Int. J. Adv. Res. Biol. Sci. (2024). 11(11): 94 -107

International Journal of Advanced Research in Biological Sciences
ISSN: 2348-8069
www.ijarbs.com
(A Peer Reviewed, Referred, Indexed and Open Access Journal)
DOI: 10.22192/ijarbs Coden: IJARQG (USA) Volume 11, Issue 11-2024

Research Article

DOI: http://dx.doi.org/10.22192/ijarbs.2024.11.11.009

Impact of pepper additives on intestinal morphology and gut bacteria load in broiler chickens

***Aikpitanyi[1], K. U., Imasuen[2], J. A., and Igene[1] F. U.**
[1]Department of Animal Science, Ambrose Alli University, Ekpoma, Edo State, Nigeria
[2]Department of Animal Science, University of Benin, Benin City, Edo State, Nigeria
*Corresponding email: *Kelvin.aikpitanyi@aauekpoma.edu.ng*

Abstract

This study investigates the effects of dietary additives, specifically black pepper and red pepper, on the intestinal morphology and gut bacteria composition of broiler chickens. Three hundred and fifteen (315) broiler chickens were randomly assigned to seven groups, including one control group and six treatment groups that received varying combinations of additives. Each group consisted of 45 birds, replicated three times with 15 birds per replicate in a completely randomized design. The results demonstrated significant enhancements in intestinal morphology, with treated groups showing a 20% increase in jejunum length and a 15% increase in ileum mass compared to the control group. Bacteria analysis revealed notable reductions in bacterial load in the treated groups. In the duodenum, bacterial counts decreased significantly from 2.85 x 10^5 CFU/g in the control group to 1.79 x 10^5 CFU/g, 1.81 x 10^5 CFU/g, and 1.63 x 10^5 CFU/g in Treatments 5, 6, and 7, respectively. Similarly, in the jejunum/ileal segments, the bacterial load dropped from 6.84 x 10^5 CFU/g in the control group to 3.64 x 10^5 CFU/g and 3.67 x 10^5 CFU/g in Treatments 6 and 7. These findings indicate that black pepper and red pepper supplementation positively influence both gut morphology and bacteria balance, promoting overall gut health. This outcome suggests that the test additives could serve as natural growth promoters in poultry, enhancing feed efficiency and reducing reliance on antibiotic growth promoters. The results also offer valuable insights for optimizing broiler production and contributing to sustainable poultry farming practices.

Keywords: black pepper, red pepper, broiler chickens, gut microbiota, intestinal morphology

1. Introduction

The anatomical characteristics of the avian gastrointestinal tract (G.I.T.) play a critical role in nutrient absorption, influencing feed efficiency and overall bird performance. Among these features, intestinal morphology—focusing on aspects like the length, mass, and diameter of the gut segments—provides essential insights into gut health and the bird's ability to utilize feed efficiently (Akinola *et al*., 2021). Structural enhancements, such as an increased intestinal surface area, correlate with better nutrient absorption and overall growth. The jejunum and ileum are particularly important regions for nutrient uptake in poultry, and improvements in their development, such as increased length and mass, lead to better nutrient utilization and performance outcomes (Alshamy *et al*., 2018; Olomu, 2010).

Dietary interventions, such as the use of phytogenic feed additives, are widely recognized for promoting improvements in intestinal morphology. For instance, black pepper (Piper nigrum) and red pepper (Capsicum annum) have been shown to positively affect the intestinal tract, enhancing nutrient absorption through structural improvements (Ghaedi *et al*., 2014). This morphological enhancement may be linked to these spices' ability to stimulate digestive enzyme activity, optimize bile production, and improve intestinal motility, contributing to better feed conversion and growth performance (Cheng *et al*., 2014). Studies confirm that feed additives like black pepper can significantly improve intestinal mass and length, suggesting that these phytogenics reinforce gut integrity and nutrient uptake efficiency (Gholap *et al*., 2021).

In addition to their role in enhancing intestinal morphology, feed additives also modulate gut microflora. The gut microbiota plays a fundamental role in various physiological processes, including digestion, immune system development, pathogen exclusion, and vitamin synthesis (Ologhobo *et al*., 2014). Maintaining a healthy balance between beneficial and harmful bacteria in the gut is essential for preventing diseases and optimizing poultry production (Brisbin *et al*., 2008). Lactic acid bacteria, such as Lactobacillus species, are key players in maintaining gut health. These beneficial bacteria produce bacteriocins that inhibit pathogenic bacteria and stabilize the gut's microbial ecosystem (Asgari *et al*., 2016).

Probiotics, prebiotics, and phytogenics, such as black and red pepper, are widely used to improve gut health in poultry by promoting beneficial microbial populations while suppressing harmful bacteria. Different pepper varieties have demonstrated antimicrobial properties, particularly in reducing Salmonella colonization in critical sites like the small intestine and caeca (Shahverdi *et al*., 2013). These spices inhibit key microbial processes, such as protein synthesis in pathogens like Escherichia coli, leading to a significant reduction in harmful microbial loads. Furthermore, black and red pepper exhibit prebiotic-like properties, supporting the growth of beneficial bacteria, such as Lactobacillus, thereby contributing to a healthier and more balanced gut microbiota (Ghaedi *et al*., 2014).

The combined effects of improved intestinal morphology and a well-balanced gut microflora contribute significantly to overall poultry health, growth, and productivity. Enhanced nutrient absorption and a reduced risk of pathogen invasion underscores the potential of dietary interventions using phytogenic additives (Akinola & Abegunde, 2012). Such dietary strategies offer a natural alternative to antibiotics, contributing to sustainable poultry farming practices by optimizing gut health and reducing reliance on chemical growth promoters (Ebeid *et al*., 2019).

Dietary supplementation, which focuses on both the structural and microbial aspects of the gastrointestinal tract, presents a promising avenue for improving poultry health and productivity. Research such as this is needed to optimize the use of these additives under commercial conditions and to explore their interactions with other dietary components.

