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Dietary clove (*Syzygium aromaticum)* powder enhanced broiler chicken performance, gut histomorphometry and health-associated whilst decreasing pathogenic microbiota

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ABSTRACT

This study evaluated the impact of dietary clove powder on growth performance, carcass traits, nutrient digestibility, jejunal histomorphometry and ileal microbiota in broiler chickens. A total of 250 *Chikun* day-old broiler chicks were allocated to 5 dietary groups with 5 replicates of 10 birds each for 6 weeks in a completely randomized design. The groups were fed diets containing clove powder at 0, 0.5, 1.0, 1.5 and 2 g/kg diet. Results showed that dietary clove supplementation improved final body weight and feed conversion ratio (P < 0.001) while reducing total feed intake. Also, it increased weights of live chickens at slaughter, breast meat, shank and drumstick/thigh ratio (P < 0.001). Further, it linearly increased apparent digestibility of DM, CP, and EE (P < 0.01) as it quadratically decreased CF (P < 0.01). Furthermore, it linearly increased jejunal villus length, crypt depth and thickness of muscularis (P < 0.001) whilst it quadratically decreased thickness of the epithelium (P < 0.01). Moreover, it increased ileal *Lactobacilli* whilst decreasing *Salmonella* and *E. coli* (P < 0.05) bacterial species. In conclusion, dietary clove powder improved growth performance, carcass characteristics, nutrient digestibility, gut histomorphometry and *Lactobacilli* while it decreased *Salmonella* and *E. coli* bacteria.

Keywords: Carcass characteristics, Clove, Gut microbiota, Histomorphometry, Nutrient digestibility.

INTRODUCTION

In Nigeria, South Africa, and other Sub-Saharan African (SSA) countries, broiler production provides a viable means of ensuring food security and enhancing the economic prospects of the burgeoning human population. To limit pathogenic disease incidences and mortality, as well as improve appetite and growth performance in broilers, antibiotics have long been utilized in their production. However, concerns about the development of antibiotic-resistant microbes, the occurrence of antibiotic residues in animal products, as well as environmental pollution, have culminated in the banning of in-feed antibiotics in many countries including the USA, EU, China, and other regions of the world (Abdel-Moneim *et al*., 2020). Consequently, this has necessitated research into sustainable and environmentally friendly alternatives to conventional antibiotics.

Natural feed additives have been increasingly investigated as alternatives to conventional antibiotics. In particular, phytogenics (phytobiotics) including herbs are preferred for their inherent antioxidant, anti-inflammatory, and antimicrobial functionalities and for inducing no antibiotic-resistance or residues in animal products and the environment (Suliman *et al*., 2021). They are also relatively cheap and readily available which makes them beneficial in lowering the cost of production particularly among resource-limited small-scale farmers (Nahed *et al*., 2022).

Clove (*Syzygium aromaticum*; family *Myrtaceae*) flower buds contain high amounts of volatile essential oils, with eugenol being the dominant component, in addition to other phytochemicals that account for their numerous health benefits (Arif *et al*., 2022). Dietary clove supplementation improves nutrient absorption and utilization, growth performance, meat yield, immunity, beneficial intestinal bacteria (Elbaz *et al*., 2022) as well as protein digestibility, nutrient availability and utilization in broilers (Kunnumakkara *et al*., 2018). However, negative effects on growth performance with increasing inclusion levels of clove seed (Suliman *et al*., 2021) or clove buds (Othman *et al*., 2022) have also been reported. Given these inconsistencies in previous studies, it has been necessary to further elucidate the effects of dietary clove supplementation in broiler nutrition. Additionally, there is paucity of information on the impact of cloves on gut populations of health-associated and pathogenic bacteria. Thus, this study aimed to address these gaps by comprehensively examining the effects of dietary clove powder supplementation on growth performance, carcass characteristics, nutrient digestibility, gut histomorphometry as well as health-associated and pathogenic gut microbiota in broiler chickens. Our hypothesis posits that feeding broilers with clove-containing diets from day-old to the finisher phase will yield improvements in these parameters, ultimately enhancing gut health and overall performance.

MATERIALS AND METHODS

Study Location

The study was carried out at the Poultry Section of the University of Nigeria, Nsukka Research Farm, Enugu State, Nigeria. The study site typically experiences rainy (May – October) and dry (December – March) seasons with average temperatures of 25.6– 34.0°C and annual average rainfall between 1680 and 1700mm.

Ethical Statement

The study was conducted in compliance with ethical guidelines to ensure adherence to both national and international standards for research involving animals. Approval for the use of animals in the research was granted by the Ethical Clearance Committee of the University of Nigeria, Nsukka, Nigeria.

Sourcing and Preparation of Materials

Dried clove flower buds were collected from Nsukka in the Nsukka Local Government Area of Enugu State (Nigeria), ground into powder, packaged and stored whilst *Chikun* broiler chicks were sourced from Agritted Hatchery (Ibadan, Nigeria).

