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Research Article
Impact of Aqueous *Ocimum gratissimum* (Lyn) Leaf Extract on Growth Performance, Gut pH and Bacterial Counts in Broiler Chickens

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Abstract

Background and Objectives: Phytobiotics are included in poultry diets as suitable alternatives to antibiotic growth promoters. Their impact on growth promotion and intestinal health as natural growth promoters also varies. This experiment was conducted to determine the impact of aqueous *Ocimum gratissimum* leaf extract on growth performance, gut pH and gut bacterial counts in broiler chickens. **Materials and Method:** One-hundred-day-old broiler chicks were randomly distributed into two treatment groups of fifty birds each with five replicates of ten birds per replicate. The control group was not administered aqueous *O. gratissimum* leaf extract, while the treatment group was administered aqueous *O. gratissimum* leaf extract. The experiment lasted six weeks and was arranged as a completely randomized design. Data on feed intake and weight gain were collected and the feed conversion ratio (FCR) was determined by calculation. The pH of the crop, proventriculus, ileum and caecum was measured and recorded and *Escherichia coli* (*E. coli*) and *Lactobacillus* counts in these gut sections were determined. The data were subjected to one-way analysis of variance and significant differences were identified using Tukey's post hoc test. **Results:** Aqueous *O. gratissimum* leaf extract significantly ($p<0.05$) enhanced the final live weight (1900.4±39.5 g bird^{-1}), weight gain (1780.4±39.5 g bird^{-1}) and FCR (2.05±0.02) of broiler chickens compared to the control group. Gut pH was significantly ($p<0.05$) reduced in the ileum (6.36±0.03) and caecum (6.46±0.01) of the birds in the treatment group. *Escherichia coli* counts were also significantly ($p<0.05$) reduced in the ileum (2.18±0.02 Log CFU) and caecum (4.40±0.11 Log CFU) in the treatment group compared to the control group (6.81±0.13 Log CFU in the ileum and 6.73±0.18 Log CFU in the caecum). **Conclusion:** The results showed that aqueous *O. gratissimum* leaf extract improved growth performance and reduced gut pH and *E. coli* counts.

Key words: Broiler chicken, feed conversion ratio, gut bacteria, gut pH, *Ocimum gratissimum*, weight gain

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Herbs, spices and essential oils contain active phytochemicals that can be used as an alternative to antibiotic growth promoters (AGPs) in poultry nutrition. A number of herbs and spices can be used as phytogenic feed additives. The use of herbs, spices and their essential oils is aimed primarily at harnessing their antimicrobial potential, safeguarding gut health and ultimately boosting performance. Biogenic additives and phytogenic feed additives such as herbs, spices, plant extracts and essential oils have been used for natural therapy as pharmaceuticals. They have also been used to preserve food and to make it more appetizing. Recently, aromatic plants and their extracts have been introduced into animal feeding. Some of these have been reported to improve feed intake and secretion of digestive juices[1]. They can also act as natural antimicrobials[2,3]. According to a recent report by Nte *et al*.[4], they can act as natural growth promoters (NGPs). In comparison to synthetic antibiotics or inorganic chemicals, plant-derived products have been proven to be natural, less toxic and residue-free and are thought to be ideal feed additives in food animal production[5]. Typical examples of herbal plants utilized in poultry nutrition include *Vernonia amygdylina*,[6,7] *Moringa oleifer*,[8] *Rosemary officinalis*,[9] *Leucaena leucocephala*[10] and *Azadirachta indica*,[11] to mention only a few.

Ocimum gratissimum (Lyn) is an herbal plant whose benefits can be harnessed in poultry nutrition. Otherwise known as African basil or scent leaf, it is widely grown as a perennial herb in tropical Africa, southeast Asia, India and Hawaii. As reported earlier Gill[12], it is highly recognized worldwide due to its versatile nutritional, anaesthetic and medicinal uses. The presence of alkaloids, tannins, phytates, flavonoids, oligosaccharides, terpenoids, thymol and saponin along with tolerable levels of cyanogenic glucoside in scent leaf[13] has been noted. The essential oil (eugenol) present in scent leaf exhibits antimicrobial activities against pathogenic strains of gram-negative and gram-positive bacteria and pathogenic fungi[14]. Previous *in vitro* studies reported the antibacterial effect of *O. gratissimum* leaf extract against *Salmonella* sp. and *Escherichia coli* isolated from the ileum of broiler birds[15]. In the light of the above-described properties of *O. gratissimum* and the results of *in vitro* studies published earlier, this study was conducted to determine the impact of aqueous *O. gratissimum* leaf extract (AOGLE) on growth performance, gut pH and gut bacterial populations in broiler chickens. Aqueous *O. gratissimum* leaf extract positively enhanced the live weight, weight gain and feed conversion ratio (FCR) of broilers. Aqueous *O. gratissimum* leaf extract administration also resulted in reduced *Escherichia coli* counts in the ileum of broilers while increasing *Lactobacillus* counts.