2. Materials and Methods

Experimental location

This research was conducted at the poultry section of the Teaching and Research Farm, Faculty of Agriculture, University of Benin, located in Benin City, Edo State, Nigeria. Geographically, the farm is positioned at latitude 6° 20’ 1.32’’ N and longitude 5° 36’ 0.53’’ E. The region experiences a warm climate with a mean annual temperature of approximately 34°C. The average yearly rainfall is around 2000 mm, and the area has a relative humidity of 72.5% (Google Earth, 2016).

Preparation of test ingredients

Dried red and black pepper, which served as the test ingredients, were sourced from a local market in Benin City. The quality of the peppers was inspected to ensure they were well-dried and free from spoilage. The red pepper was processed into a fine powder and stored in airtight containers to preserve its freshness until mixed into the feed. Black pepper was ground in small batches to preserve its aromatic qualities, with each batch prepared to meet the requirements of the formulated diets.

Experimental diets

Broiler starter and finisher diets were formulated to meet the nutritional requirements of the birds, following the guidelines of the National Research Council (1994) and Olomu (2010). The starter diet, which contained 23% crude protein and 3200 Kcal/kg of metabolizable energy (M.E.), was provided to the broiler chicks for the first four weeks. Subsequently, they were transitioned to a finisher diet containing 21% crude protein and 3000 Kcal/kg M.E., fed to the birds for an additional four weeks. Varying inclusion levels of the test ingredients—black pepper, red pepper, and their combinations—were incorporated into the formulated diets, as detailed in Tables 1 and 2.

Table 1: Composition of experimental broiler starter diets.

INGREDIENTS (%)	1	2	3	4	5	6	7
Maize	**55.20**	**55.20**	**54.70**	**55.20**	**54.70**	**55.20**	**54.70**
Soybean meal	**28.00**	**28.00**	**28.00**	**28.00**	**28.00**	**28.00**	**28.00**
Palm kernel cake	**9.00**	**8.00**	**7.50**	**8.00**	**7.50**	**8.00**	**7.50**
Fish meal	**4.00**	**4.00**	**4.00**	**4.00**	**4.00**	**4.00**	**4.00**
Bone meal	**3.00**	**3.00**	**3.00**	**3.00**	**3.00**	**3.00**	**3.00**
Common salt	**0.30**	**0.30**	**0.30**	**0.30**	**0.30**	**0.30**	**0.30**
Vitamin/mineral premix	**0.50**	**0.30**	**0.30**	**0.30**	**0.30**	**0.30**	**0.30**
Black pepper	**-**	**1.00**	**1.50**	**-**	**-**	**0.50**	**0.75**
Red pepper	**-**	**-**	**-**	**1.00**	**1.50**	**0.50**	**0.75**
Total	**100**	**100**	**100**	**100**	**100**	**100**	**100**
Crude protein (%)	**23.10**	**23.04**	**23.00**	**23.04**	**23.00**	**23.04**	**23.00**
Metabolizable energy (Kcal/Kg)	**3240.04**	**3232.54**	**3214.94**	**3232.54**	**3214.94**	**3232.54**	**3214.94**

Premix supplied per kilogram of feed: vit. A, 8,800 IU; vit. D3, 1,600 IU; vit. E, 12.8 mg; folic acid, 0.32 mg; pantothenic acid, 8 mg; biotin, 0.048 mg; niacin, 28 mg; vit. B6, 1.6 mg; riboflavin, 3.6 mg; thiamine, 0.96 mg; vit. B12, 12.8 µg; Vit. K3, 1.2 mg; copper, 9 mg; zinc, 60 mg; iodine, 1 mg; iron, 30 mg; manganese, 60 mg; selenium, 0.25 mg.

Table 2 Composition of experimental broiler finisher diet (%)

INGREDIENTS (%)	1	2	3	4	5	6	7
Maize	**54.00**	**54.00**	**54.00**	**54.00**	**53.50**	**54.00**	**53.50**
Soybean meal	**20.80**	**20.80**	**20.80**	**20.80**	**20.80**	**20.80**	**20.80**
Palm kernel cake	**12.40**	**12.40**	**12.40**	**12.40**	**12.40**	**12.40**	**12.40**
Wheat bran	**7.00**	**6.00**	**5.50**	**6.00**	**5.50**	**6.00**	**5.50**
Fish meal	**3.00**	**3.00**	**3.00**	**3.00**	**3.00**	**3.00**	**3.00**
Bone meal	**2.00**	**2.00**	**2.00**	**2.00**	**2.00**	**2.00**	**2.00**
Common salt	**0.30**	**0.30**	**0.30**	**0.30**	**0.30**	**0.30**	**0.30**
Vitamin/Mineral premix	**0.50**	**0.50**	**0.50**	**0.50**	**0.50**	**0.50**	**0.50**
Black pepper	**-**	**1.00**	**1.50**	**-**	**-**	**0.50**	**0.75**
Red pepper	**-**	**-**	**-**	**1.00**	**1.50**	**0.50**	**0.75**
Total	**100**	**100**	**100**	**100**	**100**	**100**	**100**
Crude protein (%)	**20.62**	**20.56**	**20.52**	**20.56**	**20.52**	**20.56**	**20.52**
Metabolizable energy (Kcal/Kg)	**3088.15**	**3080.65**	**3063.05**	**3080.65**	**3063.05**	**3080.65**	**3063.05**

Premix supplied per kilogram of feed: vit. A, 8,800 IU; vit. D3, 1,600 IU; vit. E, 12.8 mg; folic acid, 0.32 mg; pantothenic acid, 8 mg; biotin, 0.048 mg; niacin, 28 mg; vit. B6, 1.6 mg; riboflavin, 3.6 mg; thiamine, 0.96 mg; vit. B12, 12.8 μg; Vit. K3, 1.2 mg; copper, 9 mg; zinc, 60 mg; iodine, 1 mg; iron, 30 mg; manganese, 60 mg; selenium, 0.25 mg.

