



Research Article

Effects of Graded Dietary Inclusion of *Zanthoxylum chalybeum* Leaf Meal on Feed Intake, Feed Conversion Ratio and Growth Performance of Ross 308 Broiler Chickens

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Abstract | The use of natural feed additives in poultry production has gained increasing attention as a strategy to improve growth performance and feed efficiency in broiler chickens. This study evaluated the effects of graded dietary inclusion of *Zanthoxylum chalybeum* leaf meal (ZCLM) on growth performance, feed intake, and feed conversion ratio of Ross 308 broiler chickens. Ninety-six day-old broiler chicks were brooded for seven days and thereafter randomly allocated to four dietary treatments containing 0, 0.6, 0.8, or 1.0% ZCLM. Each treatment consisted of three replicate pens with eight birds per pen, and the replicate pen was the experimental unit. The experimental feeding period lasted 35 days, from 7 to 42 days of age. Body weight, feed intake, weight gain, average daily weight gain, and feed conversion ratio were evaluated. One-way analysis of variance (ANOVA) was used for feed intake and feed conversion ratio, while analysis of covariance (ANCOVA), with Day-7 body weight as a covariate, was used for growth performance variables. After adjustment for Day-7 body weight, ANCOVA detected no statistically significant treatment effects on final body weight, total weight gain, or average daily weight gain ($p > 0.05$), and ANOVA detected no significant treatment effects on feed intake or feed conversion ratio ($p > 0.05$). The numerically highest adjusted final body weight (2,731 g/bird), adjusted total weight gain (2,570 g/bird), and adjusted average daily weight gain (73.4 g/bird/day) occurred at 0.8% ZCLM inclusion. However the difference among the treatments were not statistically significant. Feed conversion ratio was numerically lowest in the control group (1.61) and was not improved by ZCLM inclusion. Overall, inclusion of ZCLM at levels up to 1.0% was not associated with an apparent growth depression under the conditions of this preliminary study. The findings should be interpreted cautiously because of the limited replication and the absence of laboratory chemical characterization of ZCLM and the complete diets. Further studies with greater replication, chemically characterized leaf meal, and experimentally determined diet composition are warranted.

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Introduction

Broiler production has become an important component of the global livestock sector because of the relatively rapid growth rate, efficient feed conversion, and short production cycle of modern broiler chickens. However, maintaining high productivity requires effective control of enteric diseases and optimization of nutrient utilization. For many decades, antibiotics have been used in poultry production for therapeutic purposes and, in some production systems, as growth-promoting agents. Antibiotic growth promoters have historically been associated with improved growth performance and feed efficiency, partly through their effects on the intestinal microbial environment and the control of subclinical infections (Gadde *et al.*, 2017). Nevertheless, the prolonged and inappropriate use of antibiotics in animal production has contributed to concerns regarding antimicrobial resistance, which represents an important One Health challenge because resistant microorganisms and resistance determinants can circulate among animals, humans, food systems, and the environment (Van Boeckel *et al.*, 2019). These concerns, together with increasing regulatory restrictions and consumer demand for poultry products produced with reduced antibiotic inputs, have intensified the search for effective alternatives to antibiotic growth promoters.

Among the alternatives being investigated, phytogetic feed additives have received considerable attention in poultry nutrition. Phytogetics comprise plant-derived materials, including herbs, spices, leaves, extracts, essential oils, and other plant products containing biologically active secondary metabolites. Their potential value in animal production has been associated with a range of biological activities, including antimicrobial, antioxidant, anti-inflammatory, immunomodulatory, and digestive effects (Windisch *et al.*, 2008; Mahfuz and Piao, 2019). In broiler chickens, phytogetic additives have been investigated for their potential to influence feed intake, gastrointestinal function, nutrient utilization, intestinal microbial populations, oxidative status, and growth performance. However, the responses reported in the literature are not always consistent. A meta-analytic study concluded that the effects of phytogetic additives on broiler performance can vary considerably among experiments, indicating that their efficacy depends on factors such as the

plant species, plant part, method of preparation, bioactive compounds present, inclusion level, basal diet, bird age, and production conditions (Polycarpo *et al.*, 2022). Thus, the identification and evaluation of locally available plants with potentially useful biological properties remains an important area of research.