MATERIALS AND METHODS

Preparation of AOGLE: Freshly harvested *Ocimum gratissimum* leaves were washed, plucked from the stem and finely chopped. Five hundred grams of the finely chopped leaves were weighed and milled in 1500 mL of clean aseptic water in an electric blending machine. The filtrate was obtained with the aid of cheesecloth and an additional 1800 mL of water was added to ensure that most if not all the extract was removed. The volume of filtrate obtained was 3200 mL. This was divided equally (640 mL) with the aid of a measuring cylinder and poured into 5 drinkers for the 5 replicates of birds to be administered AOGLE in the treatment group. The drinkers containing AOGLE were placed in the pens of the replicates allocated to the treatment group and left in the pens for 4 h to allow the birds to voluntarily consume the AOGLE. This procedure was repeated each day that the AOGLE was administered to the birds in the treatment group.

Animal experiment: One hundred-day-old ANAK 2000 broiler chicks were purchased and brooded for 7 days. The chicks were randomly distributed into two treatment groups with five replicates of ten birds per replicate. They were fed a standard broiler grower diet (Table 1) throughout the experimental period. Feed and water were provided *ad libitum*. The birds in the control group (CG) were not administered AOGLE. Broiler birds administered AOGLE were used as the treatment group (TG). Administration of AOGLE was performed twice. The first administration was performed on days 8 and 10 and the second administration was performed on days 28 and 30. The pH of various gut sections (crop, proventriculus, ileum and caecum) was determined on day 8 prior to the administration of AOGLE. The purpose of this was to determine the gut pH on day 8 prior to the establishment of normal gut microflora in the absence of any treatment effect. The pH of these four gut sections was determined again on day 28 prior to the administration of AOGLE. The purpose of this was to ascertain the effect of AOGLE in modulating the gut bacterial population via its effect on pH after the establishment of normal gut flora (14-21 days), allowing a period of three weeks post-hatching for adult caecal flora development. Digesta samples were also collected from the crop, proventriculus, ileum and caecum on days 8 and 28 (prior to the administration of AOGLE) and again on

Table 1: Gross and analysed nutrient concentrations in the diet (all values are presented in g kg^{-1} DM unless otherwise indicated)

Feed ingredients	Concentration
Maize	550.00
SBM	330.00
Fish meal	40.00
Cassava starch	42.00
Constant ingredients*	38.00
Total	1000.00
Calculated nutrient composition	
M.E. (kcal kg^{-1} DM	3028.50
Crude protein	214.94
Analysed composition	
Crude protein	210.85
Ash	46.80
Ether extract	69.80
Crude fibre	65.70
Nitrogen-free extract	609.20
Dry matter	947.60

*Bone meal (21 g), oyster shell (10 g), vitamin/mineral premix (2.5 g), DL-methionine (1.5 g), common salt (3 g)

day 35. Digesta collection on day 8 for *Escherichia coli* and *Lactobacillus* counts was performed for the purpose of ascertaining bacterial counts of *Escherichia coli* and *Lactobacillus* prior to the administration of AOGLE and prior to the establishment of normal gut microflora in the birds in both treatment groups. This provided preliminary information on bacterial counts before the administration of AOGLE. Collection of digesta on day 28 was performed to establish the treatment effect of AOGLE administration on gut bacterial counts, while digesta collection on day 35 was performed to show the effectiveness of AOGLE in modulating *Escherichia coli* and *Lactobacillus* counts in the gut of animals approaching slaughter weight at six weeks since bacterially induced diseases typically occur between 2 and 4 weeks of life in broiler chickens. The experiment lasted for 35 days, excluding the 7-day brooding period. The gross and analysed nutrient composition of the experimental diet is presented in Table 1. The diet was a standard maize/soybean meal-based diet. The experiment was arranged as a completely randomized design.

Digesta collection: On day 8 prior to AOGLE administration, one bird per replicate in each treatment group was slaughtered by severing the jugular vein. Digesta were collected from the crop, proventriculus, ileum and caecum. The collected digesta were placed in sterile sample containers and transported to the laboratory for enumeration of bacteria (*Escherichia coli* and *Lactobacillus*) using bacteria-specific agar. This was repeated on day 28 prior to the administration of AOGLE. Digesta samples were also collected on day 35 at the end of the experimental period.

Gut pH determination: One bird per replicate in each treatment group was slaughtered by severing the jugular vein. The pH of the gastrointestinal tract of each bird (crop, proventriculus, ileum and caecum) with digesta intact was determined using a pH meter (Model: HANNA Instruments Hi 9024 microcomputer pH meter). The pH values were read immediately after slaughter prior to AOGLE administration on days 8 and 28.

