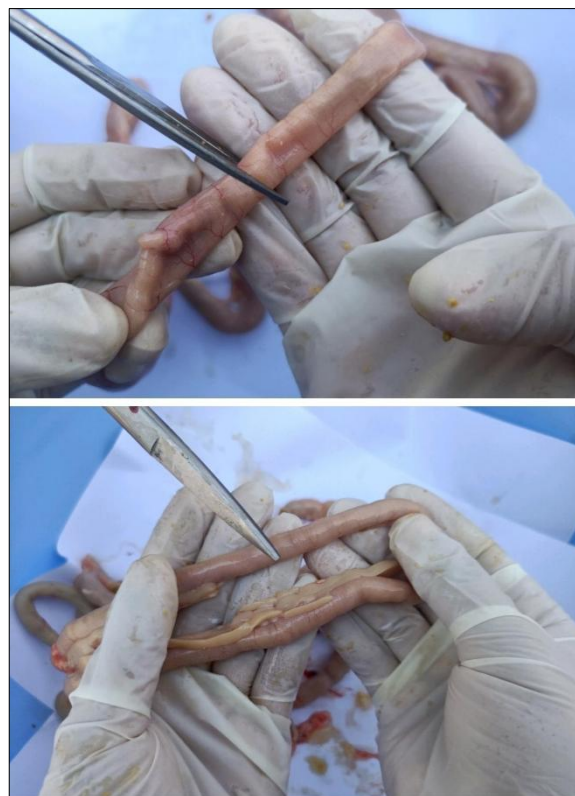


**Fig 1:** Samples collection for histomorphological examination and total viable counts of *Escherichia coli* and *Lactobacilli spp*

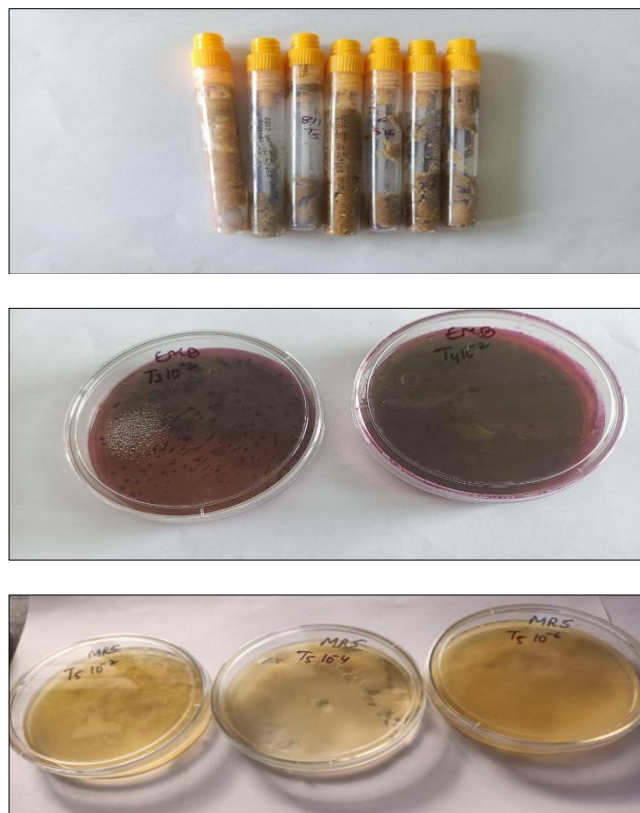


**Fig 2:** Collection of excreta from intestine for total viable counts of *Escherichia coli* and *Lactobacilli* spp

## Result and Discussion

### Effect of herbal growth promoters on nutrient digestibility

The effects of herbal growth promoters (HGP) and their combinations on nutrient digestibility in broilers are presented in Table 2. Dry matter digestibility (DMD), crude protein digestibility (DCP), and crude fibre digestibility (DCF) did not differ significantly ( $P>0.05$ ) among the treatment groups. Numerically, the highest DMD was observed in  $T_2$  ( $69.81\pm0.88\%$ ) which was fed with antibiotic, followed by  $T_4$  ( $68.32\pm0.66\%$ ), whereas the lowest value was recorded in  $T_7$  ( $66.52\pm1.23\%$ ). Similarly, DCP ranged from  $63.45\pm1.34\%$  ( $T_7$ ) to  $65.01\pm0.73\%$  ( $T_4$ ), while DCF ranged from  $30.56\pm3.11\%$  ( $T_6$ ) to  $34.26\pm1.36\%$  ( $T_4$ ).