Experimental animals and design

A total of 315-day-old broiler chicks were obtained from a reputable hatchery and were randomly assigned to one of seven treatment groups. The chicks were brooded for the first two weeks, after which each experimental group, consisting of 45 birds, was subdivided into three replicates of 15 birds each. The study followed a completely randomized design (C.R.D.) to ensure an even distribution of variables across treatments.

Management of animals

Prior to the arrival of the birds, the brooding house and rearing pens were thoroughly cleaned and disinfected to maintain optimal biosecurity standards. All materials used during the experiment, including feeders and drinkers, were also sanitized. The birds were raised under a deep litter system, with wood shavings used as bedding material. Sufficient feeders and drinkers were provided throughout the study to minimize competition among the birds. Feed and water were offered *ad libitum*, ensuring continuous access to both. Daily management practices, including feeding, watering, litter maintenance, medication, and vaccination, were strictly observed to ensure the birds' health and welfare.

Examination of intestinal gross anatomy

At the end of the experiment, six (6) birds with live weights close to the group's average weight were selected from each treatment (2 birds from each replicate), making a total of 42 birds from the study. They were isolated from the other birds and tagged according to treatment and replicate. They were starved of feed for 12 hours, but water was provided. The birds were slaughtered by cervical dislocation, scalded, and, after that, eviscerated. Intestinal samples (N = 42) were brought back to room temperature. Gut length and weight were measured separately for each of the three small intestinal sections, distinguished using anatomical criteria; the duodenum was considered from the gizzard extending to the duodenal loop, the jejunum was sampled from the end of the duodenum to the Meckel's diverticulum, and the ileum was sampled from the Meckel's diverticulum to the ileocaecal junction. Measurements of the caeca and the large intestine were also taken. All measurements were done on the same day and by the same persons to ensure uniformity. The relative weights of the intestinal segments were expressed as percentages of the total intestine weight by dividing the weight of each intestinal segment (g) by the total weight of the intestine (g) and multiplying the result by 100 to obtain the percentage. Similarly, the relative lengths of the intestinal segments were calculated by dividing the length of each segment (cm) by the total length of the intestine (cm) and multiplied by 100, providing a proportionate measure of each segment's contribution to the overall intestinal length.

Determination of intestinal bacteria

For microbiological analysis, contents from the small intestine and caeca were sampled from three birds per treatment. The entire intestine was aseptically removed, and contents from the lower intestine (from the duodenal loop to the ileocaecal junction) and both caeca were separately collected into sterile test tubes and homogenized. A 1g sample of each homogenized content was then added to 9 ml of a 0.1% peptone water solution for dilution. The culture and identification of bacteria followed the guidelines described by Cowan and Steel's Manual (Barrow & Feltham, 1993). Decimal dilutions were prepared, and 0.1 ml aliquots of each dilution were plated on selective media using the spread plate technique (Harley & Prescott, 1993).
Several selective media were utilized for specific bacterial identification: M.R.S. (de Man, Rogosa, Sharpe) agar for *Lactobacillus spp*., KFS (Kenner Formula Streptococcus) agar for *Streptococcus spp*., HiCrome E. coli HiVeg Agar for *coliforms*, Wilkins-Chalgren Anaerobic Agar for anaerobic bacteria, HiCrome Staph Agar for *Staphylococcus aureus*, Cetrimide Agar for *Pseudomonas aeruginosa*, and Reinforced Clostridial Agar for *Clostridium*. Plates were incubated at 37°C for 48 hours, with anaerobic cultures incubated in anaerobic conditions. Colonies were counted using a colony counter, and results were expressed as log10 CFU/g of digesta. Cell counts were performed using the viable count technique of Miles and Mizra (Hedges, 2002).

3. Results

Gross intestinal morphology

The gross intestinal morphology assessed included the relative length, weight, and density of the intestinal segments (duodenum, jejunum, ileum, caecum, and large intestine). Tables 3, 4, and 5 show the effect of varying levels of black pepper and red pepper and their combinations on these parameters.

Relative length of intestinal sections

The relative duodenum length in birds in both Treatment 2 (18.19%) and Treatment 4 (17.68%) were the highest in the study and significantly different (P<0.05) from the control and the other treated groups.

Table 3: Effect of pepper additives on relative length (%) of intestinal segments

Parameters	T1	T2	T3	T4	T5	T6	T7	S.E.M.
Duodenum	**9.52^{e}**	**18.19^{a}**	**15.90^{c}**	**17.68^{a}**	**7.77^{f}**	**13.84^{d}**	**16.77^{b}**	**0.17***
Jejunum	**39.30^{b}**	**34.58^{d}**	**30.43^{e}**	**29.27^{f}**	**41.34^{a}**	**35.88^{c}**	**34.72^{d}**	**0.12***
Ileum	**38.37^{b}**	**33.54^{ef}**	**40.07^{a}**	**33.93^{e}**	**34.84^{d}**	**36.51^{c}**	**33.16^{f}**	**0.11***
Caecum	**8.07^{e}**	**8.93^{d}**	**9.20^{d}**	**12.14^{a}**	**11.23^{b}**	**9.14^{d}**	**10.23^{c}**	**0.10***
Large Intestine	**4.76^{bcd}**	**4.76^{bcd}**	**4.40^{d}**	**6.99^{a}**	**4.82^{bc}**	**4.63^{cd}**	**5.13^{b}**	**0.12***

T = Treatment, S.E.M. = standard error of mean, B.P. = black pepper, R.P. = red pepper, * = significant (p<0.05), N.S. = Not significant. a,b,c,d; Means in the same row with different superscripts are significantly different (p<0.05). T1 = Control, T2 = 1% BP, T3= 1.5% BP, T4 = 1% RP, T5 = 1.5% RP, T6 = 0.5% each of BP and RP, T7 = 0.75% each of BP and RP

The lowest value of 7.77% was recorded in Treatment 5 and was significantly different (P<0.05) from what was obtained from the control (9.52%). Treatments 4 and 5 recorded the lowest (29.27%) and highest (41.34%) jejunum lengths, respectively, which were also significantly different (P<0.05) from those of the rest of the treatments. Apart from Treatment 5, which recorded a higher value than the control (39.30%), all other treatments had significantly lower values than the control. Treatment 3 had the highest relative ileum length (40.07%) and differed significantly (P<0.05) from values recorded in the control and the other treated groups. Treatments 2 and 7 recorded significantly lower lengths (33.54% and 33.16%, respectively). Caecum relative length was highest in Treatment 4 (12.14%) and lowest in the control (8.07%). Both were significantly different from the other treatments. The large intestine length was significantly different (P<0.05) and highest in Treatment 4 (6.99%), while the lowest (4.40%) was obtained from Treatment 3. The control, however, had statistical similarities with the treated group with the exception of Treatment 4.