Microbial analysis

Isolation, identification and enumeration of bacteria: The populations of microorganisms in the different samples were isolated, identified and enumerated using the 10-fold serial dilution pour plate method[16]. De Man Rogosa and Sharp (MRS) agar was used to enumerate total *Lactobacillus* spp. on a replicate basis. The plates were incubated at 37°C for 24-72 h. *Escherichia coli* was enumerated after incubation on MacConkey agar at 37°C for 48 h. The presence of pinkish to red colonies with a metallic sheen indicated *Escherichia coli* and colonies with a clear appearance on MRS agar indicated *Lactobacillus* sp. Colonies counted were expressed as colony forming units CFU g^{-1} of the samples.

The data on bacterial counts were log transformed prior to statistical analysis. Data on gut bacterial counts were expressed as Log CFU.

Statistical analysis: The collected data were subjected to one-way analysis of variance (ANOVA) using Tukey's post hoc test and significant means ($p<0.05$) were evaluated using Minitab v.17 software

RESULTS AND DISCUSSION

Growth performance indices: The results obtained on growth performance parameters are presented in Table 2. The results showed that there was significant ($p<0.05$) improvement in final live weight, weight gain and feed conversion ratio (FCR) of broilers administered aqueous *Ocimum gratissimum* leaf extract compared to birds in the control group. The recorded values were 1900.4 g $bird^{-1}$, 1780.4 g $bird^{-1}$ and 2.05, respectively. There was no significant difference ($p>0.05$) in feed intake in the treated and untreated birds but the results indicated that the feed intake of birds administered AOGLE was numerically higher (3655.30 g $bird^{-1}$) than that of birds in the control group without *Ocimum* administration (3644.00 g $bird^{-1}$). An earlier report Hashemi *et al.*[17] indicated that dietary supplementation of broiler diet with a combination of herbal plant extract and acidifier resulted in

Table 2: Performance of broilers administered aqueous *Ocimum gratissimum* leaf extract (g bird^{-1})

	Treatments	
Performance parameters	CG	TG
Initial live weight	120.00	120.00
Final live weight	1750.20±84.2^{a}	1900.40±39.5^{b}
Weight gain	1630.20±84.2^{a}	1780.40±39.5^{b}
Total feed intake	3644.00±80.7^{a}	3655.30±82.3^{a}
Feed conversion ratio	2.24±0.07^{a}	2.05±0.02^{b}

Values are mean±SD (5 replicates). Different letters in the same row indicate significant differences between treatment levels ($p<0.05$). CG (control group); TG (group treated with aqueous *O. gratissimum* leaf extract)

enhanced maintenance and function of the small intestine and enhanced broiler performance. This is a probable mode of action of aqueous *Ocimum gratissimum* leaf extract in bringing about the improved weight gain observed in the current study. Administration of 3 g of neem leaf[11] per litre of water to broiler birds encouraged healthy growth in the birds and thus could serve as an alternative to antibiotics in areas with limited access to veterinary services. D-cymene and linalool, the major constituents of coriander (*Coriandrum sativum*), were reported to elicit significant effects on performance and blood biochemical parameters in broilers[18]. According to the author, coriander also has appetizing qualities and stimulates the process of digestion. Allicin from garlic (*Allium sativum*) was also reported to improve growth performance and increase the beneficial gut microbial population[19]. Utilization of ginger (*Zingiber officinale*) was stated to result in improved growth performance and beneficial gut microbial populations[19].

Earlier *in vitro* studies of *O. gratissimum*[15] reported an antibacterial effect against *E. coli* that could lead to a reduction in the gut population of *E. coli* an enteropathogenic bacteria. Such a reduction is one of the likely modes of action of AOGLE in this *in vivo* study. This can be attributed to the fact that when fewer enteropathogenic bacteria are present in the gut, there is less competition for nutrients in the gut for growth and maintenance. In addition, fewer gut-related issues will mean better nutrient digestion and assimilation and invariably better growth performance. The feed conversion ratio was significantly improved ($p<0.05$) with administration of aqueous *O. gratissimum* leaf extract from a value of 2.24 in the control group to 2.05 in the treatment group. The increased weight gain of the birds is probably due to the antimicrobial properties of *O. gratissimum* and its impact on gut function. Another reason for the improvement in the growth performance of birds administered aqueous *O. gratissimum* could be the reduced pH of the digesta in the proventriculus, ileum and caecum; the reduction in pH might control the pathogen populations in those parts of the intestine. A similar finding was reported earlier Dono *et al.*[20] in a study which suggested that improvement in the growth performance of broilers given garlic meal and turmeric meal mixture in their diets might be related to control of the populations of pathogens in the crop, jejunum and caecum due to a reduction in the pH of the digesta in those parts of the intestine. This finding indicates that the pH reduction was not simply due to supplementation with benzoic acid[21] but that the additives also increased intestinal acidity, consequently encouraging the growth of beneficial microflora. The effect of the additives on the intestinal microflora should create a gut environment that is more suitable for optimum nutrient digestion and absorption, resulting in greater nutrient and energy utilization efficiency. Our results showed that aqueous *O. gratissimum* leaf extract improved the growth performance of broilers, in agreement with earlier reports from studies with other phytobiotics.

