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Evaluation of the impact of *Acacia nilotica* and *Annona senegalensis* as potential sources of phytogenic compounds on performances of commercial broiler chickens

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Abstract
The study investigated the impact of Acacia nilotica and Annona senegalensis leaves, as potential sources of phytogenics in broiler diets through a completely randomized design experiment. For both plants, chickens in control were fed diets without phytogenic sources, while the chicken in the test groups T2, T3, and T4, were fed with diets containing 200 g leaves per kg of feed, 200 g seed (ANS) per kg of feed, and 100 g each of both leaves and seeds per kg of feed, respectively. Performance parameters including body weight, feed intake, weight gain, feed conversion ratio was recorded while haematological parameters including white blood cells, haematocrit, platelets, haemoglobin, and red blood cells of the chickens were assessed at the end of the study. The results showed that broilers fed diet containing both Acacia nilotica leaves and seeds (T4) had the highest body weight at 8 weeks (5011.10g, $p<0.05$), total feed intake (4100.00g, $p<0.05$), weight gain (4298.70g, $p<0.05$), and the most efficient feed-to-gain ratio (0.95, $p<0.05$). The broiler chickens fed diets containing Annona senegalensis leaves and seeds showed no significant differences in final body weight or weight gain compared to the control group ($p = 0.414$), with T3 having the highest average final body weight (3480g). However, feed intake patterns varied significantly, with T2 having the lowest average feed intake (2993g, $p<0.05$), and the control group having the highest feed intake, while no significant differences were observed in feed conversion ratios (FCR) among all groups, indicating similar feed efficiency across diets. Supplementation with Acacia nilotica leaves and seeds significantly increased white blood cell counts ($p<0.05$) in broiler chickens, with T2, T3, and T4 showing higher values ($98.78 \times 10^6/\mu L$, $101.08 \times 10^6/\mu L$, and $101.24 \times 10^6/\mu L$, respectively) compared to control ($82.98 \times 10^6/\mu L$). Conversely, Annona senegalensis leaves and seeds did not significantly affect white blood cell counts or other haematological parameters ($p>0.05$). This study demonstrates the potential of Acacia nilotica and Annona senegalensis as promising phytogenic alternatives for enhancing broiler chicken performance. However further research is warranted to explore the specific bioactive compounds in the test ingredients for these effects and optimize their use in poultry diets for sustainable and productive poultry production.

***Keywords:** Broiler chickens, phytogenics, Acacia nilotica, Annona senegalensis, broiler haematology and performance.*

Introduction

In the pursuit of sustainable and effective strategies to enhance the performance and health of commercial broiler chickens, the utilization of phytogenic compounds has emerged as a promising avenue (Yang *et al*., 2015). These phytogenic compounds, derived from plants offer a natural and holistic approach to improving various aspects of poultry

performance production including the growth and physiological performance (Shehata *et al*., 2022). *Acacia nilotica*, and *Anonna senegalensis* are common tree species abundant in tropical regions, representing potential sources of bioactive compounds beneficial to poultry health and productivity (Gothiori *et al*., 2005).

The global demand for poultry meat is projected to continue rising in the coming years, placing immense pressure on the industry to adopt sustainable and ethical production practices (Hafez and Attia, 2020). However, conventional methods of poultry production are often relied on the use of antibiotics as growth promoters as well as the use of synthetic feed additives which are currently raising concerns about antibiotic resistance, feed safety, and animal welfare (Haque *et al*., 2020). In this context, the exploration of natural alternatives like phytogenics has gained significant momentum as part of livestock production science and research (Zhao *et al*., 2023; Mnisi *et al*., 2022). These phytogenics, derived from various plants, offer a promising approach to improve poultry performance and health through their diverse biological properties acting as antimicrobial agents, enhancing gut health, modulate immune responses, and promote nutrient digestion, ultimately leading to improved growth, feed utilization, and disease resistance (El-Sabrout *et al*., 2023; Anagnostopoulos *et al*., 2023).