Birds, Diets, Design and Management

Two hundred and fifty male *Chikun* strain day-old chicks (44.15 g ± 0.7) were randomly assigned to 5 experimental maize-soybean-based diets respectively supplemented with 0, 0.5, 1.0, 1.5, and 2.0 g clove bud powder per kg feed (designated CLO0, CLO5, CLO10, CLO15, and

CLO20), each with 5 replicate pens (2.6m width x 3m length) of 10 birds in a completely randomized design, for 6 weeks. Both the diets and clean water were offered *ad libitum*. The diets were formulated to meet nutritional requirements of broilers as recommended by the NRC (1994). The birds were raised through the starter (d1 – 21) and finisher (d22 – 42) phases on concrete floors with wood shavings as litter material. For brooding, the housing temperature was maintained at 32°C for the first 5 daysafter which it was gradually lowered to 25°C for the rest of the study. Vaccinations were carried out according to the recommendations of Afrimash (2019). The chemical composition of the experimental diets (Tables 1 and 2) was analyzed using standard methods (AOAC, 2005).

Growth Performance

Live body weights were taken weekly and used to calculate body weight gains (BWG). Feed offered and refusals were also recorded for calculation of feed intake (FI) and feed conversion ratio (FCR) using the formula:

$$Feed\ Conversion\ Ratio = \frac{Average\ Feed\ Intake}{Average\ Weight\ Gain}$$

Carcass Measurements

At the end of the study, 2 birds were randomly selected from each replicate, weighed, and slaughtered using the cervical dislocation technique. Feathers were then plucked off and heads, shanks, and viscera removed. The carcasses and different cuts were weighed and expressed as absolute values.

Nutrient Digestibility Trial

Two birds randomly selected from each pen were placed in cleaned metabolism cages a week before the end of the feeding trial. They were adapted for 3 days prior to 4-day sample (droppings) collection. Samples were dried at room temperature, ground, and analyzed for dry matter (DM; method 930.15), crude protein (CP; method 954.01), ether extract (EE; method 920.39), and crude fibre (CF) (AOAC 2005). The digestibility of each of the measured nutrients was calculated using the formula:

$$Nutrient\ digestibility\ (\%) = \frac{Nutrient\ in\ feed\ - Nutrient\ in\ faeces}{Nutrient\ in\ feed} \times 100$$

Gut Histology

At the end of the feeding trial, approximately 2cm of the jejunum was collected from each of 2 birds randomly selected from each pen, rinsed with 0.9% saline, fixed in 10% buffered formalin, and stored at room temperature until analysis. The samples were then washed, trimmed, dehydrated with alcohol, cleared with xylene, and impregnated with paraffin wax. The jejunum was then cut into 2 µm sections, fixed onto slides, stained with haematoxylin and eosin, and examined under a Zeiss Axio light microscope equipped with a digital camera using a 2.5x magnification objective lens. Images were analysed for morphometric characteristics using Axio vision image-analysis software version 4.7.2 (Carl Zeiss microscopy).

Gut Microbiota

At the end of the feeding trial, ileal contents aseptically collected from 2 birds randomly selected from each pen were placed into sterile glass bags and placed on ice. They were then transferred to fresh, sterile bags, immediately diluted with sterile ice-cold anoxic phosphate buffered saline (0.1 M; pH 7.0) in a 10% wt/vol ratio, and homogenized in a stomacher (Bagmixer 100 Minimix, Interscience, Arpents, France) for 3 min. Subsequently, the ileum digesta was subjected to serial dilution from 10^{-1} to 10^{-7}, and the dilutions plated on duplicate selective agar plates. As described by Tuohy *et al.* (2002), specific strains of *Lactobacilli*, *E. coli*, and *Salmonella* were enumerated using VRB agar (MERCK, 1.01406), Rogosa agar (MERCK, 1.10660), and Beerens agar, respectively. The plates were then incubated anaerobically for 48 to 120 hrs at 39 °C (Beerens, Rogosa agars) or for 24– 48hrs at 37 °C (VRB agar). Bacterial colonies were recorded, average live bacteria calculated based on the weight of original ileal contents, and data was converted into logarithmic colony-forming units (cfu/g) as described by (Koç *et al.*, 2010).

Table 1. Ingredient and Chemical Composition (%, Unless Stated Otherwise) of the Experimental Diets for Broiler Chickens at The Starter Phase (d1 – 21)

Ingredients	CLO0	CLO5	CLO10	CLO15	CLO20
Yellow maize	53.98	53.98	53.98	53.98	53.98
Groundnut cake	9.50	9.50	9.50	9.50	9.50
Wheat offal	4.75	4.75	4.75	4.75	4.75
Soybean meal	23.48	23.48	23.48	23.48	23.48
Fish meal	3.15	3.15	3.15	3.15	3.15
Limestone	2.50	2.50	2.50	2.50	2.50
Bone meal	1.64	1.64	1.64	1.64	1.64
Salt	0.27	0.27	0.27	0.27	0.27
Vitamin-mineral premix[1]	0.26	0.26	0.26	0.26	0.26
Lysine	0.23	0.23	0.23	0.23	0.23
Methionine	0.24	0.24	0.24	0.24	0.24
Total	100.00	100.00	100.00	100.00	100.00
Clove	0.00	0.05	0.10	0.15	0.20
Calculated Composition					
Metabolizable energy (Mcal/kg)	2950	2950	2950	2950	2950
Crude protein	23.00	23.00	23.00	23.00	23.00
Crude fibre	5.00	5.00	5.00	5.00	5.00
Calcium	1.54	1.54	1.54	1.54	1.54
Phosphorus	0.71	0.71	0.71	0.71	0.71
Chemical composition (%)					
Crude matter	88.84	88.81	89.56	89.84	88.96
Crude protein	22.56	22.29	22.37	22.36	22.45
Crude fat	3.00	3.02	3.02	3.02	3.02
Crude fibre	5.03	5.11	5.11	5.01	5.01
Ash	4.00	3.98	3.97	4.03	4.03
Nitrogen free extract	66.41	66.69	66.69	66.89	66.89