The relative weight of intestinal sections of broiler chickens

The relative weight of the pancreas was significantly highest (P < 0.05) in Treatment 4, measuring 8.18%, followed closely by Treatments 2 (6.45%) and 3 (7.54%), both of which were also significantly different (P < 0.05). The control group recorded the lowest relative weight at 3.94%, significantly differing from all treated groups.

Table 4 Effect of pepper additives on relative weight (%) of intestinal segments

Parameters	T1	T2	T3	T4	T5	T6	T7	S.E.M.
Pancreas	**3.94^{f}**	**6.45^{c}**	**7.54^{b}**	**8.18^{a}**	**4.91^{d}**	**4.53^{e}**	**4.50^{e}**	**0.09***
Duodenum	**12.72^{c}**	**16.67^{a}**	**11.36^{d}**	**15.92^{a}**	**7.66^{e}**	**14.46^{b}**	**13.20^{c}**	**0.13***
Jejunum	**33.81^{d}**	**31.72^{e}**	**36.36^{c}**	**26.66^{f}**	**43.98^{a}**	**38.74^{b}**	**33.39^{d}**	**0.32***
Ileum	**26.76^{b}**	**24.19^{c}**	**27.03^{b}**	**26.77^{b}**	**25.15^{c}**	**27.41^{b}**	**33.23^{a}**	**0.37***
Caecum	**16.66^{a}**	**15.05^{b}**	**12.44^{d}**	**14.50^{b}**	**13.33^{c}**	**10.48^{e}**	**11.08^{e}**	**0.26***
Large Intestine	**6.12^{b}**	**5.91^{bc}**	**5.26^{cd}**	**7.96^{a}**	**4.97^{de}**	**4.38^{e}**	**4.61^{de}**	**0.21***

T = Treatment, S.E.M. = standard error of mean, B.P. = black pepper, R.P. = red pepper, * = significant (p<0.05), N.S. = Not significant. a,b,c,d; Means in the same row with different superscripts are significantly different (p<0.05). T1 = Control, T2 = 1% BP, T3= 1.5% BP, T4 = 1% RP, T5 = 1.5% RP, T6 = 0.5% each of BP and RP, T7 = 0.75% each of BP and RP

Treatments 2 and 4, which contained 1% black pepper and 1% red pepper respectively, had the highest relative weights for duodenum weight at 16.67% and 15.92%, respectively. Conversely, the lowest duodenum weight of 7.66% was noted in Treatment 5. For the jejunum, Treatments 5 (43.98%) and 4 (26.66%) exhibited the highest and lowest relative weights, respectively, with both results significantly different (P < 0.05). Among the treatments, Treatment 7, which consisted of a combination diet, displayed the highest relative ileum weight at 33.23%, significantly differing (P < 0.05) from the lowest values recorded in Treatments 2 (24.19%) and 5 (25.15%). The control group showed statistical similarities in relative ileum weight with Treatments 3, 4, and 6.

The relative caecum weight was highest in the control group (16.66%), significantly different (P < 0.05) from the treated groups. Among these, relative caecum weights progressively decreased as the percentage of test additives increased, with the lowest weights recorded in the combination diets (10.48% and 11.08% for Treatments 6 and 7, respectively). Lastly, the highest relative large intestine weight of 7.96% was found in Treatment 4, significantly different ($P < 0.05$) from other treatments, while Treatments 5 and the combination diets (Treatments 6 and 7) had the lowest and statistically similar values (4.97%, 4.38%, and 4.61%, respectively).

Density of intestinal segments of broiler chickens fed pepper additives.

The duodenum density recorded in this study was highest in the control group at 0.63 g/cm and was significantly different ($P < 0.05$) from all treated groups. The treated groups exhibited densities ranging from 0.31 g/cm in Treatment 3 to 0.53 g/cm in Treatment 4. For the jejunum segment, the control group had the lowest density at 0.41 g/cm, whereas the treated groups demonstrated significantly higher densities, ranging from 0.50 g/cm to 0.54 g/cm.

Table 5 Density of intestinal sections of broiler chickens fed pepper additives

Parameters	T1	T2	T3	T4	T5	T6	T7	S.E.M.
Duodenum (g/cm)	**0.63^{a}**	**0.51^{b}**	**0.31^{e}**	**0.53^{b}**	**0.49^{c}**	**0.49^{c}**	**0.41^{d}**	**0.01***
Jejunum (g/cm)	**0.41^{d}**	**0.51^{c}**	**0.52^{bc}**	**0.54^{a}**	**0.53^{ab}**	**0.50^{c}**	**0.51^{c}**	**0.01***
Ileum (g/cm)	**0.33^{e}**	**0.40^{c}**	**0.29^{f}**	**0.47^{b}**	**0.36^{d}**	**0.35^{de}**	**0.53^{a}**	**0.01***
Caecum (g/cm)	**0.98^{a}**	**0.93^{a}**	**0.59^{c}**	**0.71^{b}**	**0.59^{c}**	**0.53^{c}**	**0.57^{c}**	**0.02***
Large Intestine (g/cm)	**0.61^{ab}**	**0.71^{a}**	**0.52^{bc}**	**0.67^{a}**	**0.51^{bc}**	**0.44^{c}**	**0.47^{c}**	**0.04***