The search for plant-based alternatives may be particularly relevant in sub-Saharan Africa, where a diverse range of indigenous and naturalized medicinal plants are traditionally used by local communities but remain insufficiently investigated for applications in animal nutrition. Medicinal plants are of interest not only because of their potential biological activities but also because some may be locally available and adaptable to production systems where access to commercial feed additives is constrained by cost or availability (Alowanou *et al.*, 2026). Nevertheless, the traditional medicinal use of a plant should not, by itself, be interpreted as evidence of its nutritional value or suitability as a feed ingredient. Before a medicinal plant can be considered for use in animal feeding, its chemical composition, biological activity, safety, appropriate inclusion level, and effects on animal performance require systematic evaluation.

One plant that warrants investigation in this context is *Zanthoxylum chalybeum* Engl. (Rutaceae), commonly known as knobwood. *Z. chalybeum* is a medicinal plant native to Eastern and Southern Africa and is traditionally used in several countries, including Kenya, Tanzania, Uganda, Zambia, and Zimbabwe. The species has a long history of ethnomedicinal use, with different plant parts being employed traditionally for the management of conditions including malaria, respiratory ailments, digestive disorders, and other health problems (Mguni *et al.*, 2023). In Zambia, the plant occurs in several regions and forms part of the broader indigenous plant resources used by local communities. This established ethnomedicinal importance has stimulated scientific interest in the species, particularly in relation to its phytochemical composition and pharmacological properties.

Phytochemical investigations indicate that *Z. chalybeum* contains a diverse range of secondary metabolites and other bioactive constituents. The genus *Zanthoxylum* is recognised for its chemically diverse constituents, including alkaloids, amides, flavonoids, phenolic compounds, terpenoid-related constituents,

and other secondary metabolites, although the composition varies among species and plant parts (Okagu *et al.*, 2021). Specifically, studies reviewed by Mguni *et al.* (2023) have identified several chemical constituents in *Z. chalybeum*, while pharmacological investigations have reported biological activities including antibacterial, antimalarial, and antidiabetic effects. Phytogetic feed additives have been proposed to influence gastrointestinal microbial populations, digestive secretions, intestinal integrity, antioxidant status, and immune responses, although the magnitude and direction of these effects vary substantially among plant materials and experimental conditions (Mahfuz and Piao, 2019; Polycarpo *et al.*, 2022; Mufungwe *et al.*, 2025). Consequently, the biological properties reported for *Z. chalybeum* provide a rationale for investigation but should not be extrapolated directly to expected improvements in broiler performance without empirical feeding studies. Consequently, the biological properties reported for *Z. chalybeum* provide a rationale for investigation but should not be extrapolated directly to expected improvements in broiler performance without empirical feeding studies.

Despite the extensive ethnomedicinal history and growing scientific interest in *Z. chalybeum*, available research has focused predominantly on its traditional uses, phytochemistry, pharmacological properties, and toxicological characteristics rather than its potential application in poultry nutrition (Mguni *et al.*, 2023). In particular, there is limited information on the effects of incorporating *Z. chalybeum* leaf meal (ZCLM) into diets of growing broiler chickens. This represents an important knowledge gap because the leaves constitute a potentially accessible plant material, yet their suitability as a dietary ingredient cannot be inferred solely from the medicinal properties reported for extracts or other plant parts. Moreover, the effects of ZCLM may differ according to dietary inclusion level, and the response of broilers in terms of feed intake, growth, and feed efficiency remains insufficiently characterized.