Organic matter digestibility (DOM) was significantly influenced by dietary treatments ( $P<0.05$ ). The highest DOM was recorded in  $T_2$  ( $72.69\pm0.80\%$ ), which was significantly higher than  $T_7$  ( $69.04\pm1.14\%$ ), while  $T_1$ ,  $T_3$ ,  $T_4$ ,  $T_5$ , and  $T_6$  remained statistically comparable. Ether extract digestibility (DEE) also differed significantly ( $P<0.05$ ), with  $T_4$  exhibiting the highest value ( $75.81\pm0.50\%$ ) and  $T_1$  the lowest ( $68.53\pm1.17\%$ ). Nitrogen-free extract digestibility (DNFE) was significantly higher in  $T_2$  ( $78.39\pm0.63\%$ ) compared with most other groups, whereas the lowest value was observed in  $T_7$  ( $73.86\pm0.96\%$ ). However, DNFE values of  $T_2$  and  $T_4$  ( $76.28\pm0.49\%$ ) were statistically comparable.

The present study evaluated a polyherbal phytobiotic comprising *Andrographis paniculata*, *Boerhaavia diffusa*, *Achyranthes aspera*, and *Azadirachta indica* as a potential alternative to antibiotic growth promoters (AGPs) in broilers. Although the antibiotic-fed group ( $T_2$ ) recorded the highest DOM and DNFE, the phytobiotic-supplemented group  $T_4$  (400 g/ton feed) exhibited statistically comparable values and achieved the highest DCP and DEE.

Furthermore, the absence of significant differences between  $T_2$  and the phytobiotic-supplemented groups for DMD, DCP, and DCF indicates that the herbal formulation maintained nutrient utilization efficiency comparable to the antibiotic treatment.

The improved nutrient digestibility observed in  $T_4$  may be attributed to the synergistic action of bioactive compounds present in the constituent herbs. Collectively, these phytochemicals may enhance digestive enzyme secretion, improve intestinal microbial balance, and increase nutrient absorption. The results of the present study are in agreement with the findings of Singh *et al.* [26] and Kulhrey *et al.*, [16] who reported significant improvements in nutrient digestibility, particularly of organic matter (OM), ether extract (EE), and nitrogen-free extract (NFE), following herbal supplementation. Similarly, Bhattacharyya *et al.* [6], Assunção *et al.* [4], Tomar *et al.* [29], and Prakash *et al.* [23] reported higher values for dry matter digestibility (DMD), crude protein digestibility (DCP), and crude fibre digestibility (DCF) in supplemented groups, although the differences were not statistically significant. Mahmood *et al.* [18] also reported a non-significant improvement in crude fibre digestibility in broiler chicks. Similar improvements in nutrient digestibility following phytochemical supplementation have been attributed to enhanced digestive function and improved gut health in broilers (Amad *et al.*, and Aruwa *et al.*) [1, 3]. In contrast, the highest supplementation level ( $T_7$ ) resulted in lower digestibility values, suggesting that excessive phytobiotic inclusion may reduce the beneficial effects of phytochemicals on nutrient utilization.

### Gut health

#### Gross morphology (villus height and crypt depth)

The effect of dietary supplementation of herbal growth promoters and their combinations on intestinal morphology in broiler chickens is presented in Table 4 and figure 3. A marked improvement in villi length and crypt depth was

observed across different segments of the small intestine in treated groups compared to the control. In the duodenum, the highest villi length was recorded in T<sub>4</sub> (1406.70±11.15), followed by T<sub>5</sub> (1371.54±9.29) and T<sub>3</sub> (1366.51±7.96), while the lowest value was observed in T<sub>1</sub> (1150.40±8.99). Duodenal crypt depth was significantly higher in T<sub>6</sub> (178.86±1.54), whereas the lowest value was noted in T<sub>4</sub> (161.73±1.50). In the jejunum, T<sub>4</sub> exhibited the maximum villi height (1261.06±9.59), followed by T<sub>6</sub> (1207.26±13.10), while T<sub>7</sub> recorded the minimum (1131.92±12.89). Jejunal crypt depth was highest in T<sub>4</sub> (164.83±1.58) and lowest in T<sub>7</sub> (142.71±1.36). In the ileum, significantly greater villi length was observed in T<sub>4</sub> (913.25±14.97), followed by T<sub>3</sub> (893.95±7.06), whereas the lowest value was recorded in T<sub>1</sub> (702.19±10.09). Ileal crypt depth was highest in T<sub>2</sub> (161.46±2.84) and lowest in T<sub>7</sub> (145.10±1.94). Overall, dietary inclusion of herbal growth promoters, particularly in T<sub>4</sub>, resulted in improved intestinal morphology characterized by increased villi length and favorable crypt architecture across all intestinal segments, indicating enhanced absorptive surface area and better gut health compared to the control group. These results of the present study are in agreement with the growth promoting effect of supplementation of *Azadirachta indica* reported by Tamilvanam *et al.* [28] revealed that neem and Aloe vera supplementation significantly (P<0.05) improved the height of villi in duodenum, jejunum and ileum. crypt depth was (P<0.05) significantly reduced by Aloe and neem supplementation in duodenum and ileum except jejunum. Similarly, Liu *et al.* [17] reported that supplementation of unfermented and fermented *A. paniculata* improved intestinal morphology (villi height, intestinal thickness and villi surface area) and intestinal microbiota composition.

