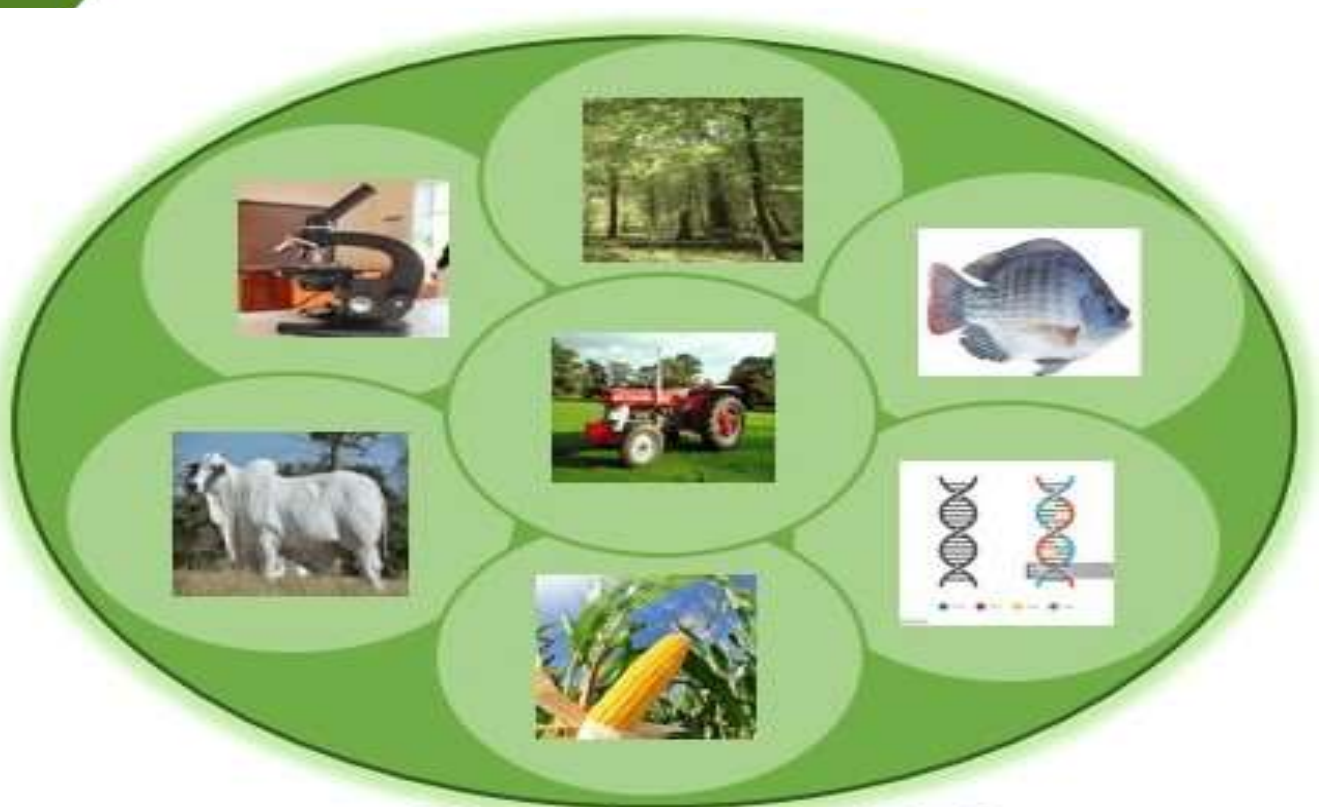




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The Kebbi Journal of Agriculture and Natural Sciences has the sole aim of providing an intellectual platform and ideas for scholars, by promoting interdisciplinary studies related to agriculture and natural science through publishing the latest scientific research findings that are of direct policy implications and beneficial to the research community. Consequently, the journal covers all aspects of Crop Science, Animal Science, Agricultural Economics, Agricultural Extension and Rural Development, Food Science, Fisheries and Aquaculture, Biotechnology, Soil Science and Agricultural Engineering, Forestry and Environment, Wildlife, Agricultural Education, Agro-allied Industries as well as all Natural Science researches related to Agriculture.