Therefore, the objective of the present study was to evaluate the effects of graded dietary inclusion of *Z. chalybeum* leaf meal at 0, 0.6, 0.8, and 1.0% on growth performance and feed utilisation of Ross 308 broiler chickens from 7 to 42 days of age. Specifically, the study assessed final body weight, total weight gain, average daily weight gain, feed intake, and feed conversion

ratio. The study was intended to provide preliminary evidence on the suitability of ZCLM as a potential plant-derived feed resource for broiler chickens and to generate information that could guide further research on its nutritional value, biological activity, safety, and appropriate inclusion level in poultry diets.

Materials and Methods

Study site and experimental birds

The birds were managed under routine commercial broiler husbandry practices in accordance with the general animal welfare practices of the University of Zambia Department of Animal Science, including provision of adequate housing, continuous access to feed and clean drinking water, appropriate litter, vaccination according to the hatchery-recommended schedule, regular health monitoring, and careful handling to minimize stress. Any bird showing signs of ill health is to be promptly attended to by qualified veterinary personnel.

The experiment was conducted at the Animal Science Field Station of the University of Zambia, Lusaka, Zambia. A total of 96 day-old Ross 308 broiler chicks were obtained from a local commercial hatchery. Upon arrival, the chicks were housed in an open-sided poultry house and brooded under standard brooding conditions. During the 7-day brooding period, the chicks had ad libitum access to feed and clean drinking water. A lighting programme of 23 h light and 1 h darkness per day was provided throughout the study. The birds were vaccinated according to the vaccination programme recommended by the hatchery and were managed under similar husbandry conditions throughout the experiment.

At 7 days of age, the chicks were randomly allocated to four dietary treatments, with 24 birds assigned to each treatment. Each treatment consisted of three replicate pens, with eight birds per replicate pen. Thus, the experimental arrangement comprised four dietary treatments, three replicate pens per treatment, and eight birds per replicate pen. The birds remained in their respective treatment groups until 42 days of age.

Preparation of Zanthoxylum chalybeum leaf meal

Fresh leaves of *Z. chalybeum* Engl. (Rutaceae), commonly known as knob wood, were collected from mature plants in Luapula Province, Zambia. The leaves were thoroughly washed with clean water to remove

adhering soil and foreign materials and subsequently air-dried under shade at ambient temperature until a constant weight was attained. The dried leaves were milled using a laboratory grinder to obtain leaf meal. The resulting *Z. chalybeum* leaf meal (ZCLM) was stored in airtight containers under dry conditions until incorporation into the experimental diets.

Experimental diets and dietary treatments

Four dietary treatments were evaluated, comprising an unsupplemented control diet and diets supplemented with 0.6, 0.8, or 1.0% ZCLM. The inclusion levels corresponded to 0, 6, 8, and 10 g ZCLM per kg of complete feed, respectively. Experimental diets were formulated for the starter, grower, and finisher phases to meet or exceed the nutrient requirements of broiler chickens in accordance with the relevant recommendations of the Zambia Bureau of Standards. ZCLM was incorporated into the diets at the designated inclusion levels by proportionally reducing maize meal and soybean meal according to their respective proportions in the basal diet. The total quantity of maize meal and soybean meal removed from each diet was equivalent to the amount of ZCLM added, while the inclusion levels of the other ingredients were maintained. This approach ensured that the total ingredient composition of each experimental diet remained at 100%.

The calculated nutrient composition of the formulated diets was compared with the phase-specific nutrient specifications recommended for Ross 308 broilers (Aviagen, 2022). Because neither ZCLM nor the complete experimental diets were subjected to laboratory chemical analysis, the nutrient values in Table 1 should be interpreted as calculated formulation estimates rather than experimentally determined concentrations.

The ZCLM used in the study was not subjected to laboratory proximate analysis or phytochemical screening. Consequently, its actual nutrient composition and phytochemical profile, and their contribution to the complete diets, could not be experimentally verified. This limitation should be addressed in future studies.

Experimental design and feeding management

The experimental feeding period commenced when the birds were 7 days of age and continued for 35 days, with the trial ending when the birds reached

42 days of age. Thus, the total period from placement of the day-old chicks to the end of the study was 42 days, comprising a 7-day common brooding period followed by a 35-day experimental feeding period.