In most tropical part of Africa, there are diverse flora including the *Acacia nilotica* and *Annona senegalensis* which stand out as potential sources of valuable phytogenics suitable for use in poultry. The *Acacia nilotica*, commonly known as the gum Arabic tree, has been traditionally used in various medicinal applications exhibiting benefits including having antibacterial, antioxidant, and anti-inflammatory properties (Rather and Mohammad, 2015). Similarly, *Annona senegalensis*, known as the African custard-Apple, possessing antimicrobial and immunomodulatory properties documented in several reports (Biseko, 2019). However, despite their individual promise, the scientific evaluation of their application as poultry feed additives remains limited. Therefore, this study aims to address this gap by investigating the impact of *Acacia nilotica* and *Annona senegalensis* as tropical sources of phytogenic compounds on the performance and health of commercial broiler chickens. The study hypothesized that incorporating these plants in broiler diets could improve growth parameters ultimately contributing to sustainable and productive poultry production in tropical regions.

Materials and Method

Study Location: The research was conducted in the Poultry Unit of the Federal University of Agriculture Zuru, Nigeria. The study location is situated within the Northern Guinea Savannah agroecological zone of Nigeria, characterized by a semi-arid climate.

Experimental design: The research implemented a complete randomized design experiment with four Treatment groups including T1 (control), and test groups include T2, T3, and T4, respectively. The birds in T1 were fed diets without any phytogenic compounds while birds in the test groups T2, T3, and T4, were fed with diets containing 200 g *Acacia nilotica leaves* (ANL) per kg of feed, 200 g *Acacia nilotica seed* (ANS) per kg of feed, and 100 g each of both ANL and ANS per kg of feed, respectively. Similarly, for *Annona senegalensis*, the Treatment groups including T1 (control), and test groups include T2, T3, and T4, respectively. The birds in T1 were fed diets without any phytogenic compounds

while birds in the test groups T2, T3, and T4, were fed with diets containing 200 g *Annona senegalensis leaves* (ASL) per kg of feed, 200 g *Annona senegalensis seed* (ASS) per kg of feed, and 100 g each of both ASL and ASS per kg of feed, respectively

Experimental chickens and management: The experimental birds were 96 one-day old chicks obtained from a reputable commercial hatchery. The birds when delivered were checked for deformities and were randomly distributed in the experimental groups. In each experimental group, there were three replicates of 8 birds per replicate, giving a total of 24 birds per group. The birds were provided with water administered with anti-stress on the day of their arrival while other medications and vaccinations followed the guidelines of commercial broilers management in Nigeria. Feeds and water were provided for the birds *ad-libitum* during and after brooding which lasted for a period of 21 days after which the birds were transferred to rearing pen.

The study last for a period of 8 weeks phased into two different phases based on the type of feed supplied, the first phase of the experiment was 0-4 weeks when broiler starter feed was provided while the second phase of the experiment was 5-8 weeks when broiler finisher feed was provided for the chicken. The starter feed was supplied for a period of 4 weeks (Table 1), after which the finisher diets were supplied for another 4 weeks (Table 2).

Table 1: Composition and nutrient analysis of the feed supplied to the broilers at starting phase

Ingredients	T1	T2	T3	T4
Maize	42.00	42.00	42.00	42.00
SBM	26.00	26.00	26.00	26.00
GNC	14.50	14.50	14.50	14.50
Wheat Offal	12.00	12.00	12.00	12.00
Fishmeal	1.00	1.00	1.00	1.00
Bone meal	3.45	3.45	3.45	3.45
Broiler premix	0.30	0.30	0.30	0.30
Salt	0.20	0.20	0.20	0.20
Methionine	0.30	0.30	0.30	0.30
Lysine	0.20	0.20	0.20	0.20
Total	100	100	100	100
Analyzed Nutrients composition of the diets				
Metabolizable Energy (Kcal/kg)	2895.95	2895.95	2895.95	2895.95
Crude Protein (%)	23.86	23.86	23.86	23.86
Available calcium (%)	1.40	1.40	1.40	1.40
Phosphorus (%)	0.90	0.90	0.90	0.90

SBM: Soya Bean Meal; GNC: Groundnut Cake. Each 2.5 kg of broiler premix used contained: Vitamin A: 10000000 IU, Vitamin D3: 2000000 IU, Vitamin E: 23000 mg, Vitamin K3: 2000 mg, Vitamin B1: 1800 mg, Vitamin B2: 5500 mg, Niacin 27,500 mg, Pantothenic Acid 7500 mg, Vitamin B6: 3000 mg, Vitamin B12: 15 mg, Folic Acid : 750 mg, Biotin: 60 mg, Choline Chloride: 300000 mg, Cobalt: 200 mg, Copper: 3000 mg, Iodine: 1000 mg, Iron: 20000 mg, Manganese: 40000 mg, Selenium: 200 mg, Zinc: 30000 mg, and Antioxidant: 1250 mg.