Statistical Analysis

Data were subjected to a one-way analysis of variance using the Agricolae Statistical package (De Mendiburu, 2019) in (RStudio 2020). Mean differences were tested at 5% a probability level ($P< 0.05$) while significantly different means were separated using Tukey's test. Then, the response surface regression analysis procedure (SAS 2002) was used to evaluate the data for linear and quadratic effects using the following non-linear model:

$$y = ax^2 + bx + c$$

Where y is the dependent variable; a and b = coefficients of the model; c = intercept; x is dietary clove dosage; and $-b/2a$ is the x value for optimal response.

RESULTS AND DISCUSSION

Growth Performance

The effect of dietary clove powder inclusion on the growth performance of broiler chickens is presented in Table 3. The initial body weight of the birds upon grouping did not show significant differences across the groups ($P > 0.05$). However, adding clove powder yielded diverse responses in critica

growth parameters, including FBW, BWG, TFI, and FCR. The inclusion of dietary clove powder induced quadratic decreases in both FCR [$y = 1.40\ (\pm0.23)x - 0.23\ (\pm0.04)x^2 + 0.66\ (\pm0.30)$; $R^2 = 0.754$, $P < 0.001$] and TFI [$y = 3184\ (\pm446)x - 543\ (\pm72.9)x^2 + 3651\ (\pm585)$; $R^2 = 0.824$, $P < 0.001$]. These quadratic responses allowed us to identify the optimum clove powder inclusion level, determined to be 3.04 g/kg. At lower levels of clove powder (CLO10 and CLO15), there was a significant increase ($P < 0.001$) in the TFI and FCR, but a decrease ($P < 0.001$) in FBW and BWG. Notably, CLO15 resulted in the lowest FBW and BWG ($P < 0.001$), whilst CLO0, CLO5 and CLO20 promoted the highest FBW and BWG ($P < 0.001$). Importantly, there were no significant differences in FBW, BWG, TFI and FCR between the CLO20 and control groups.

While our study did not reveal effects of dietary clove supplementation on FBW, BWG, and FCR in broiler chickens, a closer examination through regression analysis unveiled a potential for optimizing BWG and feed consumption when clove powder was provided at 3.04 g/kg of diet. The observed dose-dependent effect of clove supplementation was highlighted by a reduced quadratic response in both TFI and FCR, notably at the higher inclusion level of 2.0 g./kg of diet. The decreased TFI at this higher level may be attributed to reduced palatability, potentially influenced by the higher concentration of eugenol, which has been reported to decrease gut motility (Daniel *et al*., 2009). Furthermore, the lower FCR value in the group that received 2.0 g/kg of clove powder suggests potential improvements in nutrient absorption and utilization with higher clove inclusion levels, although these values remained comparable to those of the control group. This aligns with previous reports of increased BWG and feed consumption in broilers fed diets supplemented with clove powder (Islam *et al*., 2023). The enhancement in growth performance may be attributed to clove's ability to stimulate appetite and improve feed utilization efficiency (Islam *et al*., 2023; Naeem *et al*., 2022), possibly through eugenol's stimulation of digestive enzyme secretion and gastrointestinal tract motility (Ogwuegbu *et al*., 2021).

Carcass Traits

Table 2. Ingredient and Chemical Composition (%, Unless Stated Otherwise) of the Experimental Diets for Broiler Chickens at The Finisher Phase (d22 – 42)

Ingredients	CLO0	CLO5	CLO10	CLO15	CLO20
Yellow maize	62.75	63.75	63.75	63.75	63.75
Groundnut cake	8.50	9.50	9.50	9.50	9.50
Wheat offal	6.38	6.38	6.88	6.88	6.88
Soybean meal	14.98	14.98	14.98	14.98	14.98
Fish meal	2.00	2.00	2.00	2.00	2.00
Limestone	2.50	2.50	2.50	2.50	2.50
Bone meal	1.89	1.64	1.64	1.64	1.64
Salt	0.27	0.27	0.27	0.27	0.27
Mineral-vitamin premix[1]	0.26	0.26	0.26	0.26	0.26
Lysine	0.23	0.23	0.23	0.23	0.23
Methionine	0.24	0.24	0.24	0.24	0.24
Total	100.00	100.00	100.00	100.00	100.00
Clove	0.00	0.05	0.10	0.15	0.20
Calculated composition					
Metabolizable energy (Mcal/kg)	3105	3105	3105	3105	3105
Crude protein	19.98	19.98	19.98	19.98	19.98
Crude fibre (%)	5.00	5.00	5.00	5.00	5.00
Calcium	1.59	1.59	1.59	1.59	1.59
Phosphorous	0.73	0.73	0.73	0.73	0.73
Chemical composition (%)					
Crude matter	90.01	90.00	90.02	90.03	90.00
Crude protein	18.08	18.25	18.11	18.05	18.20
Crude fat	5.00	5.01	5.00	4.99	4.87
Crude fibre	4.98	5.00	4.81	5.11	5.00
Ash	4.00	3.91	4.01	4.03	4.05
Nitrogen free extract	67.94	68.13	68.07	67.90	68.11