Following the brooding period, the birds were randomly assigned to the four dietary treatments. Each treatment consisted of three replicate pens containing eight birds each. The experimental diets were offered ad libitum throughout the 35-day feeding period, and clean drinking water was continuously available. Feed was offered to each replicate pen in known quantities, and feed refusals were recorded at the end of each measurement period. Feed intake was determined for each replicate pen as the difference between the quantity of feed offered and the quantity of feed remaining. Feed intake was expressed on a per-bird basis using the number of birds represented in each replicate. The replicate pen was considered the experimental unit for variables measured at pen level, including feed intake and feed conversion ratio (FCR). Although body weight was measured individually, the eight individual bird measurements within each replicate pen were averaged to obtain a single replicate-pen mean before statistical analysis. Thus, the replicate pen, rather than the individual bird, represented the experimental unit for growth performance analysis.

Growth performance measurements

Individual body weights were recorded at 7 days of age, after allocation of the birds to their replicate pens and immediately before the experimental diets were introduced. Day-7 body weight therefore represented the starting body weight for the 35-day experimental feeding period rather than a measurement obtained before the common brooding period.

Statistical analysis

All data were analyzed using the General Linear Model (GLM) procedure of SAS software, version 9.4 (SAS Institute Inc., 2013). The replicate pen was the experimental unit, giving three independent experimental observations per treatment. One-way ANOVA was used for Day-7 body weight, feed intake, and feed conversion ratio. Growth performance variables measured at the end of the feeding period were analyzed using analysis of covariance (ANCOVA), with Day-7 body weight as the covariate, to account statistically for differences in starting body weight among treatment groups.

Table 1: *Ingredient composition and calculated nutrient composition of starter, grower and finisher diets containing graded levels of Zanthoxylum chalybeum leaf meal (ZCLM).*

Ingredient composition (%)	Level of inclusion of ZCLM (%)											
	Starter				Grower				Finisher			
	0	0.6	0.8	1.0	0	0.6	0.8	1.0	0	0.6	0.8	1.0
Maize meal	48.0	47.7	47.6	47.5	51.1	50.8	50.7	50.6	56.2	55.9	55.8	55.7
Soybean meal	43.6	43.3	43.2	43.1	39.4	39.1	39.0	38.9	34.6	34.3	34.2	34.1
Soybean oil	3.8	3.8	3.8	3.8	5.7	5.7	5.7	5.7	5.9	5.9	5.9	5.9
Dicalcium phosphate	2.0	2.0	2.0	2.0	1.7	1.7	1.7	1.7	1.5	1.5	1.5	1.5
Limestone flour	1.25	1.2	1.2	1.2	0.9	0.9	0.9	0.9	0.7	0.7	0.7	0.7
Lysine	0.15	0.15	0.15	0.15	0.09	0.09	0.09	0.09	0.07	0.07	0.07	0.07
DL-Methionine	0.33	0.33	0.33	0.33	0.29	0.29	0.29	0.29	0.26	0.26	0.26	0.26
Broiler premix	0.50	0.50	0.50	0.50	0.40	0.40	0.40	0.40	0.30	0.30	0.30	0.30
Salt (NaCl)	0.37	0.37	0.37	0.37	0.37	0.37	0.37	0.37	0.37	0.37	0.37	0.37
ZCLM	0	0.6	0.8	1.0	0	0.6	0.8	1.0	0	0.6	0.8	1.0
Calculated nutrient composition												
Crude protein (%)	23.3	23.2	23.2	23.2	21.7	21.6	21.6	21.6	20.0	19.9	19.9	19.9
ME (MJ/kg)	12.7	12.7	12.7	12.7	13.4	13.4	13.4	13.3	13.7	13.7	13.6	13.6
Crude fibre (%)	3.5	3.5	3.6	3.6	3.3	3.4	3.4	3.4	3.1	3.2	3.3	3.3

Note: Nutrient values are calculated formulation estimates based on ingredient database values and were not experimentally determined by laboratory analysis of the complete diets.