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PHYTOBIOTIC EFFECT OF LEMON GRASS (*Cymbopogon citratus*) LEAF AND ORANGE (*Citrus sinensis*) PEEL MEALS ON PERFORMANCE AND ILEAL BACTERIAL COUNT OF NOILER CHICKENS

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ABSTRACT

The experiment was conducted to evaluate the phytobiotic effect of feeding lemon grass leaf meal (LGM) and orange peel meal (OPM) on growth performance indices of Noiler chicken in Kebbi State, Nigeria. Their effects on bacterial populations were also investigated. Ninety (90) 1-day old Noiler birds were randomly assigned to five (5) treatment groups with three (3) replicates of six (6) birds each in a completely randomized design (CRD). The treatments included: Birds fed basal diet without additive (negative control; T0); Birds fed basal diet with antibiotics (positive control; T1); Birds fed basal diets and 4g/L each of LGM and OPM as T2 and T3 respectively; while T4 received 4g/L of LGM+OPM on 1:1 ratio. Ileal digesta of slaughtered birds were collected for bacterial population analysis. The results on the feeding trial showed significant ($P < 0.05$) difference in weight gain (WG), feed intake (FI), feed conversion ratio (FCR) and mortality at starter phase though BWG, FCR and mortality did not differ significantly ($P > 0.05$) for finisher birds. Noiler birds fed LGM+OPM had higher BWG (148.07g/d) and best FCR (1.39g:g) compared to other treatments. For bacterial populations, no significant ($P > 0.05$) variation was observed among the treatments. However, birds fed LGM+OPM (T4) diet had lowest bacterial load (2.2×10^6 cfu/g) compared with the antibiotic (14.2×10^6 cfu/g) and control (18.8×10^6 cfu/g) groups. In conclusion, combined LGM and OPM improved WG and feed efficiency of Noiler birds at starter and finisher phases of growth and reduced intestinal bacterial populations hence, can be used in place of conventional antibiotic.

Keywords: Performance, Noiler, Phytobiotic, Lemon grass leaf, Orange peel

Introduction

Antimicrobial growth promoters (AGP) have been used in the past to enhance poultry growth and health (Paul *et al.*, 2022, Attia *et al.*, 2023, Murshed *et al.*, 2024). The AGP's mode of action alters gut microbiome thereby favouring bacteria that promote growth and health while inhibits the growth of pathogens (Brown *et al.*, 2017). Despite the growth-promoting potential and medicinal effects, there were issues with AGP use including emergence of resistance bacteria, residual effects, toxicity, cost and regulatory bans. This has stimulated increased interest in the usage of consumer accepted natural alternatives such as phytobiotics.

Phytobiotics are plant-derived substances used in animal diets to boost the performance. Nowadays, these additives have known to show antimicrobial, antioxidants, anthelmintic, immuno-modulatory, detoxifying, digestion-stimulating and flavouring properties (Grashorn, 2010). Phytobiotics have an important benefit over AGP, because they are natural, less toxic, residue free, generally recognized as safe and commonly used as food substances (Ayodele *et al.*, 2021). They comprise of spices, herbs and essential oils (Khafaga *et al.*, 2019; Abd El-Hack *et al.*, 2020; Alagawany *et al.*, 2021). Lemongrass (*Cymbopogon citratus*) and orange peels (*Citrus sinensis*) are included in the list of phytobiotics due to their antimicrobial and phytogetic composition that promote the growth and health of poultry (Majekodunmi *et al.*, 2021; Aderibigbe *et al.*, 2024).