CLO0: Basal diet only (Control), CLO5: Diet with 0.5 g/kg clove powder, CLO10: Diet with 1.0 g/kg clove powder, CLO15: Diet with 1.5 g/kg clove powder, and CLO20: Diet with 2 g/kg clove powder.

Table 3. Effect of Dietary Clove Powder on Growth Performance of Broiler Chickens

Parameters	Treatments					SEM	Significance	
	CLO0	CLO5	CLO10	CLO15	CLO20		*P*- Linear	*P*- Quadratic
Initial body weight (g)	43.98	44.28	44.27	44.61	43.60	0.18	0.743	0.150
Final body weight (g)	3276.67^{a}	3460.67^{a}	3033.00^{b}	2750.00^{c}	3366.00^{a}	74.20	0.298	0.105
Body weight gain (g/d)	3232.69^{a}	3416.39^{a}	2988.73^{b}	2705.39^{c}	3322.40^{a}	74.25	0.298	0.105
Total feed intake (g/d)	6320.92^{b}	7753.51^{a}	8427.08^{a}	7639.76^{a}	5999.16^{b}	269.52	0.398	<.0001
Feed conversion ratio	1.96^{c}	2.27^{b}	2.82^{a}	2.82^{a}	1.81^{c}	0.12	0.577	<.0001

^{a,b,c} Row means with a common superscript do not differ differ ($P \geq 0.05$); SEM= Standard error of the mean; CLO0 (only basal diet; BD), CLO5 (BD + 0.5 g clove kg^{-1} diet), CLO10 (BD + 1 g clove kg^{-1} diet), CLO15 (BD + 1.5 g clove kg^{-1} diet), and CLO20 (BD + 2 g clove kg^{-1} diet).

Table 4. Effect of Dietary Clove Powder on Carcass Characteristics of Broiler Chickens

Parameters	Treatments					SEM	Significance	
	CLO0	CLO5	CLO10	CLO15	CLO20		*P*- Linear	*P*- Quadratic
Live weight (g)	3276.67^{a}	3460.67^{a}	3033.00^{b}	2750.00^{c}	3366.00^{a}	74.20	0.298	0.105
Dressed weight (g)	2550.00	2786.67	2583.33	2270.00	2533.00	67.11	0.281	0.923
Drumstick/thigh (g)	334.67^{b}	388.67^{a}	342.0^{b}	257.33^{c}	323.67^{b}	11.57	0.068	0.885
Shank (g)	48.67^{b}	55.00^{a}	45.00^{b}	55.67^{a}	48.30^{b}	1.26	1.000	0.563
Back cut (g)	423.33^{c}	502.12^{bc}	701.67^{a}	593.67^{ab}	637.00^{a}	31.23	0.001	0.021
Wings (g)	123.33	126.33	135.0	121.33	134.00	2.82	0.452	0.906
Breast-meat (g)	942.00^{a}	1095.37^{a}	685.67^{b}	646.69^{b}	981.69^{a}	50.49	0.277	0.080

^{a,b,c} Row means with a common superscript do not differ differ ($P \geq 0.05$); SEM= Standard error of the mean; CLO0 (only basal diet; BD), CLO5 (BD + 0.5 g clove kg^{-1} diet), CLO10 (BD + 1 g clove kg^{-1} diet), CLO15 (BD + 1.5 g clove kg^{-1} diet), and CLO20 (BD + 2 g clove kg^{-1} diet).