Previous studies have shown that phytobiotics improve the growth performance of broiler chickens, similar to AGPs[22-23]. Contrasting results were reported by other workers Ocak *et al.*,[24,] Karimi *et al.*[25] and Al-Mufarrej[26] who did not observe any effect of the inclusion of oregano or black cumin (*Nigella sativa* L.) powder in the diet on the performance of broiler chickens. Differences in the dietary ingredients and in the type and dosage of phytobiotics used in different studies may explain the observed discrepancies. Antimicrobial activity and immune enhancement are generally considered the two major mechanisms through which phytobiotics exert bene cial effects on the health and growth performance of chickens[27].

Notwithstanding the existing discrepancies, it is reasonable to state that the ef cacy of phytobiotics as feed additives and their impact on gut health and growth performance may vary as a result of variation in their composition due to biological factors (plant species, growing location and harvest conditions), processing methods (extraction/distillation, stabilization) and storage conditions (light, temperature, oxygen tension and time)[28].

Gut pH: Accurate determination of the digesta pH in broilers could serve as a tool to indicate the potential for optimum gut health and hence maximal nutrient absorption[29]. The results obtained in this study on the gut pH of broiler birds administered aqueous *Ocimum gratissimum* leaf extract are presented in Table 3. Although, the pH of gut sections was determined prior to the administration of AOGLE on day 8, significant differences ($p<0.05$) were observed in the gut pH values of the crop (5.95), the proventriculus (5.96), the ileum (6.31) and the caecum (6.30) of the birds in the control group. As expected, the crop and proventriculus were more

Table 3: pH of gut sections on various sampling days

	Day 8		Day 28	
Gut section	CG	TG	CG	TG
Crop	5.95±0.11[a]	6.10±0.08[a]	6.23±0.04[a]	6.03±0.18[ab]
Proventriculus	5.96±0.06[a]	5.71±0.51[a]	6.23±0.32[a]	5.89±0.30[a]
Ileum	6.31±0.12[b]	6.00±0.03[a]	6.41±0.13[a]	6.36±0.03[b]
Caecum	6.30±0.11[b]	6.11±0.13[a]	6.52±0.19[a]	6.46±0.01[b]

Values are Mean±SD (5 replicates). Different letters in the same column indicate significant differences between gut sections ($p<0.05$), CG (control group), TG (treatment group)

acidic; however, this was not the case in the crop of the treatment group, which were generally more neutral than acidic (pH 6.10). The results show that the pH of the crop and proventriculus did not differ significantly ($p>0.05$) in either treatment group. The pH values recorded in the ileum and caecum were significantly ($p<0.05$) lower in broilers administered AOGLE at day 8 than they were prior to administration of AOGLE. This may be attributed to chance when compared to birds in the control group since no factor with a capacity to impact the gut pH had been administered at day 8. The pH values in the ileum and caecum on day 28 after administration of AOGLE did not follow this trend. This can be attributed to the establishment of normal bacterial flora in the gut; the bacterial flora can be modulated depending on the type of feed and the feed additives administered. In this study, feed can be readily ruled out as a factor because the birds in both treatment groups received the same type of feed. It appears that administration of AOGLE tended to maintain the gut pH during the establishment of normal gut microflora as well during the period in which the birds were susceptible to enteropathogenic bacteria-induced diseases.

The pH data obtained on day 28 revealed the opposite with respect to the values recorded in the crop and proventriculus. Administration of aqueous *O. gratissimum* leaf extract resulted in significant ($p<0.05$) reduction in crop and proventriculus pH compared to birds that were not administered AOGLE. The values recorded were acidic rather than tending towards neutral. A previous study Hinton *et al*.[30] showed that lower intestinal pH stimulated the growth of beneficial bacteria and inhibited the growth and colonization of enteropathogens, especially *Salmonellae* and *Enterobacterium*. Such a reduction in pH was reflected in the pH values recorded in the ileum and caecum of birds administered aqueous *O. gratissimum* leaf extract. The benefit of such a reduction in intestinal pH is that it creates a suitable environment for the growth and proliferation of beneficial microbes such as *Lactobacillus* while prohibiting the growth and proliferation of harmful microbes such as *Escherichia coli*. The results of the current study corroborate those of an earlier study[31] in which the pH of different gastrointestinal sections was measured at different stages of growth. The authors stated that intestinal pH changes with GIT section and age. The observed pH in the gut sections in our study varied significantly ($p<0.05$) in the treatment group administered aqueous *O. gratissimum* leaf extract. This variation was numerical in the control group but the difference was not significant ($p>0.05$).

