EFFECTS OF CLOVE (Syzygium aromaticum) ON GUT MICROBIOTA AND HISTOLOGICAL TRAITS OF BROILER BIRDS

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ABSTRACT
An eight-week study was conducted to evaluate the carcass traits, gut microbiota and histological traits of broilers fed varying levels of clove powder. A total of 120 one-day old "Chikun strain" broilers were randomly allotted to five groups of 24 birds each. The treatments include: **T1=**Basal diet (BD; control), **T2=**BD+0.5gclove/kg diet, **T3=**BD+1.0gclove/kg diet, **T4=**BD+1.5gclove/kg diet and **T5=**BD+2.0g clove/kg diet. Each group was replicated 3 times with 8 birds per replicate in a completely randomized design (CRD). The birds were provided feed and water *ad libitum* for the period of the study. Data were collected on gut microbial count and histology. Results showed that the *Lactobacillus* counts of the caecum were highest ($p<0.05$) for birds fed dietary T4 and T5. The highest ($p<0.05$) *Salmonella* population was seen in birds fed the T1control diet, whereas birds fed T3, T4 and T5 had the lowest ($p<0.05$) *Salmonella* population. Birds fed T1 had the highest ($p<0.05$) *E.coli* counts when compared to T4 which had the lowest ($p<0.05$) counts of *E.coli.* The villus length of birds fed T3, T4 and T5 were statistically similar with those fed T2, while birds fed T1 had the lowest ($p<0.05$) villus length. The crypt depth and thickness of the muscularis were highest ($p<0.05$) for birds fed T5 while T1 had the lowest values. It was concluded that up to 2.0g/kg of clove powder can be safely included in broiler diet for improved gut integrity and histological traits of broiler birds. We therefore recommend inclusion level of 2g/kg diet of clove, having discovered that the inclusion of this additive at single level have more positive effects on the animals.
Keywords: broiler, clove, gut microbiota, histology

INTRODUCTION
Broiler production is one of the quickest ways of producing a high quality and quantity protein for human consumption in order to solve the problem kwashikor which is a resultant effect of malnutrition (Sleman *et al*., 2015). With the ever-increasing cost of poultry and poultry products and the adverse effect of synthetic antibiotics there is need to explore the use of natural alternative of antibiotics that are locally available, safe and cheap. Clove is one of such alternative antibiotics and one of the most valuable spices with some evidence of improved health and growth of animals (Ashan *et al.,* 2018). It contains carnosol and their acid forms, or flavonoids as its natural major active compounds (Wankhede, 2015). This in-feed phytogenics (clove) been reported to possess trivial health implications, leaving no arsenic residues in meat or the rearing environment (Wankhede, 2015). It is generally believe to be healthier, with no dangerous residue found in meat, and these features have positioned phytogenics as indispensible substitutes/analogues to antibiotics in poultry production. Therefore, this study aimed at investigating the effect of clove inclusion on carcass traits, gut microbial count and histological traits of broiler birds.

MATERIALS AND METHODS
The study was carried out at the Department of Animal Science Teaching and Research Farm, University of Nigeria Nsukka. One hundred and twenty (120) day-old broilers (Chikun strain) were randomly divided into five groups of 24 birds each. The treatments groups were as follows: **T1=**Basal diet (BD; control), **T2=**BD+0.5gclove/kg diet, **T3=**BD+1.0gclove/kg diet, **T4=**BD+1.5gclove/kg diet and **T5=**BD+2.0gclove/kg diet. Each treatment was replicated three times with eight birds per replicate placed in 2.6 x 3m deep litter pens with fresh wood shavings. Feed and water were offered *ad libitum* every

morning from 7.00am -8.00 am and the birds were properly vaccinated. The proximate analysis was carried out according to the procedures of AOAC (2006). Data collected were subjected to analysis of variance as described for completely randomized design (Steel and Torrie, 1980) and differences between treatment means were separated using Duncan's New Multiple Range Test (Duncan, 1955).

RESULTS AND DISCUSSION

Table 2 shows the effect of clove powder supplementation on the gut microbial count of broiler birds. All the parameters measured differed significantly ($p<0.05$) among treatment in the *Lactobacillus, E.coli* and *Salmonella* counts. Birds fed T4 (1.5 g/kg) and T2 (2.0 g/kg) had the highest ($p<0.05$) population of the *Lactobacillus spp* while decreasing the *Salmonella*and *E.coli spp* count in the caecum section of the intestine. *Lactobacillus* have a number of biochemical properties, including the production of antibacterial compounds and bile salt hydrolase compounds, as well as their probiotics roles that help to maintain gut integrity (Ogwuegbu *et al*.,2021). This result is in agreement with the report of Ashan, (2018), who reported a decreaseing *Salmonella* and *E.coli spp* as well as *Lactobacillus* increase in broilers fed phytogenic feed additives. Again, Clove possesses antimicrobial properties due to its important role in the gut and it has a favourable effect on broiler health by controlling the population of pathogenic bacteria and inducing the proliferation of beneficial bacteria by creating an acidic environment in the gut which represents a selective environment that hinders the production of pathogenic bacteria (Kim *et al.,* 2009).