The morphological structure of the intestinal mucosa has a direct bearing on the growth performance of poultry, and the health of the birds depends on the integrity of its structure and function (Wang *et al.*) [30] due to changes in the architecture of the gut caused by increased intercellular space, fast cell division, and elongation, which improved the surface area available for nutrient absorption as shown by improvements in growth performance and feed efficiency. It has been observed that extracts from herbs and spices may increase enzyme activity and digestive secretions, which may have positive effects on the digestive system (Platel *et al.*) [22]. It is well documented that microbes can cause a change in the intestinal architecture. Improved villus height and crypt depth ratio was reported by (Amad *et al.*) [1] in broilers. An enhanced degree of epithelial turnover may be correlated with the growth of the intestinal villi and the villus crypt. Herbal growth enhancers have a good effect on broiler chickens, as evidenced by the enlarged intestinal dimensions in these animals.

However, the group supplemented with herbal growth promoters @ 1000 g/ton of feed (T<sub>7</sub>) recorded the lowest villi length and crypt depth (duodenum, jejunum and ileum) Olorunbohunmi, [20] revealed that supplemented with moringa and neem had an increased villus height and crypt depth, than that of negative control.

### Microbial load of GIT

The results on the *Escherichia coli* (*E. coli*) and *Lactobacillus spp* counts (expressed in log<sub>10</sub> CFU/mL) in the small intestine as influenced by different dietary treatments at 42<sup>nd</sup> day of age and the same are presented in

Table 5. The *E. coli* counts for T<sub>1</sub>, T<sub>2</sub>, T<sub>3</sub>, T<sub>4</sub>, T<sub>5</sub>, T<sub>6</sub> and T<sub>7</sub> group's was 4.96±0.06, 4.71±0.03, 4.86±0.03, 4.56±0.04, 4.84±0.10, 5.13±0.09 and 4.97±0.05 (CFU/mL) respectively. Significantly lowest (P<0.05) *E. coli* counts were observed in T<sub>4</sub> compared to T<sub>1</sub> (Control) and T<sub>6</sub>. However, there was no significant influence of dietary supplementation of herbal growth promoters among other treatment groups (T<sub>3</sub> and T<sub>5</sub>). The *Lactobacilli* counts for T<sub>1</sub>, T<sub>2</sub>, T<sub>3</sub>, T<sub>4</sub>, T<sub>5</sub>, T<sub>6</sub> and T<sub>7</sub> group's was 7.77±0.09, 8.15±0.04, 8.04±0.07, 8.24±0.05, 8.13±0.03, 7.91±0.07 and 7.70±0.04 (CFU/mL) respectively. Significantly highest (P<0.05) *Lactobacilli* counts were observed in T<sub>4</sub> compared to T<sub>7</sub>. However, there were significant effect of herbal growth promoters on *Lactobacilli* counts in all other treatment groups