Materials and Methods

Experimental Location

The study was conducted at Abdullahi Fodiyo University of Science and Technology, Aliero (AFUSTA). Aliero is located in the Southeastern part of Kebbi State, in North-

western Nigeria and lies between latitude 12°16'42"N and longitude 4°27'6"E with the altitude of 350m above sea level (Mamman *et al.*, 2000). The area has an average temperature and relative humidity of 33°C and 35% respectively (KSUSTAM, 2020). The mean annual rainfall is 733mm in the Northern part of the State and 1045mm for the Southern part (Mamman *et al.*, 2000). The State has a land mass of 37.699km² of which 36.46% is occupied by farm land with an estimated livestock population of about 1.8 million cattle, 2.2 million sheep, 2.8 million goats, 50,000 camels, 200,000 donkeys, 20,000 horses and a large number of backyard poultry (KSMOAHF&H, 2023). Kebbi State's population is estimated to be around 6 million, making it the 17th most populous state in Nigeria (Wikipedia, 2024).

Procurement and Preparation of Test ingredients

Lemon grass was purchased from commercial botanical garden, adjacent to Sir Yahaya Memorial Hospital, Birnin Kebbi, Kebbi State, Nigeria. The plant was transported and transplanted in a farm located at the Faculty of Agriculture Laboratories complex, AFUSTA and all agronomic practices were followed to manage the plants. After five (5) weeks of planting, the leaves were harvested, wash with distilled water to remove all unwanted materials; cut at 2cm; air-dried for 3 days (72 hours), ground with electric blender and sieved using a 0.2 mm laboratory seiver. The resulting fine powder was kept under room temperature in sealed plastic containers until required for further use. Similarly, fresh sweet orange peels were sourced at AFUSTA Minimart, Aliero Local Government, Kebbi State, Nigeria. The fresh orange peels were sliced at 2cm, cleaned and air-dried for 3 days (72 hours). They were grounded using electric blender and sieved using a 0.2mm laboratory seiver and kept in

sealed plastic containers until required for further use. The resulting leaf and peel powder were used as feed additives in Noiler chickens diets.

Experimental birds, diets and design

A total of ninety (90) 7-day-old Noiler chicks were used for the study. They were randomly allocated on weight equalization basis to five treatment groups of 18 birds. Each group had three replicates with six birds per replicate in a completely randomized design (CRD). The treatments included: birds fed basal diet without additive (negative control; T1) and birds fed basal diet with Gentadox (positive control; T2). Birds fed basal diet and 4g/L each of LGM and OPM as T3 and T4 respectively while T5 received 4g/L of LGM+OPM on 1:1 ratio. The LGM and OPM were mixed in 1 litre of warm water and left overnight for proper mixing. The birds were fed Chikum commercial corn-soybean meal diets in pellet form that met the nutrient requirements of broiler chickens (NRC, 1994).

Determination of growth performance

The initial body weight was taken at the beginning of the experiment and weekly thereafter. Feed intake was measured as the difference between the feed offered and leftovers on daily basis. Body weight gain was calculated as final weight minus initial weight by number of birds. Feed conversion ratio was determined as feed intake divided by weight gain while mortality was calculated as number of birds remains divided by number of birds used multiply by 100.

Intestinal microbial population

At the end of the trial (12 weeks), 15 birds (one bird per replicate) were slaughtered and ileal digesta was collected and placed on plastic tubes, then immediately transferred to the refrigerator for bacterial population analysis. Bacterial count was done using methods

defined by Barrow and Feltham (1993). For determination of *Lactobacillus* and *E. coli* population, 1g of digesta was diluted with 9 mL of 10g/L peptone broth, homogenized and then incubated for 1 hour at room temperature. Viable bacterial count in the ileum samples were determined by plating serial 10-fold dilutions (in 10 g/L peptone solution) on MacConkey agar plates to isolate *E. coli* and de Man Rogosa and Sharpe (MRS) agar to isolate *Lactobacillus*. The MacConkey agar plates were incubated for 24 hours at 37°C under aerobic conditions. The MRS agar plates were incubated in an anaerobic jar at 37°C for 48 hours. The *E. coli* and *Lactobacillus* colonies were counted immediately after removal from the incubator. Colonies with smooth convex circles and a pink colour were counted as *E. coli*. While colonies appear as white and yellow were counted as *Lactobacillus* and *Salmonella* respectively. Results were expressed as log¹⁰ of Colony Forming Units (CFU) per gram of digesta (Loh *et al.*, 2014).