Table 2: Composition and nutrient analysis of the feed supplied to the broilers at finishing phase

Ingredients	T1	T2	T3	T4
Maize	56.00	56.00	56.00	56.00
SBM	19.00	19.00	19.00	19.00
GNC	8.00	8.00	8.00	8.00
Wheat Offal	10.90	10.90	10.90	10.90
Fishmeal	1.00	1.00	1.00	1.00
Bone meal	4.00	4.00	4.00	4.00
Broiler premix	0.40	0.40	0.40	0.40
Salt	0.20	0.20	0.20	0.20
Methionine	0.30	0.30	0.30	0.30
Lysine	0.20	0.20	0.20	0.20
Total	100	100	100	100
Analyzed Nutrients composition of the diets				
Metabolizable Energy (Kcal/kg)	2998.95	2998.95	2998.95	2998.95
Crude Protein (%)	19.30	19.30	19.30	19.30
Available calcium (%)	1.60	1.60	1.60	1.60
Phosphorus (%)	0.90	0.90	0.90	0.90

SBM: Soya Bean Meal; GNC: Groundnut Cake. Each 2.5 kg of broiler premix used contained: Vitamin A: 10000000 IU, Vitamin D3: 2000000 IU, Vitamin E: 23000 mg, Vitamin K3: 2000 mg, Vitamin B1: 1800 mg, Vitamin B2: 5500 mg, Niacin 27,500 mg, Pantothenic Acid 7500 mg, Vitamin B6: 3000 mg, Vitamin B12: 15 mg, Folic Acid : 750 mg, Biotin: 60 mg, Choline Chloride: 300000 mg, Cobalt: 200 mg, Copper: 3000 mg, Iodine: 1000 mg, Iron: 20000 mg, Manganese: 40000 mg, Selenium: 200 mg, Zinc: 30000 mg, and Antioxidant: 1250 mg.

Blood sample collection and analysis: Blood samples were collected from 15 birds in each group through random selection of 5 birds per replicate, the birds were bled for blood which was collected into EDTA anticoagulant bottles to prevent coagulation. Blood samples were collected in triplicates and were immediately transported on ice to the laboratory where they were subjected to analysis using the Sysmex Auto Haematological Parameter Analyzer (Sysmex Corporation, Japan). The analyzer was programmed to measure various haematological parameters, including complete blood count (CBC), haemoglobin concentration, haematocrit level, red blood cell count, white blood cell count, and platelet count.

Data collection and Statistical analysis: There was daily collection of feed intakes, weekly measurement of body weight gain, while feed conversion ratio was computed on 4^{th} and 8^{th} weeks of the study, respectively. The data obtained were subject to one-way analysis of variance using the phytogenic additive as the factor, significant differences in means of the parameters were determined at $p<0.05$ degree of freedom.

Results and Discussion

Effect of *Acacia nilotica* leaves and seeds as phytogenic sources on performance of the broiler chickens

The study involved four experimental groups: T1 (control group without any phytogenic source), T2 (diet containing 200 g Acacia nilotica leaves per kg of feed), T3 (diet containing 200 g Acacia nilotica seeds per kg of feed), and T4 (diet containing 100 g each of Acacia nilotica leaves and seeds