Table 3 shows the effect of clove powder supplementation on the histological traits of broiler birds. All the parameters measured differed ($p>0.05$) significantly among the treatments. The highest villus length was recorded for birds fed T3 (1.0 g/kg), T4 (1.5 g/kg) and T5 (2.0 g/kg) of clove. Birds fed T4 (1.5 g/kg) and T5 (2.0 g/kg) of clove had the highest crypt depth, thickness of epithelium and thickness of the muscularis. This result is in collaboration with the report of Dialoke, (2020) who reported that the inclusion of phytogenic in the diet of broiler birds increased the villus height and crypt depth of broiler birds. The inclusion of clove accelerates the growth of enterocytes and villus elongation that resulted in the increased villus height and deeper crypts, and this suggests that improved digestive tract maintenance. In addition, clove as a phytogenic feed additive have a role in the development of intestinal epithelium, villi growth and also as a major source of enterocytes, which is essential to the health of intestinal mucosa. Higher intestinal villi are associated with increased absorptive surface area of the intestine and thus an increased absorptive capacity with resultant higher body weight gain (Dialoke *et al*., 2020).

CONCLUSION

The result of the study obtained that up to 2.0 g/kg clove can be included in broiler diet for improved gut integrity and histological traits of broilers. We therefore recommend that 2g/kg diet of clove, having discovered that the inclusion of this additive at single level have more positive effects on the animals and also increased income over expense. This additive can be successfully used as a potent alternative to antibiotics without any deleterious effect on the animal and consumers.

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Table 1: Ingredient (%) and chemical composition of experimental diets for broiler starter and finisher birds

Ingredients (%)	**Starter**	**Finisher**
Maize	44	54.04
Wheat	5.00	5.14
Soybean meal	14.00	12.42
Groundnut cake	24.00	16.00
Palm kernel	5.00	5.4
Fish meal	3.00	2.00
Bone meal	4.00	4.00
Salt	0.25	0.25
Vitamin + mineral premix*	0.25	0.25
Methionine	0.25	0.25
Lysine	0.25	0.25
Total	100	100
Calculated composition:		
Crude protein (%)	22.67	18.00
Metabolizable energy(Mcal/kg)	3000	2900
Chemical Composition		
Dry matter	90.00	91.03
Crude protein	21.56	18.08
Crude fat	3.00	5.00
Crude fibre	5.03	4.98
Ash	4.00	4.00
Nitrogen free extract	66.41	67.94

Table 2: Effects of clove powder counts of bacteria in the ileum (log_{10}cfu/g) of broiler birds

Parameters	**T1**	**T2**	**T3**	**T4**	**T5**	**SEM**
Lactobacillus	6.08^{d}	8.67^{c}	10.16^{b}	13.02^{a}	12.92^{a}	0.710
Salmonella	8.60^{a}	6.07^{b}	5.28^{c}	5.16^{c}	5.04^{c}	0.36
E.coli	7.46^{a}	6.71^{ab}	5.14^{bc}	4.63^{c}	5.35^{bc}	0.35

^{a,b,c,d}Means on the same row with different superscripts are statistically different ($p<0.05$). SEM: Standard Error of Mean,**T1**=control,**T2**=0.5g clove/kg diet,**T3**=1.0g clove/kg diet,**T4**=1.5g clove/kg diet and **T5**=2.0g clove/kg diet.

Table 3: Effects of cloves on histological traits of samples of jejunum of broiler starter birds

Parameters	**T1**	**T2**	**T3**	**T4**	**T5**	**SEM**

Villus length (nm)	1183.64[b]	1277.76[ab]	1347.71[a]	1425.63[a]	1391.40[a]	28.94
Crypt depth (nm)	144.60[c]	173.73[bc]	191.49[bc]	215.29[ab]	252.42[a]	11.28
Thickness of epithelium (nm)	38.32[c]	42.51[c]	56.09[b]	62.14[a]	59.33[ab]	2.61
Thickness of muscularis (nm)	127.37[d]	141.91[c]	150.53[bc]	158.40[ab]	166.30[a]	3.75

[a,b,c,d]Means on the same row with different superscripts differ significantly ($p<0.05$). SEM: Standard error of the mean, **T1 =** Control diet, **T2 =** 5g cloves/kg diet, **T3 =** 10g cloves/kg diet, **T4 =** 15g cloves/kg diet and **T5=** 20g cloves/kg diet.