These results of the present study are in agreement with the (Tamilvanam *et al.*, Assuncao *et al.*, Park and Kim, Chachar *et al.*, Jahja *et al.*, Liu *et al.*, and Rehman *et al.*) [28, 4, 21, 8, 12, 17, 24]. The study suggested that the lowest pathogenic bacteria (*E. coli*) and highest non-pathogenic bacteria (*lactobacillus*) observed in broiler chickens supplemented with herbal growth promoters at 400 g/ton of feed could be attributed to several factors. Trillions of bacteria inhabit the gastrointestinal tract of animals. In most hosts, these symbionts play a large role in promoting microbe-host internal environmental balance (Wang *et al.*) [30]. As the microbial population in the gastrointestinal tract of poultry plays an important role in normal digestive processes and health maintenance. This microbial shift might be due to the presence of huge numbers of chemically diverse and biologically active ingredients like Andrographolides active ingredient of *A. paniculata* function as antimicrobials which help to maintain the balance of microflora within the digestive tract. Ideally, more than 90% of the microflora population in the digestive tract should be beneficial bacteria, such as *Lactobacilli* and Bifido bacteria and only a very small proportion of potentially damaging bacteria should be present, such as *E. coli* and *Staphylococci*. A previous study has shown that the active ingredients of many medicinal plants inhibit growth of food-borne and other pathogens and fungi in the digestive tract (Guthter and Ulfah,) [10]. Hidanah *et al.* [11] also reported that feed supplemented with aqueous *A. paniculata* extract could reduce *E.coli* bacterial infection. This is in accordance with the statement of Aruwa *et al.* [3], that beneficial bacteria such as *Lactobacillus spp.* was predominant in small intestine, especially in the ileum. Herbal growth promoters has ability to decrease harmful bacteria and also might be because of establishment of beneficial *Lactobacillus* sps. in that intestine. The final fermentation product of *Lactobacillus* sps. is lactic acid that might have made the gut environment unfavorable for pathogens and modified harmful microbial population. However, the group supplemented with herbal growth promoters @ 800 g/ton of feed (T<sub>6</sub>) recorded the highest *E. coli* count and herbal growth promoters @ 1000 g/ton of feed (T<sub>7</sub>) recorded the lowest *lactobacillus* count as reported by Andrew *et al.* [2] observed that there was no significant difference in aerobic bacteria, lactic acid-producing bacteria and coliform bacteria (p>0.05) count across the experimental treatments containing Superliv powder®, SP (*Andrographis paniculata*, *Boerhaavia diffusa*, *Achyranthus aspera*, *Azadirachta indica* etc).

**Table 3:** Effect of herbal growth promoters and their combination on nutrient digestibility percentage in broilers

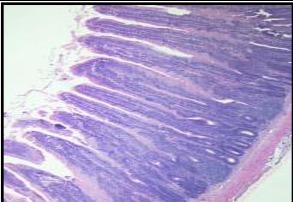
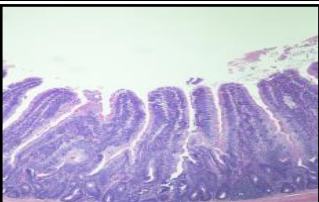
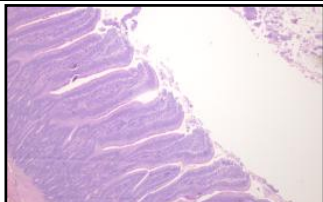
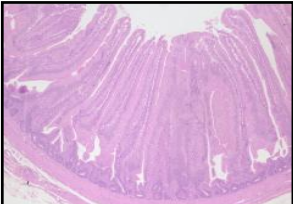
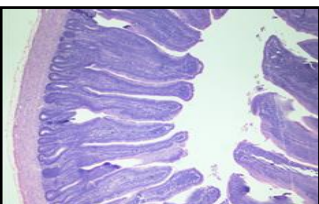
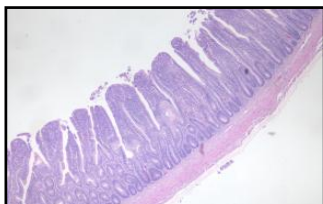
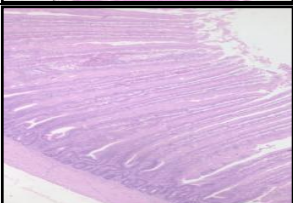
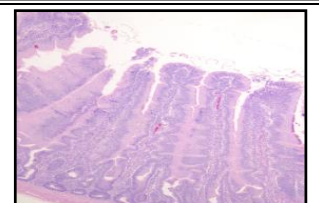
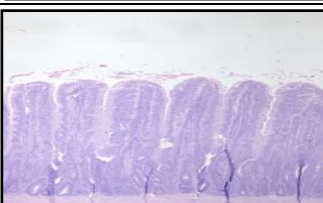
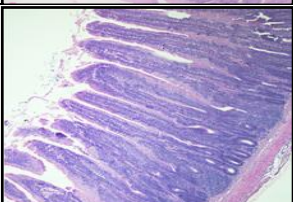
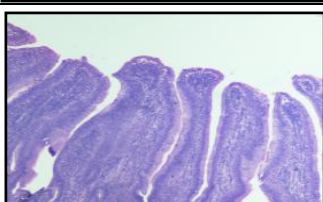
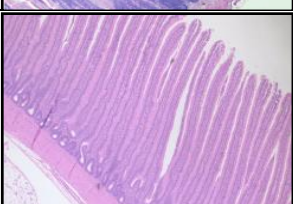
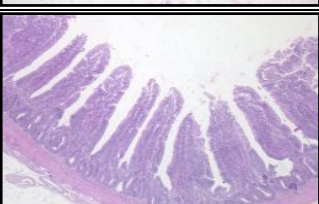
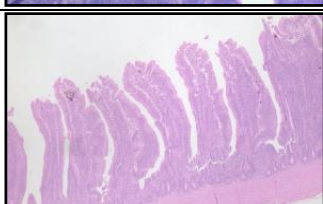
| Treatment      | DMD %      | DOM%                     | DCP%       | DEE%                      | DCF%       | DNFE%                    |
|----------------|------------|--------------------------|------------|---------------------------|------------|--------------------------|
| T <sub>1</sub> | 66.64±1.24 | 69.24±1.14 <sup>ab</sup> | 63.51±1.36 | 68.53±1.17 <sup>d</sup>   | 31.20±2.56 | 74.33±0.95 <sup>b</sup>  |
| T <sub>2</sub> | 69.81±0.88 | 72.69±0.80 <sup>a</sup>  | 64.61±1.03 | 74.13±0.75 <sup>ab</sup>  | 34.01±1.93 | 78.39±0.63 <sup>a</sup>  |
| T <sub>3</sub> | 67.19±1.44 | 70.15±1.31 <sup>ab</sup> | 63.76±1.59 | 72.26±1.22 <sup>bc</sup>  | 31.39±3.01 | 75.27±1.08 <sup>b</sup>  |
| T <sub>4</sub> | 68.32±0.66 | 71.46±0.59 <sup>ab</sup> | 65.01±0.73 | 75.81±0.50 <sup>a</sup>   | 34.26±1.36 | 76.28±0.49 <sup>ab</sup> |
| T <sub>5</sub> | 67.55±1.12 | 70.60±1.01 <sup>ab</sup> | 64.90±1.21 | 72.19±0.96 <sup>abc</sup> | 33.43±2.30 | 75.42±0.85 <sup>b</sup>  |
| T <sub>6</sub> | 67.42±1.46 | 70.05±1.34 <sup>ab</sup> | 63.48±1.64 | 70.05±1.34 <sup>cd</sup>  | 30.56±3.11 | 75.47±1.10 <sup>b</sup>  |
| T <sub>7</sub> | 66.52±1.23 | 69.04±1.14 <sup>b</sup>  | 63.45±1.34 | 71.06±1.06 <sup>abc</sup> | 30.94±2.53 | 73.86±0.96 <sup>b</sup>  |