Earlier studies postulated that lower digesta pH in the gut is associated with a reduction in the growth and colonization of pathogenic organisms, thus permitting greater partitioning of nutrients for optimal growth and nutrient utilization[32]. The significantly better ($p<0.05$) growth performance recorded in broiler chickens administered aqueous *O. gratissimum* leaf extract could also be related to the lower gut pH, as lower gut pH has been reported to be associated with greater weight gain[20]. Lower pH in the intestine inhibits the growth of certain microorganisms, especially pathogenic organisms and favours the growth of beneficial organisms[33]. Furthermore, reduction in the digesta pH is associated with a reduction in the growth and colonization rates of intestinal pathogenic microflora[32,34]. Such a reduction in pH might stimulate the growth and proliferation of beneficial species[35,36], reduce competition for nutrients between intestinal pathogens and the host[30] and stimulate the proliferation of intestinal absorptive cells[37-39] as well as enhance pancreatic secretion[40]. A more favourable intestinal environment is presumed to enhance nutrient uptake by absorptive cells in the gut[39]. This stimulates greater nutrient digestibility[32,41] and aids in shunting more nutrients towards growth rather than maintenance activities. All of these factors should result in better growth performance. It is thought that any condition that favours lower intestinal pH, which is usually associated with colonization by beneficial microbes, might also correlate with greater energy and nutrient utilization efficiency and ultimately more rapid growth[36,42,43].

Gut microbial count: A healthy intestine is one of the most important requirements for enhancing the productivity of broiler chickens. Favourable changes in the intestinal

Table 4: Bacterial count (Log colony-forming units (Log CFU)) on various sampling days

		Day 8		Day 28		Day 42	
Sample	Gut section	*Escherichia coli*	*Lactobacillus*	*Escherichia coli*	*Lactobacillus*	*Escherichia coli*	*Lactobacillus*
CG	Crop	6.86±0.20^{a}	6.89±0.17^{a}	6.79±0.16^{a}	7.00±0.30^{a}	4.44±3.85^{a}	6.88±0.24^{a}
	Proventriculus	6.91±0.12^{a}	7.04±0.13^{a}	6.92±0.12^{a}	7.00±0.09^{a}	6.79±0.10^{a}	6.97±0.12^{a}
	Ileum	6.90±0.05^{a}	6.95±0.04^{a}	6.68±0.03^{a}	6.90±0.11^{a}	6.81±0.13^{a}	7.00±0.72^{a}
	Caecum	6.83±0.13^{a}	6.96±0.10^{a}	6.85±0.21^{a}	7.05±0.05^{a}	6.73±0.18^{a}	7.09±0.09^{a}
TG	Crop	7.05±0.26^{ab}	6.94±0.15^{a}	6.58±0.06^{a}	6.91±0.09^{a}	6.40±0.17^{a}	6.71±0.24^{b}
	Proventriculus	7.00±0.16^{ab}	7.11±0.13^{a}	6.70±0.00^{a}	6.89±0.15^{a}	6.37±0.07^{a}	6.75±0.05^{b}
	Ileum	7.17±0.18^{ab}	7.03±0.17^{a}	6.86±0.17^{a}	7.06±0.06^{a}	2.18±0.02^{ab}	6.83±0.06^{b}
	Caecum	7.08±0.21^{ab}	7.06±0.07^{a}	6.77±0.11^{a}	7.01±0.06^{a}	4.40±0.11^{ab}	6.85±0.07^{b}

Values are Mean±SD (5 replicates). Different letters in the same row and/or column indicate significant differences among bacteria (t-test with Tukey's post hoc test, $p<0.05$). CG: Control group, TG: Treatment group

environment will provide better conditions for the bird to approach its optimum performance level. The pH and transit times of different sections of chicken gut allow the establishment of specific microbial populations. Previous reports Albazaz *et al*.[44] indicated that the composition of microorganisms in the gut is not stable over time and not homogeneous among species, individuals and different parts of the intestine. Table 4 shows bacterial counts (Log CFU) in broilers administered aqueous *O. gratissimum* leaf extract. The bacterial counts of *Escherichia coli* and *Lactobacillus* were not significantly different ($p>0.05$) across the treatment groups on day 8 prior to administration of aqueous *O. gratissimum* leaf extract. This was the case for all of the gut sections, irrespective of the gut pH at day 8. Gut bacterial count at day 28 also followed a similar trend. *Lactobacillus* counts were numerically higher in the ileum (7.06 Log CFU) and caecum (7.01 Log CFU) of broilers administered aqueous *O. gratissimum* leaf extract than in the same gut sections of birds in the control group not administered aqueous *O. gratissimum* leaf extract. This may be a reflection of the positive effect of significantly lower gut pH recorded in these sections of the gut at day 28.