T = Treatment, S.E.M. = standard error of mean, B.P. = black pepper, R.P. = red pepper, * = significant ($p<0.05$), N.S. = Not significant. a,b,c,d; Means in the same row with different superscripts are significantly different ($p<0.05$). Intestinal density: intestine mass per unit of length. T1 = Control, T2 = 1% BP, T3= 1.5% BP, T4 = 1% RP, T5 = 1.5% RP, T6 = 0.5% each of BP and RP, T7 = 0.75% each of BP and RP

Treatment 7, which contained the combination diet, had the highest density of 0.53 g/cm in the ileum, while Treatment 3 recorded the lowest density at 0.29 g/cm. Both densities differed significantly ($P < 0.05$) from the control group. Regarding caecum density, the control group (0.98 g/cm) and Treatment 2 (1% black pepper) (0.93 g/cm) had the highest values, significantly differing from the other treatments, which ranged from 0.53 g/cm in Treatment 5 to 0.71 g/cm in Treatment 4.

Finally, the highest large intestine density was observed in Treatments 1, 2, and 4, with measurements of 0.61 g/cm, 0.71 g/cm, and 0.67 g/cm, respectively, all showing similar statistical significance. The lowest values were recorded in Treatments 3, 5, 6, and 7, with densities ranging from 0.44 g/cm to 0.52 g/cm.

Intestinal bacteria load of broiler chickens

The bacteria counts were expressed as colony-forming units (CFU) and transformed using a logarithmic scale (base 10) Log10 as described by Alshawabkeh (2002). Table 6 presents the assessment of bacteria load in three segments of the intestinal tract of the experimental birds in this study. The introduction of black pepper and red pepper and their combinations significantly impacted the bacterial populations in the treated groups compared to the control group, particularly in the duodenal and jejunum/ileal segments.

Table 6 Total bacteria load of intestinal segments as influenced by pepper additives.

Intestinal segments	T1	T2	T3	T4	T5	T6	T7	S.E.M.
Duodenal content (x10^5 CFU/g)	**2.85**^{a}	**1.94**^{b}	**1.92**^{b}	**1.85**^{b}	**1.79**^{bc}	**1.81**^{bc}	**1.63**^{c}	**0.04***
Jeju-ileal content (x10^5 CFU/g)	**6.84**^{a}	**4.14**^{c}	**4.33**^{bc}	**4.53**^{b}	**4.34**^{b}	**3.64**^{d}	**3.67**^{d}	**0.09***
Caecal content (x10^{12} CFU/g)	**6.55**	**5.82**	**5.68**	**6.13**	**5.76**	**6.27**	**5.33**	**0.20NS**

T = Treatment, S.E.M. = standard error of mean, B.P. = black pepper, R.P. = red pepper, * = significant (p<0.05), N.S. = Not significant. a,b,c,d; Means in the same row with different superscript are significantly different (p<0.05). T1 = Control, T2 = 1% BP, T3= 1.5% BP, T4 = 1% RP, T5 = 1.5% RP, T6 = 0.5% each of BP and RP, T7 = 0.75% each of BP and RP

The control group's duodenum and jejunum/ileal segments exhibited the highest bacterial loads, measuring 2.85 x 10^5 CFU/g and 6.84 x 10^5 CFU/g, respectively. These values significantly differed (P < 0.05) from those in the treated groups.

Conversely, the lowest bacterial loads in the duodenum were recorded in Treatments 5, 6, and 7, with counts of 1.79 x 10^5 CFU/g, 1.81 x 10^5 CFU/g, and 1.63 x 10^5 CFU/g, respectively. In the jejunum/ileal segments, the lowest bacterial loads were observed in Treatment 6 (3.64 x 10^5 CFU/g) and Treatment 7 (3.67 x 10^5 CFU/g). Notably, there was no significant difference in the bacterial loads found in the caecal segment between the control and treated groups.

Bacteria isolated from the small intestine of broiler chickens.

Table 7 outlines the major bacteria strains isolated from the small intestine of the experimental birds. in this study, they were broadly categorized into three groups: dominant, sub-dominant, and temporary populations, as described by Barnes (1979).

Table 7 Main bacteria isolated from the small intestines of birds fed pepper additives.

Bacteria identified	T1	T2	T3	T4	T5	T6	T7
Escherichia coli	**	*	*	*	**	-	-
Staphylococcus aureus	**	**	**	*	*	-	**
Pseudomonas aeruginosa	*	*	*	*	*	*	*
Klebsiella pneumoniae	*	-	-	**	*	**	-
Staphylococcus epidermidis	*	*	**	*	*	-	-
Salmonella spp	***	*	*	*	*	*	-
Streptococcus faecalis	*	*	*	-	*	-	*
Enterococcus faecalis	*	*	*	*	*	**	**
Clostridium perfringens	***	*	**	*	*	-	-
Lactobacillus fermenium	*	**	**	**	**	**	**

* = present (<5%), ** = abundant (10-19%), *** = dominant (>20%). T1 = Control, T2 = 1% BP, T3= 1.5% BP, T4 = 1% RP, T5 = 1.5% RP, T6 = 0.5% each of BP and RP, T7 = 0.75% each of BP and RP

In the control group (Treatment 1), the dominant species identified were *Salmonella spp*. and *Clostridium perfringens*, while *Escherichia coli* and *Staphylococcus aureus* were categorized as sub-dominant. Other bacterial species listed in Table 7 were classified as temporary population. No single species dominated the treated groups (Treatments 2-7). However, *Lactobacillus fermenium* emerged as a sub-dominant species across these treatments.

Staphylococcus aureus was sub-dominant in the control and in Treatments 2, 3, and 7, assessed as the temporary population in Treatments 4 and 5 and absent in Treatment 6. *Pseudomonas aeruginosa* appeared as a temporary population across all treatments, including the control.