Results

Performance of Noiler birds fed test ingredients (1-12 weeks)

Table 1 showed the results on growth performance of Noiler chickens fed LGM, OPM and LGM+OPM. The supplementation of LGM, OPM and their combination have significant ($p < 0.05$) effects on total feed intake (FI), body weight gain (BWG), feed conversion ratio (FCR) and mortality rate. Noiler birds fed antibiotic supplemented diets (T1) had a higher final body weight of 268.48g/bird compared to other treatment groups, but the difference was not significant ($p > 0.05$). The results however showed that Noiler birds offered LGM + OPM consumed more feed compared to other treatment groups. There was mortality across the treatments. Noiler birds served LGM+OPM had the lowest



(2.50%) mortality rate with highest value of 11.67% was recorded in T0 (negative control).



Table 1: Performance of Noiler birds fed test ingredients (1-12 weeks)

Parameter	Treatment					SEM
	T0 NCN	T1 PCN	T2 LGM	T3 OPM	T4 LGM+OPM	
Starter phase						
IBW (g/b)	40.50	40.17	40.37	40.53	40.70	3.13
FBW (g/b)	242.77	268.48	245.87	241.07	262.65	65.13
TFI (g/b)	2154.33 ^c	2206.00 ^c	2196.33 ^c	2407.67 ^b	2707.67 ^a	110.45
DFI (g/d)	52.30	51.22	52.52	66.85	81.65	2.63
BWG(g/b)	128.93 ^b	131.38 ^b	130.122 ^b	132.03 ^b	148.07 ^a	144.37
FCR (g:g)	2.32 ^a	2.04 ^{ab}	1.63 ^{bc}	1.51 ^c	1.39 ^c	1.19
Mort. (%)	11.67 ^a	7.64 ^b	5.30 ^c	6.00 ^{bc}	2.50 ^d	0.02
Finisher phase						
IBW (g/b)	242.77	268.48	245.87	241.07	262.65	65.13
FBW (g/b)	1414.67 ^d	1543.75 ^c	1617.51 ^b	1559.00 ^c	1810.75 ^a	157.94
TFI (g/b)	2930.56 ^d	3085.00 ^c	3150.56 ^c	3266.81 ^b	3429.33 ^a	59.64
DFI (g/d)	104.66	110.18	106.66	115.50	116.67	2.36
BWG (g/b)	1290.09	1313.09	1300.00	1240.85	1300.06	172.43
FCR (g:g)	3.73	3.07	3.44	2.99	2.36	0.33
Mort. (%)	1.50	0.00	0.00	0.00	0.00	0.01

abc= means in the same row with different superscript are significantly ($p < 0.05$) different at 5%

KEY: NCN=negative control, PCN=positive control, AIW=average initial weight, AFW=average final weight, ABWG=average body weight gain, TFI=total feed intake, ADFI=average daily feed intake, FCR=feed conversion ratio

At finisher phase, final body weight (FBW) values indicates growth differences, with the highest weight in T4 (1810.75g/bird) and the lowest in T0 (1414.67g/bird). T2 (1617.51g/bird) and T2 (1559.00 g/bird) also show substantial growth but are slightly more than

T1 (1543.75 g/bird) while T0 (negative control) has moderate FBW compared to treated groups, highlighting the possible positive effect of treatments (T3 and T4 seem most effective). The FI was higher in T4 (3429.33g/bird) and lower in T0

(2930.56g/bird). Initial body weight (IBW), BWG, FCR and mortality did not differ significantly ($P>0.05$). It could be observed however, that the untreated Noiler at finisher phase which had slightly lower IBW (242.77g/bird) gained slightly more body weight (1290.09g/bird) than those in T3 which gain 1240.85g/bird with an IBW of 245.87g/bird. The untreated birds (T0) also consumed less feed (2930.56g/bird) with poorer FCR (3.73g feed g^{-1} gain). Noiler birds in T1 (antibiotic) maintained the lead in BWG (1313.09g/bird) though LGM+OPM (T4) recorded best FBW (1810.75g/bird) with total FI of 3429.33g/bird and FCR of 2.36 g feed g^{-1} gain.