The one-way ANOVA model was:

$$Y_{ij} = \mu + T_i + \varepsilon_{ij}$$

Where Y_{ij} is the observed value for the j th replicate pen receiving the i th dietary treatment, μ is the overall mean, T_i is the fixed effect of the i th dietary treatment ($i = 1, 2, 3, 4$), and ε_{ij} is the random error term. The error term was assumed to be independently and normally distributed with a mean of zero and constant variance. Treatment effects were considered statistically significant at $p < 0.05$. Because no statistically significant treatment effects were detected, post-hoc mean separation was not performed. Treatment means are therefore presented without mean-separation superscripts.

For final body weight, total weight gain, and average daily weight gain, ANCOVA was performed using Day-7 body weight as a covariate. This approach adjusted the treatment means for differences in starting body weight among the groups.

$$Y_{ij} = \mu + T_i + \bar{\beta}(X_{ij} - \bar{X}) + \varepsilon_{ij}$$

Where; Y_{ij} is the observed value for the j th replicate pen receiving the i th dietary treatment; μ is the

overall mean; T_i is the fixed effect of treatment i ($i = 1, 2, 3, 4$); $\bar{\beta}$ is the regression coefficient for the covariate; X_{ij} is the Day-7 body weight of replicate j in treatment i ; $\bar{X}$ is the overall mean Day-7 body weight; and ε_{ij} is the random error term. Treatment effects were considered statistically significant at $p < 0.05$. Because no statistically significant treatment effects were detected, post-hoc mean separation was not performed. Adjusted treatment means from ANCOVA are presented with their SEM and treatment p -values.

It is important to note that the use of three replicate pens per dietary treatment provided limited statistical power to detect small to moderate treatment effects. The limited replication increases the risk of Type II error (Roush and Tozer, 2004), and means that the absence of statistical significance should not be interpreted as proof that the treatments are biologically identical. Accordingly, the present experiment is considered a preliminary baseline or feasibility study rather than a definitive efficacy trial.

Results

Experimental diets

Four dietary treatments were evaluated, comprising an

unsupplemented control diet and diets supplemented with 0.6, 0.8, or 1.0% ZCLM. The inclusion levels corresponded to 0, 6, 8, and 10 g ZCLM per kg of complete feed, respectively. Experimental diets were formulated for the starter, grower, and finisher phases to meet or exceed the nutrient requirements of broiler chickens in accordance with the relevant recommendations of the Zambia Bureau of Standards. ZCLM was incorporated into the diets at the designated inclusion levels by proportionally reducing maize meal and soybean meal according to their respective proportions in the basal diet. The total quantity of maize meal and soybean meal removed from each diet was equivalent to the amount of ZCLM added, while the inclusion levels of the other ingredients were maintained. This approach ensured that the total ingredient composition of each experimental diet remained at 100%.

The calculated nutrient composition of the formulated diets was compared with the phase-specific nutrient specifications recommended for Ross 308 broilers (Aviagen, 2022). Because neither ZCLM nor the complete experimental diets were subjected to laboratory chemical analysis, the nutrient values in Table 1 should be interpreted as calculated formulation estimates rather than experimentally determined concentrations.

The ZCLM used in the study was not subjected

to laboratory proximate analysis or phytochemical screening. Consequently, its actual nutrient composition and phytochemical profile, and their contribution to the complete diets, could not be experimentally verified. This is a primary limitation of the study and should be addressed in future work.

Growth performance

Growth performance results are presented in Table 2. Mean Day-7 body weight ranged from 149 to 161 g/bird, with the highest starting weight in the group later receiving 0.8% ZCLM. ANCOVA-adjusted final body weight ranged from 2,531 to 2,731 g/bird. Dietary treatment had no significant effect on adjusted final body weight ($p = 0.17$), adjusted total weight gain ($p = 0.15$), or adjusted average daily weight gain ($p = 0.15$). Day-7 body weight was a significant covariate in the growth models ($p < 0.001$).