per kg of feed). There was no significant difference observed in the initial body weight of the broilers among the groups ($p > 0.05$), the body weight at 4 weeks showed significant differences among the groups ($p < 0.05$). The birds in T4 groups exhibited the highest body weight (754.54g), followed by T3 (558.32g) and T2 (510.60g), while T1 had the lowest body weight (445.45g). Similarly, significant differences were also observed in the body weight at 8 weeks among the groups ($p < 0.05$). The birds in the group T4 had the highest body weight (5011.10g), followed by T3 (4766.60g) and T2 (3911.10g), with T1 showing the lowest body weight (3544.40g). There was significant difference in the total feed intake among the groups ($p < 0.05$). T4 consumed the most feed (4100.00g), followed by T3 (3900.00g) and T2 (3233.33g), while T1 had the lowest feed intake (2900.00g). The body weight gained also showed significant differences among the groups ($p < 0.05$). T4 had the highest weight gain (4298.70g), followed by T3 (4250.28g) and T2 (3443.20g), with T1 showing the lowest weight gain (3140.55g). Furthermore, the feed-to-gain ratio was also significantly different among the groups ($p < 0.05$). T4 exhibited the most efficient feed conversion (0.95), followed by T2 and T3 (0.92 each), while T1 had a less efficient ratio (0.92) (Table 3).

Table 3: Effect of *Acacia nilotica* leaves and seed as phytogenic sources on performance traits of broiler chickens

Parameters	T1	T2	T3	T4	Mean	SEM	p-values
Initial body weight (g)	42.60	42.70	42.50	42.50	42.58	0.22	0.10
Final body weight (g)	3544.40^{d}	3911.10^{c}	4766.60^{b}	5011.10^{a}	4308.30	607.91	0.0001
Total feed intakes (g)	2900.00^{d}	3233.33^{c}	3900.00^{b}	4100.00^{a}	3533.35	267.70	0.001
Body weight gain (g)	3140.55^{c}	3443.20^{b}	4250.28^{a}	4298.70^{a}	3781.93	316.29	0.0001
Feed to gain ratio (g)	920.00^{b}	930.00^{ab}	920.00^{b}	950.00^{a}	930	10.00	0.001

^{abc}Means with different superscript in the same row are significantly different for the parameters measured ($p<0.05$). T1 birds fed diets without any phytogenic source, while birds in the test groups T2, T3, and T4, were fed with diets containing 200 g *Acacia nilotica leaves* (ANL) per kg of feed, 200 g *Acacia nilotica seed* (ANS) per kg of feed, and 100 g each of both ANL and ANS per kg of feed, respectively.

Effect of *Acacia nilotica* leaves and seeds as phytogenic sources on haematological parameters of the broiler chickens

There was significant difference in the White Blood Cells (WBC) among the groups ($p < 0.05$), T2, T3, and T4 had significantly higher white blood cell counts (98.78 x 10^6/µL, 101.08 x 10^6/µL, and 101.24 x 10^6/µL, respectively) compared with T1 (82.98 x 10^6/µL), indicating potential positive effects of the phytogenic sources on the immune system (Table 4). There also significant differences observed in the haematocrit among the groups ($p < 0.05$). T1 showed the highest haematocrit value (36.50%), while T3 had the lowest (29.68%). T2 and T4 exhibited intermediate values, suggesting a potential effect of the phytogenic sources on blood composition. Furthermore, platelet count was also significantly different among the groups ($p < 0.05$). T2 had the highest platelet count (28.40 x 10^6/µL), followed by T3 (25.60 x 10^6/µL) and T4 (18.80 x 10^6/µL), while T1 had the lowest (17.80 x 10^6/µL). There was no significant difference in the Red Blood Cells (RBC) observed among the groups ($p > 0.05$) (Table 4).

Table 4: Effect of *Acacia nilotica* leaves and seed as phytogenic sources on haematological parameters of broiler chickens

Parameters	T1	T2	T3	T4	Mean	SEM	p-values
White Blood Cells ($x10^9$/µL)	8.28^b	9.88^a	10.10^a	10.12^a	9.60	0.87	0.01
Haematocrit (%)	36.50^a	37.32^a	29.68^c	33.04^b	34.14	1.19	0.001
Platelet ($x10^6$/µL)	17.80^b	28.40^a	25.60^a	18.80^b	22.65	2.23	0.001
Haemoglobin (g/dL)	12.42^a	9.84^b	10.96^{ab}	10.22^{ab}	10.86	0.49	0.05
Red Blood Cells ($x10^6$/µL)	3.01	2.65	2.97	3.68	3.08	0.43	0.412

^{abc}Means with different superscript in the same row are significantly different for the parameters measured ($p<0.05$). T1 birds fed diets without any phytogenic source, while birds in the test groups T2, T3, and T4, were fed with diets containing 200 g *Acacia nilotica leaves* (ANL) per kg of feed, 200 g *Acacia nilotica seed* (ANS) per kg of feed, and 100 g each of both ANL and ANS per kg of feed, respectively.