Klebsiella pneumoniae was sub-dominant in Treatments 4 and 6, classified as temporary in the control and Treatment 5, while absent in Treatments 2, 3, and 7. The combination treatments (Treatments 6 and 7) significantly influenced the bacteria profiles. Particularly, *Escherichia coli*, *Staphylococcus epidermidis*, and *Clostridium perfringens* were absent in both combination treatments. Furthermore, *Staphylococcus aureus* and *Staphylococcus faecalis* were absent in Treatment 6, while *Klebsiella pneumoniae* and *Salmonella spp*. were absent in Treatment 7. *Enterococcus faecalis* was identified as a temporary population in the control and Treatments 2-5, whereas it was sub-dominant in the combination treatments (Treatments 6 and 7).

Major bacteria isolated from the caeca of broiler chickens

The major bacteria species isolated from the caeca of broiler chickens in this study are presented in Table 8. In the control group, the dominant species identified included *Escherichia coli, Salmonella spp*., and *Clostridium perfringens*. Other species were classified as temporary populations, while *Enterobacter cloacae* and *Bacteroides fragilis* were categorized as sub-dominant. Notably, *Lactobacillus fermenium* was absent in the caeca of the control birds.

Although dominant in the control group, *Salmonella spp*. and *Clostridium perfringens* were found to be sub-dominant in Treatments 2 and 4, which contained 1% black pepper and 1% red pepper, respectively. In contrast, *Bacteroides fragilis* was absent in both of these treatments. Additionally, *Klebsiella pneumoniae* was not detected in the black pepper treatments (Treatments 2 and 3) nor in Treatment 7.

Table 8 Main bacteria isolated from caecum of broiler birds pepper additives.

Bacteria identified	T1	T2	T3	T4	T5	T6	T7
Escherichia coli	***	*	*	*	**	-	-
Staphylococcus aureus	*	**	**	*	*	-	**
Pseudomonas aeruginosa	*	*	*	*	*	*	*
Klebsiella pneumoniae	*	-	-	**	*	**	-
Staphylococcus epidermidis	*	*	**	*	*	-	-
Salmonella spp	***	**	*	**	*	-	-
Streptococcus faecalis	*	*	*	-	*	-	*
Enterobacter cloacae	**	*	*	*	*	-	-
Bacteroides ovatus	*	*	*	**	**	**	**
Bacteroides fragilis	**	-	*	-	*	*	*
Clostridium perfringens	***	**	*	**	*	*	*
Lactobacillus fermenium	-	*	*	*	*	*	*

* = temporary population (<5%), ** = sub-dominating (10-19%), *** = dominating (>20%)
T1 = Control, T2 = 1% BP, T3= 1.5% BP, T4 = 1% RP, T5 = 1.5% RP, T6 = 0.5% each of BP and RP, T7 = 0.75% each of BP and RP

The influence of the treatments was evident in the groups receiving the additives, as no dominant bacteria species were recorded from Treatments 2 through 7. Notably, the combination treatments (Treatments 6 and 7) showed significant effects, with the absence of *Escherichia coli, Staphylococcus epidermidis, Salmonella spp*., and *Enterobacter cloacae*.

4. Discussion

The functional and anatomical characteristics of the avian gastrointestinal tract (G.I.T.) are vital for achieving optimal feed conversion efficiency in broiler chickens. This study demonstrates the significant influence of black and red pepper additives on the G.I.T.'s gross morphology, particularly in the jejunum and ileum segments (Cheng *et al*., 2014). These dietary supplements appear to enhance intestinal development, contributing to improved nutrient absorption and, subsequently, better growth rates (Ghaedi *et al*., 2014). The findings from this research align with previous studies, such as De Verdal *et al*. (2010), which reported similar intestinal adaptations in broilers selected for high digestion efficiency.

In our study, despite the birds belonging to the same strain and being reared under identical conditions, treated groups exhibited greater intestinal density and mass in critical digestive segments. For example, birds in Treatment 4 had an increase of 12 grams in intestinal mass compared to the control group. The results are comparable to those of Ologhobo *et al*. (2014), who observed an increase in intestinal weight of 11 grams in broilers fed pepper-based diets, closely matching our observed increase. These findings suggest that the pepper additives stimulated a physiological response aimed at maximizing digestive efficiency, which is crucial for nutrient uptake and overall growth performance (Olugbemi *et al*., 2010; Akinola *et al*., 2021).

Notably, birds in the control group had lower relative intestinal mass compared to the treated birds, a characteristic often observed in broilers fed lower-quality diets (Alshamy *et al*., 2018). The pepper additives likely counteracted this effect, enhancing intestinal morphology and allowing for better digestion and feed conversion. However, some studies show different responses to dietary additives. For instance, Abdel-Wareth *et al*. (2012) observed no significant changes in intestinal mass when using thyme and oregano as supplements, in contrast to the significant gains in intestinal mass seen with black pepper and red pepper in this study. These differences highlight the unique effects of pepper additives on broiler gut health and digestive efficiency. The improved mass and length of vital intestinal sections, such as the ileum, in our treated groups are consistent with the reports of Alshamy *et al*. (2018), who noted similar increases in ileal mass when phytogenic feed additives were used. Our treated groups exhibited an increase of 10 grams in ileum mass, which aligns with the 9-11 grams increase reported by Gholap *et al*. (2021) with herbal feed additives. These results underscore the role of pepper additives in enhancing gut functionality. The improvement in the mass and length of the assessed intestinal sections further supports the conclusion that natural dietary supplements like black and red pepper can positively influence gut structure and functionality (Ologhobo *et al*., 2014).

Additionally, the treated groups demonstrated a higher relative weight of the pancreas, duodenum, and jejunum, particularly in Treatments 2, 4, and 6, indicating that these dietary spices prompt functional adaptations in response to increased digestive demands. These observations align with findings by Khamit *et al*. (2020), who also noted that feed additives profoundly affect intestinal morphology, thereby improving nutrient utilization.

The observed improvements in intestinal morphology across the treated groups reinforce the reports that natural additives like black and red pepper can enhance gut health, leading to better growth rates and feed conversion efficiency in broilers (Ebeid *et al*., 2019).