At 42 days of age, observed final body weight ranged from 2,513 to 2,754 g/bird. The highest observed value occurred in the 0.8% ZCLM group, which also had the highest Day-7 starting body weight. After adjustment for Day-7 body weight, least-squares means ranged from 2,531 to 2,731 g/bird; the treatment effect was not statistically significant ($p = 0.17$). Total weight gain and average daily weight gain showed the same general numerical pattern, but their adjusted treatment effects were also not significant ($p = 0.15$ for each).

Table 2: Growth performance of broiler chickens fed diets containing graded levels of *Zanthoxylum chalybeum* leaf meal, with growth outcomes adjusted for Day-7 body weight using ANCOVA.

Parameter	Level of inclusion of ZCLM (%)				SEM	p-value
	0	0.6	0.8	1.0		
Day-7 body weight (g/bird)	149	157	161	152	4.5	0.09
Adjusted final body weight ¹ (g/bird)	2,531	2,632	2,731	2,586	62.2	0.17
Adjusted total weight gain ¹ (g/bird)	2,382	2,475	2,570	2,434	60.8	0.15
Adjusted average daily weight gain ¹ (g/bird/day)	68.1	70.7	73.4	69.5	1.7	0.15

¹Adjusted means are least-squares means from ANCOVA with Day-7 body weight as the covariate. SEM = standard error of the adjusted mean. Treatment p -values for adjusted growth variables are from ANCOVA; the Day-7 body-weight p -value is from one-way ANOVA. The replicate pen was the experimental unit ($n = 3$ pens per treatment).

Table 3: Feed intake and feed conversion ratio of broiler chickens fed diets containing graded levels of *Zanthoxylum chalybeum* leaf meal.

Parameter	Level of inclusion of ZCLM (%)				SEM	p-value
	0	0.6	0.8	1.0		
Total feed intake (g/bird)	3,815	4,137	4,305	4,037	102.5	0.20
Average daily feed intake (g/bird/day)	109.0	118.2	123.0	115.3	2.9	0.20
Feed conversion ratio (g:g)	1.61	1.66	1.66	1.67	0.03	0.31

Feed intake and feed conversion ratio

Feed intake and feed conversion ratio (FCR) of broiler chickens fed diets containing graded levels of *Zanthoxylum chalybeum* leaf meal are presented in Table 3. Total feed intake ranged from 3,815 to 4,305 g/bird and average daily feed intake from 109.0 to 123.0 g/bird/day. One-way ANOVA showed no significant treatment effect on either measure ($p = 0.20$).

Feed conversion ratio was numerically lowest in the control group (1.61) and ranged from 1.66 to 1.67 in the ZCLM treatments. One-way ANOVA showed no significant treatment effect on FCR ($p = 0.31$). Thus, ZCLM inclusion did not improve feed efficiency under the conditions of this preliminary study.

Discussion

The principal finding of the present study is that dietary inclusion of ZCLM at 0.6–1.0% did not produce statistically significant changes in growth performance, feed intake, or feed conversion ratio under the conditions evaluated. Although the 0.8% treatment had the numerically highest ANCOVA-adjusted final body weight, total weight gain, and average daily weight gain, these differences were not statistically significant. The results therefore do not support a conclusion that 0.8% ZCLM is an optimum inclusion level. Importantly, inclusion up to 1.0% was not associated with an apparent growth depression, providing preliminary evidence that warrants further phytogenic investigation. However, the study did not include direct toxicological or health-safety endpoints; therefore, the results should not be interpreted as establishing the safety of ZCLM.