At day 42, which was day 35 of the experiment, *Escherichia coli* counts were significantly reduced in the ileum (2.18 Log CFU) and caecum (4.40 Log CFU) of broiler chickens administered aqueous *O. gratissimum* leaf extract, with a corresponding increase in *Lactobacillus* counts (6.83 Log CFU in the ileum and 6.85 Log CFU in the caecum). This was also the case in the crop and in the proventriculus. This further substantiates the impact of gut pH on the population of pathogenic gut bacteria, as reported in several studies by other authors. *Lactobacillus* counts were unexpectedly significantly lower ($p<0.05$) in all gut sections in broiler chickens administered AOGLE than the control group. This may suggest that *O. gratissimum* has both bacteriostatic and bactericidal effects on *Lactobacillus* and *E. coli*, unlike mushroom-based products, which have been shown to eliminate detrimental microbes and enhance the levels of beneficial bacteria, particularly *Lactobacillus* and *Bifidobacteria*[45]. The reason for the observed result is unknown. It may be that, as was postulated in an earlier *in vitro* study[15], *Ocimum gratissimum* does not discriminate against beneficial and pathogenic bacteria but instead decreases the population levels of both beneficial and detrimental microbes. These are questions that remain unanswered. The results of the current study corroborate earlier reports Junaid *et al*.[46] of the antimicrobial efficacy of *O. gratissimum* leaf extracts against some bacterial isolates, such as *Aeromonas hydrophila*, *Bacillus cereus*, *E. coli*, *Salmonella typhimurium* and *Yersinia enterocolitica*.

The microbial load of broilers fed a diet containing 2.0% *Moringa oleifera* leaf meal was reported to be lower than that of a control group and of a group supplemented with antibiotics[47] Furthermore, the gut health of the birds was improved thereby, suggesting that *Moringa oleifera* leaf can be used as a suitable natural alternative to antibiotics, as also indicated by the results obtained in the current study. Herbs and their mixtures can enhance the performance of birds by improving digestive tract function through their anti-inflammatory, anti-oxidative and antimicrobial effects. In addition to these functions, some herbs stimulate changes in various physiological functions. Herbs may exert multiple functions in the bird's body system[48]. Most of them act as sialagogues and stimulate the secretion of saliva, which makes swallowing easier. Extracts of *Salvia officinalis*, *Thymus vulgaris* and *Rosmarinus officinalis* and a blend of carvacrol, cinnamaldehyde and capsaicin were reported to improve feed digestibility in broilers[48]. The antimicrobial effect of *O. gratissimum*, an herbal antibacterial agent, was evident in the current study.

CONCLUSION

It is evident from the results of the present study that aqueous *O. gratissimum* leaf extract effectively enhanced the final live weight, weight gain and FCR of broiler chickens. The

observed improvement in the animals' growth could be attributed to a significant impact of aqueous *O. gratissimum* leaf extract on gut pH, particularly in the ileum and caecum on day 28 and the impact of lowered gut pH on gut bacterial counts. However, it was observed that aqueous *O. gratissimum* leaf extract elicited a bacteriostatic effect on the *Lactobacillus* population and that this effect did not limit the competitive exclusion response of *Lactobacillus* in limiting the population of *Escherichia coli* in any of the gut sections studied.

SIGNIFICANCE STATEMENTS

This study explored the possible use of aqueous *O. gratissimum* leaf extract as a natural growth promoter that can be beneficial for poultry farmers. This study will help researchers uncover the critical areas of natural growth promoters that many researchers were not able to explore. Thus, a new theory on the mode of action of natural growth promoters and possibly in combination with other alternatives to antibiotic growth promoters may be arrived at.