Values bearing different superscripts (a,b,c,d) differ significantly (P<0.05) between groups

**Table 4:** Effect of herbal growth promoters and their combination on gross morphology of intestine in broilers

| Treatment      | Duodenum                    |                           | Jejunum                     |                           | Ileum                      |                            |
|----------------|-----------------------------|---------------------------|-----------------------------|---------------------------|----------------------------|----------------------------|
|                | Villi length (µm)           | Crypt depth (µm)          | Villi length (µm)           | Crypt depth (µm)          | Villi length (µm)          | Crypt depth (µm)           |
| T <sub>1</sub> | 1150.40±08.99 <sup>d</sup>  | 172.75±1.40 <sup>ab</sup> | 1020.79±24.25 <sup>e</sup>  | 157.62±0.91 <sup>ab</sup> | 702.19±10.09 <sup>e</sup>  | 158.26±2.74 <sup>ab</sup>  |
| T <sub>2</sub> | 1219.07±14.21 <sup>c</sup>  | 171.92±2.16 <sup>ab</sup> | 1134.83±11.52 <sup>cd</sup> | 151.98±0.93 <sup>b</sup>  | 805.34±12.54 <sup>cd</sup> | 161.46±2.84 <sup>a</sup>   |
| T <sub>3</sub> | 1366.51±07.96 <sup>a</sup>  | 169.58±0.49 <sup>ab</sup> | 1158.20±15.02 <sup>bc</sup> | 161.94±3.09 <sup>a</sup>  | 893.95±07.06 <sup>ab</sup> | 148.78±1.25 <sup>cd</sup>  |
| T <sub>4</sub> | 1406.70±11.15 <sup>a</sup>  | 161.73±1.50 <sup>b</sup>  | 1261.06±09.59 <sup>a</sup>  | 164.83±1.58 <sup>a</sup>  | 913.25±14.97 <sup>a</sup>  | 157.33±1.99 <sup>ab</sup>  |
| T <sub>5</sub> | 1371.54±09.29 <sup>a</sup>  | 170.38±4.08 <sup>ab</sup> | 1092.63±16.42 <sup>d</sup>  | 161.72±1.46 <sup>a</sup>  | 843.22±18.21 <sup>bc</sup> | 152.05±2.44 <sup>abc</sup> |
| T <sub>6</sub> | 1292.62±07.00 <sup>b</sup>  | 178.86±1.54 <sup>a</sup>  | 1207.26±13.10 <sup>ab</sup> | 163.88±2.52 <sup>a</sup>  | 832.79±25.21 <sup>bc</sup> | 155.90±3.16 <sup>abc</sup> |
| T <sub>7</sub> | 1180.72±08.78 <sup>cd</sup> | 173.16±3.56 <sup>a</sup>  | 1131.92±12.89 <sup>cd</sup> | 142.71±1.36 <sup>c</sup>  | 768.79±12.83 <sup>d</sup>  | 145.10±1.94 <sup>d</sup>   |