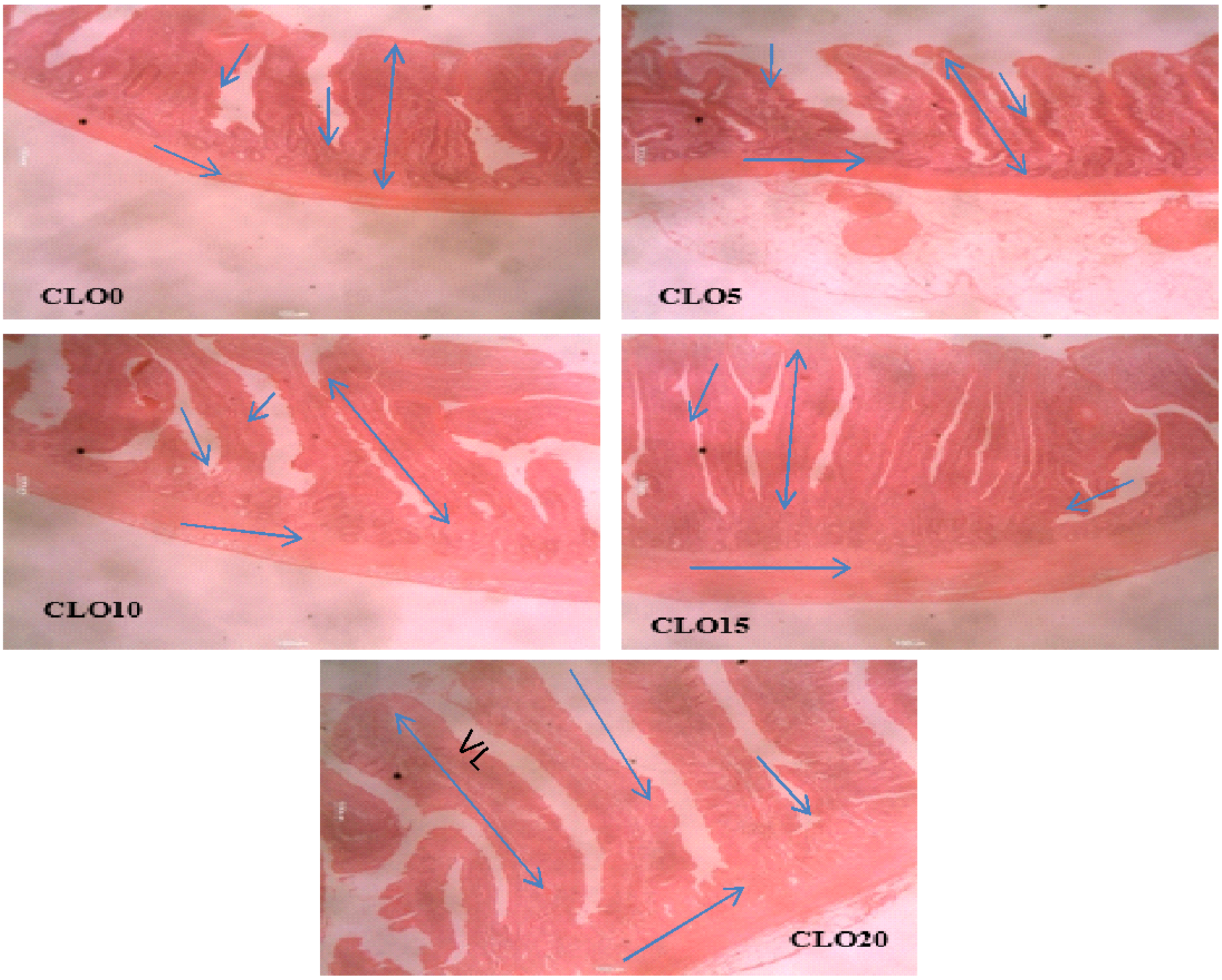

Figure 1. Histomicrograph of the jejunum of birds fed clove powder supplemented diets. CLO0 (Basal diet (BD) only; Control), CLO5 (BD + 0.5 g clove powder kg^{-1} diet), CLO10 (BD + 1 g clove powder kg^{-1} diet), CLO15 (BD + 1.5 g clove powder kg^{-1} diet), and CLO20 (BD + 2 g clove powder kg^{-1} diet). VL= Villus length; CD= Crypt depth;TM= Thickness of epithelium; TM = Thickness of muscularis.

The effect of dietary clove powder inclusion on carcass traits of broilers is shown in Table 4. Dietary inclusion of clove powder resulted in a quadratic decrease in back-cut weight [y = 216 (±63.4)x - 27.5 (10.4)x^2 + 225 (±83.2); R^2 =0.671, P < 0.05], relative dressed weight [y= 11.2(±5.34)x - 1.93(0.87)x^2+ 67.8(±7.01); R^2 = 0.294; P < 0.05], relative drumstick/thigh weight [y = 1.19(±0.64)x - 0.24(0.11)x^2 + 9.52(±0.84); R^2 = 0.492; P < 0.05] and relative back-cut weight [y = 10.0(±2.65)x - 1.35(0.43)x^2 +3.03 (±3.47); R^2 = 0.664; P <0.01], with an optimal level of 3.9 g clove powder per kg diet. Whilst there was no effect of diet (P > 0.05) on dressed weight and wing weight, dietary clove powder increased the weights of live chickens at slaughter (P < 0.001), shank (P < 0.01), breast meat (P < 0.001), and back-cut (P < 0.001), as well as the drumstick/thigh ratio (P < 0.001).

Considering that broilers nowadays are predominantly marketed in the form of meat portions rather than whole birds (Barbut 2015), it is necessary to assess the carcass and meat yield responses to dietary phytogenic additives (Ncube *et al*., 2017). In this regard, the observed increased weights of birds at slaughter, breast meat, shank, as well as drumstick/thigh ratio indicate an improvement in muscle and bone development and demonstrate clove powder as a desirable phytogenic feed additive, consistent

Table 5. Effect of Dietary Clove Powder on Apparent Nutrient Digestibility (%) in Broiler Chickens