Effect of *Anonna senegalensis* leaves and seeds as phytogenic sources on performance of the broiler chickens

The broiler chickens fed diets containing *Annona senegalensis* leaves and seeds (T2, T3, T4) showed that the experimental group T3 had the highest average final body weight (3480g), but the difference compared to other groups was not statistically significant ($p=0.414$). The experimental group T2 and T4 (leaves and seeds) had final body weights like the control group leading to highest weight gain observed in T3 although was not significantly different from other groups. The supplementation with *Annona senegalensis* leaves and seeds at the levels tested did not exert a statistically significant effect on broiler final body weight or weight gain compared to the control group. While T3 showed numerically higher values, further investigation is needed to confirm the trend holds true with larger sample sizes or different inclusion levels of the plant materials. Meanwhile the feed intake and feed conversion ratio had a different pattern, with the broiler chicken in T2 (*Annona senegalensis* leaves only) having the lowest average feed intake (2993g), which was significantly lower than T3 (330 6g) but not statistically different from T1 (3173g) and T4 (3097g). The control group had the highest feed intake compared to other groups ($p<0.05$), but there were no statistically significant differences in the FCR among all groups, indicating similar feed efficiency across all diets.

Although, while T2 showed the lowest feed intake, it did not translate to a statistically significant improvement in FCR compared to other groups. This suggests that the reduced feed intake in T2 might not be solely due to improved feed utilization but could be influenced by other factors which require further analysis into nutrient utilization within each group. Summarily, it can be deduced that supplementation with *Annona senegalensis* leaves and seeds at the tested levels did not exert statistically significant impact on the broiler chicken performance parameters measured in this study (Table 5). Hence, further research with different inclusion levels, longer study periods, or combined with other phytogenics might be necessary to elucidate any potential benefits of this plant for poultry production.

Table 5: Effect of *Annona senegalensis* leaves and seeds as alternative synthetic antibiotics on performance traits of broiler chickens

Parameters	T1	T2	T3	T4	Mean	SEM	p-values
Initial body weight (g)	61.59	62.62	61.58	61.18	61.74	0.22	0.566
Final body weight (g)	3340.00	3249.10	3480.00	3260.00	3307.50	9.00	0.414
Total feed intakes (g)	3173.00[b]	2993.00[a]	3306.00[c]	3097.00[b]	3142.25	28.71	0.002
Body weight gain (g)	3278.41[b]	3187.38[c]	3418.42[a]	3198.82[c]	3270.76	10.35	0.001
Feed to gain ratio (g)	967.00	969.00	967.11	968.16	967.56	0.61	0.698

[abc]Means with different superscript in the same row are significantly different for the parameters measured ($p<0.05$). T1 birds fed diets without any phytogenic source, while birds in the test groups T2, T3, and T4, were fed with diets containing 200 g *Annona senegalensis* leaves (ASL) per kg of feed, 200 g *Annona senegalensis* seed (ASS) per kg of feed, and 100 g each of both ASL and ASS per kg of feed, respectively.

Effect of *Annona senegalensis* leaves and seeds as phytogenic sources on haematological parameters of the broiler chickens

Contrary to the observation with *Acacia nilotica* leaves and seeds, the supplementation with *Annona senegalensis* leaves and seeds did not significantly affect white blood cell count in this study. This suggests that there may be a need for further investigation with larger sample sizes or different inclusion levels. Unlike the outcomes of the *Acacia nilotica*, the result suggests that there is no strong evidence for a consistent effect of *Annona senegalensis* compared with the *Acacia nilotica* on the haematological parameters of the commercial broilers ($p>0.05$). While T3 showed numerically higher values for platelets, further research with larger sample sizes with different inclusion levels may be crucial to confirm these observations and elucidate the potential mechanisms of action that may be associated. Additionally, the lack of significant effects on the haematological parameters suggests that *Annona senegalensis* supplementation at the tested levels did not exert major alterations blood components and their function in this study (Table 6).