Values bearing different superscripts (a,b,c,d) differ significantly (P<0.05) between groups

| Treatment      | Deodunum                                                                            | Jejunum                                                                              | Ileum                                                                                 |
|----------------|-------------------------------------------------------------------------------------|--------------------------------------------------------------------------------------|---------------------------------------------------------------------------------------|
| T <sub>1</sub> |   |   |   |
| T <sub>2</sub> |  |  |  |
| T <sub>3</sub> |  |  |  |
| T <sub>4</sub> |  |  |  |
| T <sub>5</sub> |  |  |  |

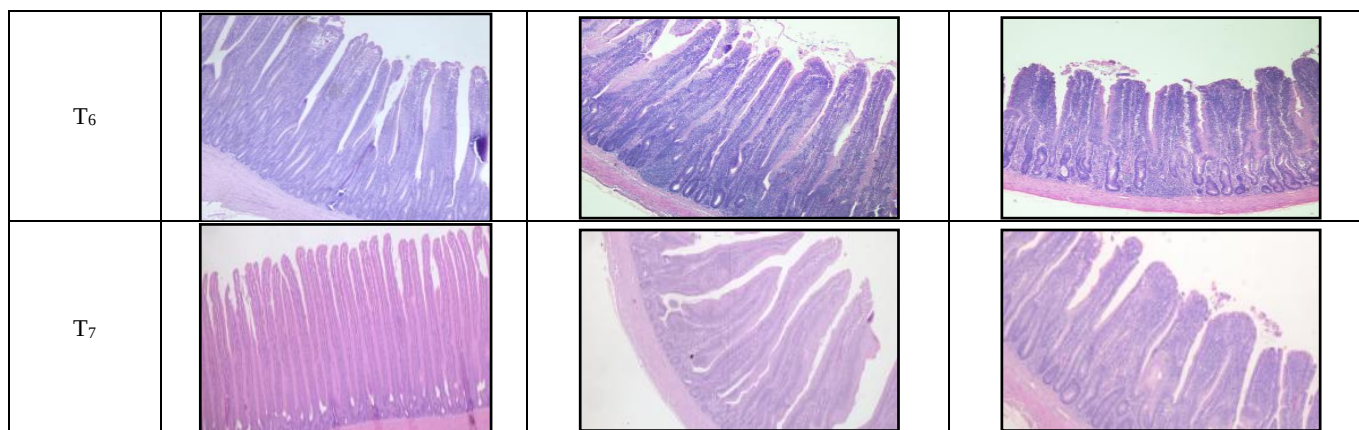


**Fig 3:** Photomicrograph of the cross section of intestine (villus length and crypt depth) in intestine of broilers

**Table 5:** Effect of herbal growth promoters and their combination on microbial load of GIT in broilers

| Treatment      | <i>E. Coli</i> (Log CFU/mL) | <i>Lactobacilli</i> (Log CFU/mL) |
|----------------|-----------------------------|----------------------------------|
| T <sub>1</sub> | 4.96±0.06 <sup>d</sup>      | 7.77±0.09 <sup>de</sup>          |
| T <sub>2</sub> | 4.71±0.03 <sup>ab</sup>     | 8.15±0.04 <sup>ab</sup>          |
| T <sub>3</sub> | 4.86±0.03 <sup>bc</sup>     | 8.04±0.07 <sup>bc</sup>          |
| T <sub>4</sub> | 4.56±0.04 <sup>a</sup>      | 8.24±0.05 <sup>a</sup>           |
| T <sub>5</sub> | 4.84±0.10 <sup>bc</sup>     | 8.13±0.03 <sup>ab</sup>          |
| T <sub>6</sub> | 5.13±0.09 <sup>d</sup>      | 7.91±0.07 <sup>cd</sup>          |
| T <sub>7</sub> | 4.97±0.05 <sup>cd</sup>     | 7.70±0.04 <sup>e</sup>           |

Values bearing different superscripts (a,b,c,d) differ significantly ( $P < 0.05$ ) between groups

### Conclusion

The present study indicates that dietary supplementation of herbal growth promoters positively influences nutrient utilization, gut health, and intestinal microbial balance in broiler chickens.