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REFERENCES

1. Faleiro, M.L., M.G. Miguel, F. Ladeiro, F. Venancio and R. Tavares *et al*., 2003. Antimicrobial activity of essential oils isolated from *Portuguese endemic* species of *Thymus*. Lett. Applied Microbiol., 36: 35-40.
2. Del Campo, J.A., J. Moreno, H. Rodriguez, M.A. Vargas, J. Rivas and M.G. Guerrero, 2000. Carotenoid content of chlorophycean microalgae: Factors determining lutein accumulation in *Muriellopsis* sp. (Chlorophyta). J. Biotechnol., 76: 51-59.
3. Hsieh, P.C., J.L. Mau and S.H. Huang, 2001. Antimicrobial effect of various combinations of plant extracts. Food Microbiol., 18: 35-43.
4. Nte, I.J., V.U. Oleforuh-Okoleh and B.S. Nwiidae, 2016. Effect of Scent Leaf (*Ocimum gratissimum*) Administration on Growth Performance and Carcass Traits of Broiler Chicks. In: Nigeria Society of Animal Science Production (NSAP) Conference FUNAAB Abeokuta, NSAP. (Ed.)., Nigeria Society of Animal Science Production, Nigeria.
5. Hashemi, S.M., M. Mokhtarnia, J.M. Erbaugh and A. Asadi, 2008. Potential of extension workshops to change farmers' knowledge and awareness of IPM. Sci. Total Environ., 407: 84-88.
6. Ofongo, R.T.S. and E.I. Ohimain, 2019. Effect of enzyme supplementation and plant extracts on villus height and microbial counts in broilers. Niger. J. Anim. Prod., 46: 137-149.
7. Oleforuh-Okoleh, V.U., S.O. Olorunleke and I.J. Nte, 2015. Comparative response of bitter leaf (*Vernonia amygdalina*) infusion administration on performance, haematology and serum biochemistry of broiler chicks. Asian J. Anim. Sci., 9: 217-224.
8. Yang, R.Y., L.C. Chang, J.C. Hsu, B.B.C. Weng, M.C. Palada, M.L. Chadha and V. Lavasseur, 2007. Nutritional and functional properties of *Moringa oleifera* leaves from Germ plasm to plant, to health in Moringa leaves: Strategies, standards and market for better impacts on nutrition in Africa. AVRDC-The World Vegetable Centre.
9. Ghazalah, A.A. and A.M. Ali, 2008. Rosemary leaves as a dietary supplement for growth in broiler chickens. Int. J. Poult. Sci., 7: 234-239.
10. Hussain, J., P.V.V.S. Reddy and V.R. Reddy, 1991. Utilisation of leucaena leaf meal by broilers. Br. Poult. Sci., 32: 131-137.
11. Nodu, M.B., M. Okpeku, Z.A. Akpoveta and D.O. Iroegbu, 2016. Evaluation of *Azadirachta indica* leave extract on hematology and biochemical profiles, organs weight and growth parameters of broiler chickens. J. New Sci., 32: 1879-1884.
12. Gill, L.S., 1992. Ethnomedical Uses of Plants in Nigeria. 1st Edn., University of Benin Press, Benin City, pp: 176-177.
13. Ijeh, I.I., O.U. Njoku and E.C. Ekenze, 2004. Medicinal evaluation of *Xylopia ethiopica* and ocimum gratissimum. J. Med. Aromatic Sci., 26: 44-47.
14. Matasyoh, L.G., J.C. Matasyoh, F.N. Wachira, M.G. Kinyua, A.W.M. Thairu and T.K. Mukiama, 2007. Chemical composition and antimicrobial activity of the essential oil of *Ocimum gratissimum* L. growing in Eastern Kenya. Afr. J. Biotechnol., 6: 760-765.
15. Ohimain, E.I., R.T.S. Ofongo-Abule and D.V. Zige, 2015. *In-vitro* antibacterial effect of *Ocimum gratissimum* on broiler gut microflora. Bull. Adv. Scient. Res., 1: 37-41.
16. Pepper, I.L. and C.P. Gerba, 2005. Environmental Microbiology: A Laboratory Manual. 2nd Edn., Academic Press, California, USA.
17. Hashemi, S.R., I. Zulkifli, H. Davoodi, M.H. Bejo and T.C. Loh, 2014. Intestinal histomorphology changes and serum biochemistry responses of broiler chickens fed herbal plant (*Euphorbia hirta*) and mix of acidifier. Iran. J. Applied Anim. Sci., 4: 95-103.
18. Jang, J.P., 2011. Effect of different levels of coriander oil on performance and blood parameters of broiler chicks. Ann. Biol. Res., 2: 578-583.
19. Dieumou, F.E., A. Teguia, J.R. Kuiate, J.D. Tamokou, N.B. Fonge and M.C. Dongmo, 2009. Effects of ginger (*Zingiber officinale*) and garlic (*Allium sativum*) essential oils on growth performance and gut microbial population of broiler chickens. Livest. Res. Rural Dev., 21: 23-32.