Table 6: Effect of *Annona senegalensis* leaves and seed as phytogenic sources on haematological parameters of broiler chickens

Parameters	T1	T2	T3	T4	Mean	SEM	p-values
White Blood Cells (x10^9/µL)	7.38	8.97	10.66	9.55	8.84	1.11	0.100
Haematocrit (%)	25.29	31.62	38.38	33.99	31.11	4.76	0.501
Platelet (x106/µL)	9.60	18.94	28.91	22.43	18.18	7.02	0.201
Haemoglobin (g/dL)	7.98	10.04	12.24	10.81	9.87	1.55	0.605
Red Blood Cells (x106/µL)	1.98	2.77	3.60	3.06	2.70	0.59	0.412

[abc]Means with different superscript in the same row are significantly different for the parameters measured ($p<0.05$).

T1 birds fed diets without any phytogenic source, while birds in the test groups T2, T3, and T4, were fed with diets containing 200 g *Annona senegalensis* leaves (ASL) per kg of feed, 200 g *Annona senegalensis* seed (ASS) per kg of feed, and 100 g each of both ASL and ASS per kg of feed, respectively.

The overuse of antibiotics as growth promoters in broilers could lead to the development of antibiotic-resistant bacteria,

with a potential of posing significant threats to human and animal health (Haque *et al*., 2020). This is because antibiotics as synthetic feed additives can raise concerns about potential residues in poultry meat, capable of affecting food safety and consumer trust. This is a production challenge especially under intensive production systems which often compromise animal welfare, raising ethical concerns and impacting production efficiency. Therefore, finding alternatives to antibiotics is crucial to combat resistance and ensure responsible use of antibiotics in human medicine. This necessitates identifying safe and natural feed additives that can promote growth and performance sustainably to address consumer concerns and ethical production standards. The results of the study elucidated significant effects of *Acacia nilotica* supplementation on the growth performance and blood parameters of broiler chickens. Notably, birds fed diets enriched with *Acacia nilotica* leaves and seeds exhibited improved growth performance metrics, including higher body weight gain and more efficient feed conversion ratios compared to the control group. These findings suggest the potential of *Acacia nilotica* as a valuable phytogenic additive for enhancing the productivity of broiler chickens.

Furthermore, haematological analysis revealed alterations in blood parameters indicative of potential physiological benefits conferred by *Acacia nilotica* supplementation. The elevated white blood cell counts observed in birds receiving *Acacia nilotica*-containing diets suggest an enhancement of the immune system, which may contribute to improved disease resistance and overall health in broiler chickens (Ahmed *et al*., 2023). Additionally, variations in the haematocrit and platelet counts among treatment groups indicate potential modulatory effects of *Acacia nilotica* on blood composition and clotting mechanisms as well as possible relationships with immune regulations which warrant further investigation into these mechanisms of action (Zahid *et al*., 2023).

The overall findings of this study provide valuable insights into the use of *Acacia nilotica* as a tropical source of phytogenic compounds to enhance the performance and physiological parameters of broiler chickens. Future research endeavors may focus on optimizing dosage levels and exploring additional health benefits of *Acacia nilotica* supplementation, ultimately contributing to the development of sustainable and efficacious strategies for poultry production in tropical regions.

The investigation of this study on *Annona senegalensis* leaves and seeds as phytogenic feed additives on broiler chickens showed no significant differences observed in body weight, weight gain, or feed conversion ratio, although the group T3 receiving both leaves and seeds showed numerically higher final body weight and lower feed intake. However, these observations need further investigation with larger sample sizes or different inclusion levels for confirmation. Haematological parameters were also not significantly affected by Annona senegalensis supplementation. These suggest that *Annona senegalensis* at the tested levels did not exert major effects on the performance or blood parameters of broiler chickens. Further research is needed to explore its potential benefits at different inclusion levels or in combination with other phytogenics.

Conclusion

Both the *Annona senegalensis* and *Acacia nilotica* could have potential of use as sources of phytogenics that can serve as alternatives to synthetic antibiotics in poultry production. However, there is need for

further research crucial to optimize inclusion levels, identify potential mechanisms of action, and compare their efficacy against other phytogenics and conventional antibiotics. Additionally, exploring their effects on gut health, immune function, and meat quality is important for a comprehensive understanding of their potential benefits.

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