Parameters	Treatments					SEM	Significance	
	CLO0	CLO5	CLO10	CLO15	CLO20		*P*- Linear	*P*- Quadratic
Dry matter	70.99^{c}	73.79^{c}	80.57^{b}	83.38^{ab}	86.12^{a}	1.59	<.0001	0.363
Crude protein	63.17^{d}	68.39^{c}	72.56^{b}	71.67^{bc}	80.85^{a}	1.61	<.0001	0.653
Crude fibre	16.49^{d}	20.67^{bc}	20.34^{c}	23.60^{a}	21.60^{b}	0.63	<.0001	0.005
Ether extract	57.78^{c}	60.29^{bc}	62.43^{b}	60.91^{b}	65.43^{a}	0.76	0.0006	0.934

^{a,b,c,d} Row means with a common superscript do not differ ($P \geq 0.05$); SEM= Standard error of the mean; CLO0 (only basal diet; BD), CLO5 (BD + 0.5 g clove kg^{-1} diet), CLO10 (BD + 1 g clove kg^{-1} diet), CLO15 (BD + 1.5 g clove kg^{-1} diet), and CLO20 (BD + 2 g clove kg^{-1} diet).

Table 6. Effect of Dietary Clove Powder on Gut Histomorphometric Traits of Broiler Chickens

Parameters	Treatments					SEM	Significance	
	CLO0	CLO5	CLO10	CLO15	CLO20		*P*- Linear	*P*- Quadratic
Villus length (nm)	1183.64^{b}	1277.76^{ab}	1347.71^{a}	1425.63^{a}	1391.40^{a}	28.94	0.001	0.150
Crypt depth (nm)	144.60^{c}	173.73^{bc}	191.49^{bc}	215.29^{ab}	252.42^{a}	11.28	<.0001	0.675
Thickness of epithelium (nm)	38.32^{c}	42.51^{c}	56.09^{b}	62.14^{a}	59.33^{ab}	2.61	<.0001	0.027
Thickness of muscularis (nm)	127.37^{d}	141.91^{c}	150.53^{bc}	158.40^{ab}	166.30^{a}	3.75	<.0001	0.654

^{a,b,c,} Row means with a common superscript do not differ ($P \geq 0.05$); SEM= Standard error of the mean; CLO0 (only basal diet; BD), CLO5 (BD + 0.5 g clove kg^{-1} diet), CLO10 (BD + 1 g clove kg^{-1} diet), CLO15 (BD + 1.5 g clove kg^{-1} diet), and CLO20 (BD + 2 g clove kg^{-1} diet).

with previous observations (Islam *et al*., 2023). The improvement observed could be translated to more cuts available for sale, signifying high profitability for farmers. Mechanistically, phytogenic additives such as clove increase carcass yield by stimulating nutrient digestion and absorption, as well as protein synthesis, resulting in increased muscular and tissue development (Abd El-Hack *et al*., 2015; Ogwuegbu *et al*. 2021). Contrary to our findings, (Suliman *et al*., 2021) found no effects of dietary clove extract on carcass yield of broiler birds. Disparities among the observations may be due to the levels of administration of the phytogenic feed additives.

Nutrient Digestibility

Table 5 presents results of apparent nutrient digestibility in broiler fed diets supplemented with clove powder. Dietary clove powder linearly increased apparent digestibility of DM[y = 5.74 (±1.89)x + 64.9 (±2.48); R^2 = 0.909; P = 0.001], CP [y = 2.65 (±2.69)x + 61.2 (±3.54); R^2 = 0.821; P = 0.001], and EE [y = 56.8 (±2.36)x +1.44 (±1.80); R^2 = 0.635; P < 0.001], with maximum responses at 2.0 g clove powder inclusion level per kg diet. However, a negative quadratic effect was noted for CF [y = 5.07 (±1.10)x-0.63 (0.18)x^2 + 12.21 (±1.44); R^2 = 0.808, P <0.01], with an optimum at 4.02 g clove powder inclusion level per kg diet.

The improvements observed in the apparent digestibility of DM, CP, EE and CF in broilers fed clove powder-supplemented diets in our study, along with CP and CF digestibility in the meat birds fed the phytogenic feed additive-supplemented diets in previous studies (Mahrous et al., 2017), suggest multiple potential mechanisms. These mechanisms may involve cloves possession of antimicrobial and antioxidant attributes (Al-Mufarrej *et al*., 2019), in addition to their potential to enhance amino acid preservation and enteric absorption (Mansoub, 2011).

Gut Histology

Table 6 presents a jejunal histomorphometry of broilers fed diets supplemented with graded levels of clove powder. Dietary clove powder linearly increased villus length [y = 163 (±70.7)x + 1032 (±92.8); R^2 0617; P = 0.001) and crypt depth [y = 16.3 (±22.4)x + 129 (±29.4); R^2 0.747; P = 0.001], whilst there was a linear decrease in the thickness of the muscularis muscle [y = 117 (±5.05) - 12.2 (±3.85)x; R^2 0.942; P = 0.001]. A negative quadratic effect was observed for the thickness of epithelium [y = 15.4 (±3.72)x -1.54 (±0.609)x^2 + 22.4 (±4.89); R^2 =0.869;P < 0.05], with an optimum at 5.0 g clove powder inclusion level per kg diet. These results were corroborated by the thinning accompanied by elongation of the villi in the jejunum of broilers as the dietary inclusion level of clove powder increased, with maximum villi heights at 2.0 g clove powder inclusion level per kg diet (Figure 1).