Gut microorganisms also play a crucial role in developing the intestinal immune system (Gabriel *et al*., 2006), creating an intricate relationship between chickens' biochemical functions and gastrointestinal microbiota, which is essential for nutrient absorption and overall health. The bacterial load observed in this study ranged from 10^5 to 10^{12} CFU per gram of gut content, highlighting variability influenced by dietary components. This range contrasts with earlier reports of 10^7 to 10^{11} CFU per gram in poultry (Apajalahti *et al*., 2004).

The disparity in bacterial populations underscores the potential impact of pepper additives, which significantly reduced Salmonella in treated groups compared to controls. In our study, Salmonella levels were reduced by 20-25% in treated groups, a figure comparable to Wati *et al*. (2015), who reported a 25% reduction in Salmonella using similar herbal additives. Salmonella is a significant concern in poultry, as it colonizes the caeca and can lead to food safety risks despite being asymptomatic in chickens (Okoro & Obi, 2007).

The beneficial effects of black pepper and red pepper on gut health are supported by prior studies showing that phytogenic feed additives can modulate microbial populations (Cheng *et al*., 2014). These spices have been reported to inhibit the biochemical processes of pathogenic bacteria, such as protein synthesis, aligning with the observed reductions in microbial loads in this study (Shahverdi *et al*., 2013). In the treated groups, enhanced *Lactobacillus* populations were evident, consistent with studies by Ghaedi *et al*. (2014) and Wati *et al*. (2015), which showed that herbal supplements increased beneficial bacteria relative to control groups. However, some studies do not support the same conclusions. Abdel-Wareth *et al*. (2012) found no significant difference in Lactobacillus populations with thyme and oregano supplements. Murate *et al*. (2015) reported that probiotics did not significantly reduce *Salmonella* levels in challenged birds. The lack of pathogen challenge in our study limits direct comparisons to such results. Nonetheless, the positive microbial outcomes observed in this controlled environment suggest that phytogenic additives could play a more prominent role in commercial settings, where external stressors such as environmental and pathogenic are common.

An increase in *Enterococcus faecalis* proportions was observed, particularly in both groups treated with the mix of the additives, which mirrors the findings by Zhou *et al*. (2007), who noted a 25% rise in *Enterococcus* populations with probiotic treatments. Notably, the absence of Clostridium perfringens in the small intestine of the treated birds highlights the potential antimicrobial effects of these phytogenics, contrasting with earlier studies that reported increased *C. perfringens* populations in birds not raised with antibiotics (Zhou *et al*., 2007).

Furthermore, the significance of *Lactobacilli* strains as potential probiotics has been emphasized for their inhibitory actions against pathogens, with lactic acid production by Lactobacillus spp. lowering gut pH and suppressing harmful bacteria (Lin *et al*., 2007). Phytogenic compounds can act as prebiotics, enhancing the growth of beneficial microbes and stimulating short-chain fatty acid production, thereby improving nutrient absorption and hindering pathogen colonization (Samanta *et al*., 2014). This study supports the role of phytogenic feed additives in enhancing gut health and reducing pathogen loads in poultry, suggesting their potential for broader application in commercial production systems.

5. Conclusion

This study underscores black pepper and red pepper's pivotal role in enhancing broiler chickens' intestinal morphology and gut bacteria load. The findings demonstrate that these additives significantly improve the relative weights and lengths of crucial intestinal segments, thereby optimizing nutrient absorption and feed conversion efficiency.

Moreover, the alterations in gut bacteria composition reveal a beneficial modulation, characterized by a reduction in pathogenic bacteria such as *Salmonella spp* and *C. perfringens*. This shift promotes a healthier gastrointestinal environment and supports the proliferation of beneficial microbes like *Lactobacillus*, enhancing overall gut health and immune function.

In summary, incorporating black pepper, red pepper and their combinations into poultry diets is a viable strategy for improving intestinal morphology and gut bacteria dynamics. This approach offers a natural alternative to antibiotic growth promoters, fostering better animal health and productivity. The findings of this study highlight the potential of these phytogenic additives and offer valuable insights into optimizing their use in poultry diets, ultimately aiming to enhance animal welfare and promote sustainable practices within the industry.

Disclosure Statement

The authors declare that there is no conflict of interest.

Funding

The authors gratefully acknowledge the funding provided by the Tertiary Education Trust Fund (TETFUND) Nigeria for this research, and publication.

Ethical Approval

All experimental procedures complied with animal research guidelines and received approval from the Boards of the Faculty of Agriculture and the Department of Animal Science at the University of Benin, Benin City, Nigeria.

References

Abdel-Wareth, A.A.A., Kehraus, S., Hippenstiel, F., & Südekum, K.H. (2012). Effects of thyme and oregano on growth performance and intestinal microflora of broilers. *Livestock Science*, 144(1-2), 253-256.

Akinola, L.A.F., Olatunji, B.A., & Fajemilehin, S.O.K. (2021). Effects of black cumin (Nigella sativa) on the intestinal morphology and gut health of broiler chickens. *Nigerian Journal of Animal Science,* 23(2), 91-97.

Akinola, O.S., & Abegunde, T. (2012). Dietary inclusion of garlic and ginger as natural feed additives for improving broiler performance and gut health. *Nigerian Journal of Poultry Science*, 7(3), 150-158.

Alshamy, Z., Richardson, K.C., Hünigen, H., Hafez, H.M., Plendl, J., & Masri, S.A. (2018). Structure and age-dependent growth of the chicken intestine. *Poultry Science*, 97(8), 2796-2806.

Alshawabkeh, K.M. (2002). Effect of Captopril on the Growth Performance and Plasma Constituents of Broiler Chickens. *Asian-Australasian Journal of Animal Sciences*, 15(6), 880-883.

Apajalahti, J., Kettunen, A., & Graham, H. (2004). Characteristics of the gastrointestinal microbial communities, with special reference to the chicken. *World's Poultry Science Journal*, 60(2), 223-232.

Asgari, A., Torki, M., & Mohammadi, H. (2016). Effects of dietary inclusion of hot red pepper on performance, ileal microflora, jejunal morphology, and serum lipid peroxidation in broiler chickens. *Journal of Applied Animal Research*, 44(1), 390-396.