1. The nutrient digestibility in terms of DM, OM, CP, EE, CF and NFE were studied. OM, EE and NFE were significantly ( $P \leq 0.05$ ) influenced by supplementation of different levels of herbal growth promoters in broilers. In the present study among the treatment groups, higher organic matter, ether extract and nitrogen free extract digestibility was observed in birds fed with T<sub>4</sub> diet containing herbal growth promoters @ 400 g/ton of feed. However, DM, CP and CF digestibility show no significant ( $P \leq 0.05$ ) difference between different levels of herbal growth promoters and control.
2. In the present study, the gut morphometric parameters were significantly influenced ( $P < 0.05$ ) by the addition of different levels of herbal growth promoters. T<sub>4</sub> group exhibited the highest mean villus length (duodenal, jejunum and ileum) and the highest mean crypt depth (jejunum and ileum). However, highest mean duodenal crypt depth were exhibited in herbal growth promoters @ 800 g/ton of feed (T<sub>6</sub>) and the lowest mean duodenal crypt depth were exhibited in T<sub>4</sub> group.
3. The microbial load of the gastrointestinal tract (GIT) were significantly influenced ( $P < 0.05$ ) by the addition of different levels of herbal growth promoters. Specifically, the *E. coli* counts in the intestine were lowest in birds fed with herbal growth promoters at 400 g/ton of feed (T<sub>4</sub>), while the *Lactobacilli* counts were highest in birds fed with the same dosage.

Thus, looking at the performance of supplementation of herbal growth promoters @ 400 g/ton of feed (T<sub>4</sub>) showed significant ( $P \leq 0.05$ ) improvement in nutrient digestibility, gut health parameters. Based on the experiment it can be concluded that *Andrographis paniculata*, *Boerhaavia diffusa*, *Achyranthus aspera* and *Azadirachta indica* in equal combination is safe to use in broilers without any harmful effect and could be used as replacement of antibiotic growth promoters in commercial broiler chicken

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### References

1. Amad AA, Wendler KR, Zentek J. Effects of a phytogenic feed additive on growth performance, selected blood criteria and jejunal morphology in broiler chickens. *Emirates Journal of Food and Agriculture*. 2013;25:549-554.
2. Andrew BF, Oloruntola OD, Olorotimi OJ. Influence of phytobiotic and synbiotic-acidifier blend supplements on haematobiochemical indices, antioxidant enzymes and gut microflora of broiler chicken. *Malaysian Animal Husbandry Journal*. 2023;3(1):1-6.
3. Aruwa CE, Pillay C, Nyaga MM, Sabiu S. Poultry gut health-microbiome functions, environmental impacts, microbiome engineering and advancements in characterization technologies. *Journal of Animal Science and Biotechnology*. 2021;12(1):1-15.
4. Assunção PDS, Mello HHD, Mascarenhas AG, Andrade MA, Teixeira KA, Oliveira HFD, et al. Use of neem (*Azadirachta indica*) as a substitute for antimicrobial drugs in broiler chickens' feed. *Ciência Animal Brasileira*. 2019;20:52588.