Intestinal Bacterial Populations of Noiler fed LGM, OPM and OPM

Results of the identification and bacterial population analysis of Noiler birds fed LGM,

OPM and LGM+OPM are presented in Table 2. From the result obtained *Escherichia coli* (*E. coli*), *Lactobacillus* and *Salmonella* were identified from the ileal fecal samples of Noiler finisher birds fed LGM, OPM and their combination as a replacement for AGP. *E. coli* and *Salmonella* were identified in the control group (T0). *Lactobacillus* was found absent in T0, while *E. coli* and *Salmonella* were not identified in T4. Total bacteria count (TBC) of 18.8×10^6 cfu/g, 14.2×10^6 cfu/g, 5.6×10^6 cfu/g, and 2.2×10^6 cfu/g were recorded for treatments 0, 1, 2, 3 and 4 respectively. No significant ($P>0.05$) variation was observed among the treatments. However, birds fed LGM+OPM (T4) had lowest bacterial count (2.2×10^6 cfu/g) compared with the antibiotic group (14.2×10^6 cfu/g). The TBC was higher for the control group (18.8×10^6 cfu/g).

Table 4.2: Identification of bacteria and ileal bacterial load

Treatment	Ileal Microbial Organism			TBC (CFU/g)
	<i>E. coli</i>	<i>S. typhumurium</i>	<i>L. plantarum</i>	
T0 (control)	+++	++	-	18.8×10^6
T1 (antibiotic)	+	-	+	14.2×10^6
T2 (LGM)	+	+	+	5.6×10^6
T3 (OPM)	-	+	+	5.5×10^6
T4 (LGM+OPM)	-	-	+++	2.2×10^6

KEY: + (trace amount growth), ++ (moderate amount growth), +++ (high amount growth) TBC (total bacterial count), CFU=(colony forming unit), LLP (lemon leaves powder) OPP (orange peels powder)

Discussion

The test ingredients (LGM and OPM) had significant effect on feed intake (Table 1). However, the results obtained showed that Noiler birds fed diets supplemented with LGM+OPM (T4) consumed significantly more amount of feed 2707.67g compared to other treatment groups at starter phases. Similar trend was also observed at finisher phase (Table 1). This might be attributed to the

biological function or pharmacological activities of the phytochemical components of the combination. The finding is in line with report of Aderibigbe *et al.* (2024) that combination of lemongrass leaves and orange peels were rich in phytochemical substances than their individuals. It could also be as a result of the availability of useful minerals and vitamins inherent in the combination that could have enhanced the palatability of feed and

consequently impacted positively on the bird's growth performance. Oluyemi and Roberts (2000) reported that the incorporation of both micro and macro nutrient in poultry diets enhances feed intake and utilization. The lower feed intake observed in T0 (negative control) at both phases could be due to non-inclusion of neither phytobiotics nor antibiotics.

There were significant differences ($P < 0.05$) in mean body weight gain (BWG) among the treatments (Table 1). At starter phase, the best BWG of 148.07g/bird was observed on the birds fed T4 diets compared to T1 (131.38g/bird) and control (128.93g/bird) groups. The higher BWG of birds in T4 could be as results of higher digestion of the nutrient consumed by the Noiler birds and greater efficiency in the utilization of feed which resulted in enhanced growth. Kamel (2001), Alcicek *et al.* (2004) and Zhang *et al.* (2005) had earlier reported that plants possess digestion stimulating properties. It could be that the phytochemicals may possess the ability to aid digestion thus enhancing the release of free amino acids and energy necessary to enhance growth. This view is in line with earlier view of Aderibigbe *et al.* (2024), that phytochemical aids break down of protein and cleanses the digestive tract. Similar findings have been reported by Alzawqari *et al.* (2016), Tiwari *et al.* (2018), Majekodunmi *et al.* (2021) and Monino *et al.* (2023). The decrease in BWG (128.93g/bird) observed in the control group could be due to none inclusion of either inorganic or organic additives in the diets. This finding is in line with the report of Majekodunmi *et al.* (2021) that pathogenic microbes damage intestinal mucosa, which is important part for absorption of nutrients thereby depressing growth.