20. Dono, N.D., N.H. Sparks and O.A. Olukosi, 2014. Association between digesta PH, body weight and nutrient utilization in chickens of different body weights and at different ages. J. Poultr. Sci., 51: 180-184.
21. Olukosi, O.A. and N.D. Dono, 2014. Modification of digesta pH and intestinal morphology with the use of benzoic acid or phytobiotics and the effects on broiler chicken growth performance and energy and nutrient utilization. J. Anim. Sci., 92: 3945-3953.
22. Windisch, W. and A. Kroismayr, 2007. Natural phytobiotics for health of young piglet and poultry: Mechanisms and application. Poult. Sci., 86: 643-643.
23. Abd El-Ghany, W.A. and M. Ismail, 2014. Tackling experimental colisepticaemia in broiler chickens using phytobiotic essential oils and antibiotic alone or in combination. Iran. J. Vet. Res., 15: 110-115.
24. Ocak, N., G. Erener, F. Burak Ak, M. Sungu, A. Altop and A. Ozmen, 2008. Performance of broilers fed diets supplemented with dry peppermint (*Mentha piperita* L.) or thyme (*Thymus vulgaris* L.) leaves as growth promoter source. Czech J. Anim. Sci., 53: 169-175.
25. Karimi, A., F. Yan, C. Coto, J.H. Park and Y. Min *et al*., 2010. Effects of level and source of oregano leaf in starter diets for broiler chicks. J. Applied Poult. Res., 19: 137-145.
26. Al-Mufarrej, S.I., 2014. Immune-responsiveness and performance of broiler chickens fed black cumin (*Nigella sativa* L.) powder. J. Saudi Soc. Agric. Sci., 13: 75-80.
27. Fallah, R., A. Kiani and A. Azarfar, 2013. A review of the role of five kinds of alternatives to in-feed antibiotics in broiler production. J. Vet. Med. Anim. Health, 5: 317-321.
28. Huyghebaert, G., R. Ducatelle and F. van Immerseel, 2011. An update on alternatives to antimicrobial growth promoters for broilers. Vet. J., 187: 182-188.
29. Morgan, N.K., C.L. Walk, M.R. Bedford and E.J. Burton, 2012. The effect of dietary calcium inclusion on broiler gastrointestinal pH: Quantification and method optimization. Proceedings of The WPSA, BSAS Annual Conference, April 24-25, 2012, Nottingham University, pp: 10.
30. Hinton, Jr., A., R.J. Buhr and K.D. Ingram, 2000. Physical, chemical and microbiological changes in the crop of broiler chickens subjected to incremental feed withdrawal. Poult. Sci., 79: 212-218.
31. Angel, R., B. Humphrey and W. Saylor, 2010. Age changes in gastrointestinal pH in broilers. J. Anim. Sci., 88: 650-650.
32. Lewis, M.R., S.P. Rose, A.M. Mackenzie and L.A. Tucker, 2003. Effects of dietary inclusion of plant extracts on the growth performance of male broiler chickens. Br. Poult. Sci., 44: 43-44.
33. Adil, S., M.T. Banday, G.A. Bhat, S.D. Qureshi and S.A. Wani, 2011. Effect of supplemental organic acids on growth performance and gut microbial population of broiler chicken. Livest. Res. Rural Dev., Vol. 23.
34. Ferket, P.R., 2004. Alternatives to Antibiotics in Poultry Production: Responses, Practical Experience and Recommendations. In: Nutritional Biotechnology in the Feed and Food Industries, Lyons, T.P. and K.A. Jacques (Eds.). Nottingham University Press, Nottingham, UK., pp: 57-67.
35. Partanen, K., J. Valaja, H. Siljander-Rasi, T. Jalava and S. Panula, 1998. Effects of carbadox or formic acid and diet type on ileal digestion of amino acids by pigs. J. Anim. Feed Sci., 7: 199-203.
36. Apajalahti, J.H. and M.R. Bedford, 1999. Improve bird performance by feeding its microflora. World Poult., 15: 20-23.
37. Visek, W.J., 1978. The mode of growth promotion by antibiotics. J. Anim. Sci., 46: 1447-1469.
38. Thomke, S. and K. Elwinger, 1998. Growth promotants in feeding pigs and poultry. III. Alternatives to antibiotic growth promotants. Ann. Zootech., 47: 245-271.
39. Niewold, T.A., 2007. The nonantibiotic anti-inflammatory effect of antimicrobial growth promoters, the real mode of action? A hypothesis. Poult. Sci., 86: 605-609.
40. Dibner, J.J. and P. Buttin, 2002. Use of organic acids as a model to study the impact of gut microflora on nutrition and metabolism. J. Applied Poult. Res., 11: 453-463.
41. Adibmoradi, M., B. Navidshad, J. Seifdavati and M. Royan, 2006. Effect of dietary garlic meal on histological structure of small intestine in broiler chickens. J. Poult. Sci., 43: 378-383.
42. Apajalahti, J., 2004. Structure and dietary modulation of intestinal microbial communities. Proceedings of the 2nd Mid-Atlantic Nutrition Conference, March 24-25, 2004, University of Maryland, College Park, MD., Maryland.
43. Apajalahti, J., A. Kettunen and H. Graham, 2004. Characteristics of the gastrointestinal microbial communities, with special reference to the chicken. World's Poult. Sci. J., 60: 223-232.
44. Albazaz, R.I. and E.B. Buyukunal Bal, 2014. Microflora of digestive tract in poultry. KSU J. Nat. Sci., 17: 39-42.
45. Willis, W.L., O.S. Isikhuemhen and S.A. Ibrahim, 2007. Performance assessment of broiler chickens given mushroom extract alone or in combination with probiotics. Poult. Sci., 86: 1856-1860.
46. Junaid, S.A., A.O. Olabode, F.C. Onwuliri, A.E.J. Okwori and S.E. Agina, 2006. The antimicrobial properties of *Ocimum gratissimum* extracts on some selected bacterial gastrointestinal isolates. Afr. J. Biotechnol., 5: 2315-2321.
47. Mandal, A.B., A. Biswas, A.S. Yadav and A.K. Biswas, 2014. Effect of dietary *Moringa oleifera* leaves powder on growth performance, blood chemistry, meat quality and gut microflora of broiler chicks. Anim. Nutr. Feed Technol., 14: 349-357.
48. Hernandez, F., J. Madrid, V. Garcia, J. Orengo and M.D. Megias, 2004. Influence of two plant extracts on broilers performance, digestibility and digestive organ size. Poult. Sci., 83: 169-174.