5. Bhaskar D, Sharma RK, Chauhan SS, Singh H, Kumar A. Effect of herbal detoxicants and liver stimulant on the performance of broiler chickens. *Indian Journal of Poultry Science*. 2003;38(2):110-114.
6. Bhattacharyya A, Garg SK, Kumar V, Roy D, Ravikanth K, Maini S. Effects of Superliv concentrate on growth, immunocompetence traits and nutrient retention of commercial broilers during extreme winter. *International Journal of Poultry Science*. 2013;12(1):51-54.
7. Buchanan NP, Hott JM, Cutlip SE, Rack AL, Asamer A, Moritz JS. Effects of natural antibiotic alternatives and growth promoters on broiler performance and carcass quality. *Journal of Applied Poultry Research*. 2008;17:202-210.
8. Chachar RA, Kamboh AA, Bakhetgul M. Effects of *Moringa* and neem leaves on immune response and gut microflora in Japanese quails. *Pakistan Journal of Zoology*. 2022;1-9.
9. Dhumal MV, Nikam MG, Nandedkar PV, Majee SB, Kotagiri R. Efficacy of herbal liver tonic in broilers. *International Journal of Livestock Research*. 2018;8(6):266-278.
10. Guthrie KD, Ulfah M. Influence of natural essential oils on digestion, metabolism and production efficiency. In: *Proceedings of the 4th Buffalo Symposium*; 2003; New Delhi, India.
11. Hidanah S, Sabdoningrum EK, Al Arif MA, Ansori ANM, Hasanah TP, Widaya LVA. *Sambiloto* (*Andrographis paniculata*) extract improves performance of broilers infected with *Escherichia coli*. *Indian Journal of Forensic Medicine and Toxicology*. 2020;14(4):3491-3496.
12. Jahja EJ, Yuliana R, Simanjuntak WT, Fitriya N, Rahmawati A, Yulinah E. Potency of *Origanum vulgare* and *Andrographis paniculata* extracts on growth performance in poultry. *Veterinary and Animal Science*. 2023;19:100274.
13. Jamroz D, Orda J, Kamel C, Wiliczekiewicz A, Wiertelcki T, Skorupinska J. Influence of phytogetic extracts on performance and gut microbial status in broilers. *Journal of Animal and Feed Sciences*. 2003;12:583-596.
14. Khan MTJ, Ahmad K, Alvi MN, Mansoor B, Saeed MA, Khan FZ, *et al.* Antibacterial activity of medicinal plant extracts. *Pakistan Journal of Zoology*. 2010;42(1):93-97.
15. Kim S, Lee K, Kang C, An B. Effects of essential oils on growth performance and gut microflora in broilers. *Asian-Australasian Journal of Animal Sciences*. 2016;29:549-554.
16. Kulhrey R, Kumar A, Mondal BC, Palod J, Singh PK. Effect of neem and curry leaf powder on broiler performance. *Indian Journal of Animal Nutrition*. 2016;33(2):208-211.
17. Liu Z, Lei X, Li J, Zhong Y, Tan D, Zhang Q, *et al.* Effects of fermented *Andrographis paniculata* on growth and intestinal health in poultry. *Poultry Science*. 2023;102(3):102461.
18. Mahmood S, Hayat K, Yousaf M, Abba G, Rehman A. Comparative effect of herbal extracts on broiler performance. *Scholar's Advances in Animal and Veterinary Research*. 2016;3(2):60-65.
19. Malahubban M, Alimon AR, Sazili AQ, Fakurazi S. Effects of *Andrographis paniculata* supplementation on broiler performance. *International Journal of Agriculture and Biology*. 2013;15(2):1-5.
20. Olorunbohunmi TO. Growth performance and gut health of broilers fed *Moringa oleifera* and *Azadirachta indica*. *Nigerian Journal of Animal Production*. 2022;49(3):186-194.
21. Park JH, Kim IH. Effects of *Achyranthes japonica* extract on broiler performance and gut health. *Poultry Science*. 2020;99(1):463-470.
22. Platel K, Srinivasan K. Digestive stimulant action of spices. *Indian Journal of Medical Research*. 2004;119:167-179.
23. Prakash B, Mondal BC, Palod J, Kumar A, Kumar R. Effect of neem leaf powder on broiler performance and nutrient utilization. *Indian Journal of Veterinary Sciences and Biotechnology*. 2022;18(4):109-113.
24. Rehman A, Hussain K, Zaman MA, *et al.* Effects of herbal extracts on broiler growth and gut microbial population. *Kafkas Üniversitesi Veteriner Fakültesi Dergisi*. 2023;29(4):407-413.
25. Shaahu DT, Uza O, Gege BM, Awua LL. Comparative efficiency of antibiotics and neem extract in broilers. *Nigerian Journal of Animal Science and Technology*. 2020;3(1):1-9.
26. Singh VK, Chauhan SS, Ravikanth K, Maini S, Rekhe DS. Effect of polyherbal supplementation on broiler performance. *Veterinary World*. 2009;2(9):350-352.
27. Song J, Xiao K, Ke YL, Jiao LF, Hu CH, Diao QY, *et al.* Effect of a probiotic mixture on intestinal microflora, morphology, and barrier integrity of broilers subjected to heat stress. *Poultry Science*. 2014;93:581-588.
28. Tamilvanan S, Sivasankar A, Jai S, Marudhai T, Anandamoy K. Effects of *Aloe vera* and neem on gut histomorphometry in chicks. *Veterinary World*. 2017;10:569-573.
29. Tomar RS, Baghel RPS, Nayak S, Khare A, Sharma P. Effect of *Boerhaavia diffusa* on broiler performance and nutrient utilization. *Intas Polivet*. 2020;21(2):518-521.
30. Wang BK, Gong L, Zhou YH, *et al.* Probiotics enhance broiler growth by improving intestinal health. *Animal Nutrition*. 2021;7:829-840.