Feed conversion ratio (FCR) measured feed efficiency; lower values indicate better feed utilization (Perez *et al.*, 2000). Significantly ($p < 0.05$) T4 (1.39 g feed g^{-1} gain) has the best

FCR, followed by T3 (1.51 g feed g^{-1} gain) and T2 (1.63 g feed g^{-1} gain), suggesting these treatments are more efficient at converting feed into body weight at starter phase. T0 (2.32 g feed g^{-1} gain) has the poorest FCR, reflecting inefficiency despite high feed intake. At finisher phase, the FCR of Noiler birds that received LGM (T2), OPM (T3), LGM+OPM (T4) were not significant ($P > 0.05$) though slightly better than the antibiotic (T1) and negative control (T0) groups. The better FCR observed in both phases for Noiler birds fed diet supplemented with LGM+OPM suggests the ability of the birds to utilize available nutrients in the feed and meals' combinations. This could be due to little or no damage to the intestinal mucosa thereby allowing regular absorption of nutrients. Alzawqari *et al.* (2016) and Aderibigbe *et al.* (2024) reported that the use of LGM+OPM helps to eliminate certain pathogenic organism and improved utilization of feeds by the birds.

Mortality was influenced by dietary treatments during the trial period. Significantly ($P < 0.05$) higher mortality (11.67%) was reported in the untreated (negative control, T0) while lower mortality (2.50%) was recorded in T4 (LGM+OPM) at starter phase. At finisher phase however, no mortality was recorded for Noiler birds fed diets supplemented with antibiotics (T1), LGM (T2), OPM (T3) and LLP+OPP (T4). This could be due to medicinal effect of lemon grass and orange. These findings coincide with the report of Doyle (2001), Bui *et al.* (2006) and Durrani *et al.* (2008) who reported that the application of medicinal substances (organic or inorganic) reduces the number of mortality.

The bacterial load is one of the factors that determine chicken's health, digestion and immune system development in poultry production. Results of the study showed that the total bacterial count (TBC) was significantly higher for the control group

($P > 0.05$). The absence of *E. coli* observed in the ileal TBC indicates the potential combined effort of LGM and OPM in controlling the growth of pathogenic bacteria. This reduction in TBC could be attributed to the pharmacological effects of lemongrass leaves and orange peels. This finding is in agreement with the report of Majekodunmi *et al.* (2021) and Aderibigbe *et al.* (2024) that lemon grass leaves and sweet orange peels are reservoir of many phytochemicals. However, phytochemicals have been reported to possess health promoting potential (Hennemen, 2016). Flavonoids, alkaloids, saponin, and glycosides are natural antimicrobial agents (Bisio *et al.*, 2017). The presence of these secondary metabolites in LGM and OPM suggests its role as a potential antimicrobial. Evidence of antibacterial function of LGM and OPM was confirmed by Joshua *et al.* (2012) and Baba *et al.* (2018) respectively. A more study also found that lemon grass leaves extract was effective in inhibiting *E. coli* using agar well diffusion method (Obi, 2022). The current work is the first to document that inclusion of combined LGM and OPM reduced intestinal digesta *E. coli* population in Noiler chickens.

Conclusion

It could be concluded from this study that Noiler birds fed combined LGM and OPM had higher BWG, FI and best FCR compared to other treatment groups; reduces intestinal bacterial populations and increased the numbers of intestinal lactic acid producing bacteria.

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