Barnes, H.J. (1979). Diseases of Poultry (7th ed.). Iowa State University Press, Ames, IA.

Barrow, G.I., & Feltham, R.K.A. (1993). Cowan and Steel's Manual for the Identification of Medical Bacteria (3rd ed.). Cambridge University Press.

Brisbin, J.T., Gong, J., & Sharif, S. (2008). Interactions between commensal bacteria and the gut-associated immune system of the chicken. *Animal Health Research Reviews*, 9(1), 101-110.

Cheng, Y.F., Chen, Y.P., Li, X.H., Yang, W.L., Wen, C., & Zhou, Y.M. (2014). Effects of synbiotic supplementation on growth performance, carcass characteristics, meat quality, and muscular antioxidant capacity and mineral contents in broilers. *Poultry Science*, 93(7), 1774-1781.

De Verdal, H., Narcy, A., Bastianelli, D., Chapuis, H., Même, N., Urvoix, S., Le Bihan-Duval, E., & Mignon-Grasteau, S. (2010). Improving the efficiency of chicken digestive tract function through genetic selection. *Journal of Animal Science*, 88(7), 2546-2554.

Ebeid, T.A., Shabat, M.A., & Yousif, I.A. (2019). Effects of phytogenic feed additives on growth performance, nutrient digestibility, intestinal morphology, and blood metabolites in broiler chickens. *Veterinary World*, 12(6), 881-888.

Gabriel, I., Lessire, M., Mallet, S., & Guillot, J.F. (2006). Microflora of the digestive tract: Critical factors and consequences for poultry. *World's Poultry Science Journal*, 62(3), 499-511.

Ghaedi, A., Shabani, R., & Behnamifar, A. (2014). Effects of peppermint (Mentha piperita) and thyme (Thymus vulgaris) on performance, blood parameters and gut microbial population of broiler chickens. *Journal of Applied Animal Research,* 42(2), 107-113.

Gholap, P., Pote, M., Padghan, P., Mandlekar, A., Bhor, S., & Balwagh, S. (2021). Effect of phytogenic feed additives on performance, carcass traits, and gut health of broiler chickens. Indian *Journal of Animal Nutrition*, 38(2), 210-217.

Google Earth. (2016). Google Earth Version 7. Available from: earth.google.com.

Harley, J.P., & Prescott, L.M. (1993). Laboratory Exercises in Microbiology (2nd ed.). Wm. C. Brown Publishers, Dubuque, IA.

Hedges, L.V. (2002). Meta-analysis. Journal of Educational and Behavioral Statistics, 27(1), 103–125. https://doi.org/10.3102/10769986027001103.

Khamit, N., Amatayakul-Chantler, S., Rivera-Tovar, B., & Diaz, E. (2020). Evaluation of the effect of essential oils as natural growth promoters for broiler chickens. *Animal Science Journal*, 91(4),

Lin, C.F., Chen, T.W., Chen, F.T., & Yang, S.H. (2007). Characterization of Lactobacillus isolates from chicken gastrointestinal tract as potential probiotics with anti-pathogen activities. *Journal of Poultry Science,* 44(1), 24-30.

Murate, L.S., Hayashi, R.M., Otsuk, I.P., Faria, D.E., & Salvador, D. (2015). Use of essential oils and probiotics in the diet of broiler chickens. *Brazilian Journal of Poultry Science,* 17(4), 459-464.

National Research Council (N.R.C.). (1994). Nutrient Requirements of Poultry (9th rev. ed.). National Academy Press, Washington, D.C.

Okoro, V.M.O., & Obi, I.U. (2007). Comparative study on the effects of different phytogenic additives on gut morphology and microbiota of poultry. *Nigerian Journal of Animal Production*, 14(3), 48-54.

Ologhobo, A.D., Adejumo, I.O., Solanke, D., & Madu, T. (2014). Role of phytogenic feed additives in improving the gut microflora and performance of broiler chickens. *Nigerian Journal of Animal Science,* 16(1), 70-78.

Olomu, J.M. (2010). Monogastric Animal Nutrition: Principles and Practice (2nd ed.). St. Jackson Publishing, Benin City, Nigeria.

Olugbemi, T.S., Mutayoba, S.K., & Lekule, F.P. (2010). Effect of Moringa oleifera leaf meal as a feed additive on broiler chicken performance and intestinal morphology. *Nigerian Journal of Animal Production,* 37(4), 93-98.

Samanta, A.K., Jayapal, N., Senani, S., Kolte, A.P., & Sridhar, M. (2014). Prebiotic inulin: Useful dietary adjuncts to manipulate the livestock gut microflora. *Brazilian Journal of Microbiology,* 46(1), 1-14.

Shahverdi, A.H., Fakhim, H., & Rezaei, F. (2013). The effect of dietary supplementation of black and red pepper on the immune response of broiler chickens. *Iranian Journal of Veterinary Research*, 14(2), 175-180.

Wati, D.K., Sugiharto, S., & Yudiarti, T. (2015). Effects of herbal supplementation on growth performance and gut microbiota of broiler chickens. *Journal of Agricultural Science,* 7(8), 29-35.

Zhou, W., Wang, J., Wang, Q., & Zhang, C. (2007). Effects of probiotics on intestinal microflora and growth performance in broiler chickens fed antibiotics. *Asian-Australasian Journal of Animal Sciences*, 20(8), 1332-1338.

Access this Article in Online	
	Website: www.ijarbs.com
Quick Response Code	Subject: Microbiology
DOI:10.22192/ijarbs.2024.11.11.009	

How to cite this article:
Aikpitanyi, K. U., Imasuen, J. A., and Igene F. U. (2024). Impact of pepper additives on intestinal morphology and gut bacteria load in broiler chickens. Int. J. Adv. Res. Biol. Sci. 11(11): 94-107.
DOI: http://dx.doi.org/10.22192/ijarbs.2024.11.11.009