The morphometric properties of the intestinal tract serve as indicators of broiler health and are closely linked to their nutrient assimilation capacity and immune functionality (Nicholson *et al*., 2012). The dietary clove powder-induced linear increase in villus length and crypt depth, accompanied by a decrease in the thickness of the epithelium, align with the findings of (Islam *et al*. 2023) who reported increased intestinal length, suggesting that clove powder enhances the development of the jejunum in meat birds. Dietary clove supplementation has previously been shown to stimulate enterocyte proliferation and promote the development of the intestinal epithelium in broiler chickens (Agostini *et al*. 2012). This stimulation also results in improved villus height and surface area, as reported by (Thuekeaw *et al*., 2022) and (Islam *et al*., 2023). Importantly, increased villus length is positively correlated with higher nutrient absorption, as it provides a larger surface area for enhanced nutrient uptake (Mohamed *et al*., 2014). However, in contrast to our data, (Taheri Gandomani *et al*., 2014) and (Al-Mufarrej *et al*., 2019), respectively, observed decreased crypt depths and villi heights whilst (Chowdhury *et al*., 2018; Jahejo *et al*., 2019), found no effects on villus height, villus width, or crypt depth in chickens fed clove-containing diets. The reason for the variation in responses is not known. Nonetheless, the clove powder-induced muscularis thickness may be due to mechanical effects of structural dietary fibre on bird intestinal wall (Jamroz *et al*., 2006) and could be attributed to clove powder enhancement of nutrient absorption, secretion of electrolytes and fluids, as well as renewal and replenishment of impaired cells or those lost to normal attribution (Yu WanWen *et al*., 2015).

Gut Microbiota

The effect of dietary clove powder inclusion on health-associated and pathogenic gut microbiota dynamics of broilers is shown in Figures 2 to 4. The result indicated that dietary clove powder inclusion increased ileal *Lactobacillus* (P< 0.05), with a decrease in the *Salmonella* (P < 0.05) and*E.coli*(P < 0.05) counts. The inclusion of clove induced negative quadratic effect on the ileal *Lactobacilli*[y = 3.52 (±0.56)x - 0.29 (±0.092)x^2 + 2.76 (±0.74); R^2 =0.959, P < 0.01] and *Salmonella* [y = 0.39 (±0.06)x^2 - 3.15 (±0.38)x + 11.2 (±0.50); R^2 0.928; P < 0.001] counts with apparent optimum responses at 6.07 g clove powder whilst it linearly decreased *E. Coli* [y 9.76 (±1.16) - 2.35 (±0.88)x; R^2 0.594; P < 0.01].

Both *Salmonella* and *E. coli* bacteria are serious public health concern whilst *Lactobacilli* are beneficial bacteria. Interestingly, our results demonstrated dietary clove powder to have increased *Lactobacilli* whilst it decreased *Salmonella* and *E. coli* bacteria in the ileum of broilers. These observations concur with previous studies that showed increased *Lactobacilli* in contrast to lowered *E. coli* populations in the gut of broilers (Islam *et al*., 2023). Conversely, no effects were observed on gut bacterial populations of chickens fed 100 – 150mg of cloves per kg diet (Eevuri and Putturu 2013), probably due to low clove inclusion levels. The observed pro-*Lactobacilli* and anti-*Salmonella* and anti-*E. coli* effects of clove powder may be attributed to the phytogenic feed additive's rich composition of eugenol, which possesses strong antibacterial and antifungal activities (El-Saber Batiha *et al*., 2020).

Notwithstanding the above-mentioned beneficial effects of clove powder, our data demonstrated that too high dietary inclusion of the phytogenic induced detrimental effects in broilers. In concurrence with (Mahrous *et al*., 2017), the optimal dietary inclusion level of clove powder was in the range of 1.0 to 1.5 g per kg diet; beyond which all measured parameters responded unfavorably. Other studies found higher dietary levels of cloves to decrease feed intake (Daniel *et al*., 2009), compromise intestinal epithelium integrity leading to inhibition of intestinal nutrient absorption by decreasing villus height, villus surface area and villus crypt (Budd *et al*., 2017; Ducatelle *et al*., 2018), inducing villus wall necrosis accompanied by luminal accumulation of mucus and desquamated intestinal sheets, swollen hepatocytes and dilated hepatic sinusoids (Al -Mufarrej *et al*., 2019). Cloves contain eugenol, a phenolic compound that protects the liver from damage (El-Hadary and Ramadan Hassanien 2016). Indeed, a previous study found the clove plant to be safe when consumed at concentrations of < 1.5 g/kg diet (Cortes-Rojas *et al*., 2014). Hence, excessive consumption of cloves induces detrimental effects on liver hepatocytes (Nwaopara *et al*., 2007). Interestingly, some studies did not observe any effects of cloves on liver histology (Chakma *et al*., 2020; Velisek *et al*., 2005) or liver enzyme activities (Kazi *et al*., 2017). It is contended that the mechanism underlying the detrimental effects of clove powder in broilers relates to the plant's containment of eugenol and tannins, high concentrations of which decrease not only feed palatability and feed intake (Islam *et al*., 2023; Naeem *et al*., 2022) but also BWG (Othman *et al*., 2022) in the meat-producing birds. Whilst beneficial to enterocytes, especially at low concentrations (Brus *et al*., 2018), tannins, particularly at high concentrations, induce perturbations on enteric wall morphology and function (Jamroz *et al*. 2006; Naeem *et al*., 2022).

CONCLUSION

Dietary clove powder improved growth performance, carcass characteristics, nutrient digestibility, gut histomorphometry and *Lactobacilli* while it decreased *Salmonella* and *E. coli* bacterial species in a dose-dependent manner, with optimal responses at 1.0 to 1.5 g per kg diet. Therefore, clove powder can be included as a phytogenic feed additive alternative to conventional antibiotics in broiler diets at these inclusion levels without adverse effects.

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CONFLICT OF INTEREST

Authors declare no conflict of interest for this article.

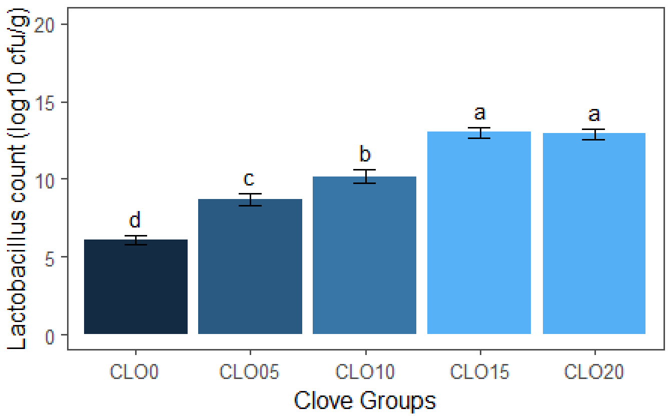

Figure 2. Effect of dietary clove powder on ileal *Lactobacillus spp.* counts (log10 cfu/g) (± SEM) ^{a,b,c,d}. Means with a common superscript do not differ ($P \geq 0.05$). CLO0 (Basal diet (BD) only; Control), CLO5 (BD + 0.5 g clove powder kg^{-1} diet), CLO10 (BD + 1 g clove powder kg-1 diet), CLO15 (BD + 1.5 g clove powder kg^{-1} diet), and CLO20 (BD + 2 g clove powder kg^{-1} diet).

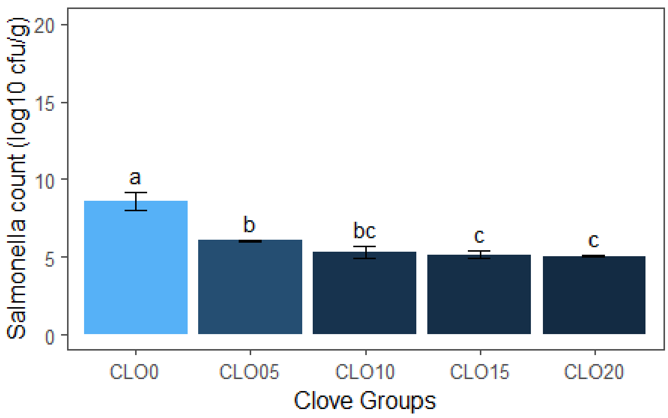

Figure 3. Effect of dietary clove powder on ileal *Salmonella spp.* counts (log10 cfu/g) (± SEM) a,b,c,d. Means with a common superscript do not differ ($P \geq 0.05$); CLO0 (Basal diet (BD) only; Control), CLO5 (BD + 0.5 g clove powder kg^{-1} diet), CLO10 (BD + 1 g clove powder kg^{-1} diet), CLO15 (BD + 1.5 g clove powder kg^{-1} diet), and CLO20 (BD + 2 g clove powder kg^{-1} diet).

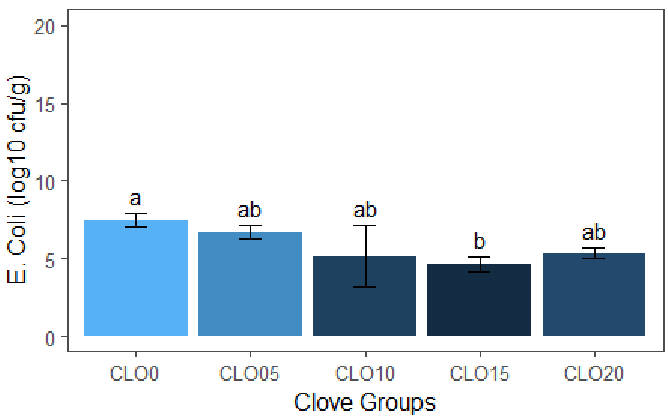

Figure 4. Effect of dietary clove powder on ileal *E. coli* counts (log10 cfu/g)
a,b,c,d. Means with a common superscript do not differ ($P \geq 0.05$). CLO0 (Basal diet (BD) only; Control), CLO5 (BD + 0.5 g clove powder kg^{-1} diet), CLO10 (BD + 1 g clove powder kg^{-1} diet), CLO15 (BD + 1.5 g clove powder kg^{-1} diet), and CLO20 (BD + 2 g clove powder kg^{-1} diet).

PREPRINT

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