Zanthoxylum species contain diverse secondary metabolites, including alkaloids and other phytochemical constituents, and several species have been investigated for their potential biological activities (Ombito, 2021; Okagu *et al.*, 2021). These phytochemical constituents could potentially contribute to biological responses when *Zanthoxylum* plant materials are included in animal diets. However, because the ZCLM used in the present study was not subjected to phytochemical analysis, the extent to which such compounds contributed to the numerical growth response observed at 0.8% ZCLM inclusion cannot be established. Accordingly, the observed growth response should be interpreted as an association

with dietary treatment rather than evidence of a demonstrated phytochemical mechanism.

Feed intake and feed conversion ratio showed numerical variation among treatments, but these differences were not statistically significant ($p = 0.20$ and $p = 0.31$, respectively). Feed intake responses in broilers may be affected by dietary composition, ingredient characteristics and feed-processing conditions (Abdollahi *et al.*, 2018). Furthermore, meta-analytical evidence indicates that the effects of leaf-meal supplementation on broiler performance vary according to the plant species, processing method, inclusion level and composition of the basal diet (Bakare *et al.*, 2022; Ogbuewu *et al.*, 2024). The numerically higher feed intake at 0.8% coincided with higher weight gain, but did not result in improved FCR compared with the control (1.66 vs 1.61). The numerical decline at 1.0% compared with 0.8% should be interpreted descriptively, as the diets were not chemically characterized. Overall, the data do not provide evidence that ZCLM enhanced feed utilization efficiency or establish an optimum inclusion level; rather, the study provides preliminary evidence that inclusion up to 1.0% was not associated with an apparent growth depression. In a recent layer study, *Zanthoxylum* was used 0, 1, 2 and 3% in feed for 8 weeks, the results exhibited no significant effects on production performance parameters (Lei *et al.*, 2026).

Several methodological limitations should be considered. The ZCLM was not subjected to laboratory proximate analysis or phytochemical screening, and the complete diets were not chemically analyzed. Consequently, Table 1 provides calculated formulation estimates rather than experimentally determined nutrient concentrations. These limitations restrict interpretation of the actual nutritional and phytochemical contribution of ZCLM and prevent attribution of the observed numerical responses to specific constituents.

The Day-7 body-weight differences among treatment groups were an important consideration in interpreting growth performance, particularly because the 0.8% treatment had the highest starting mean body weight. ANCOVA was therefore used to adjust the growth outcomes for this covariate. The adjusted treatment effects remained non-significant, indicating that the observed numerical differences in growth cannot be attributed confidently to ZCLM inclusion alone.

The present trial should therefore be viewed as a preliminary baseline or feasibility study. Future work should use adequately replicated pen-level experimental units, preferably with at least six replicates per treatment, together with ANCOVA where baseline body-weight differences remain. Chemical characterization of the ZCLM and laboratory analysis of complete diets should also be undertaken before biological responses are interpreted mechanistically.

Conclusion

Under the conditions of the present study, dietary inclusion of *Zanthoxylum chalybeum* leaf meal (ZCLM) from 0 to 1.0% did not significantly affect broiler growth performance, feed intake, or feed conversion ratio. The numerically highest adjusted growth values occurred at 0.8% inclusion, but these differences were not statistically significant and were accompanied by higher feed intake without improved FCR. Thus, the study provides no statistical evidence that ZCLM improves growth or feed efficiency and does not establish 0.8% as an optimum inclusion level. Nevertheless, inclusion up to 1.0% was not associated with an apparent growth depression. These preliminary findings justify further studies using greater replication, chemically characterized ZCLM, experimentally determined nutrient composition of complete diets, and additional health and safety endpoints are warranted.

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Novelty Statement

This study provides novel preliminary evidence on the use of *Zanthoxylum chalybeum* leaf meal (ZCLM), an indigenous African medicinal plant, as a dietary ingredient in Ross 308 broiler chickens. While previous research on *Z. chalybeum* has focused predominantly on its ethnomedicinal uses, phytochemistry, pharmacology, and toxicology, information on its application in poultry nutrition remains limited. The present study addresses this knowledge gap by evaluating graded dietary inclusion levels of ZCLM (0, 0.6, 0.8,

and 1.0%) and their effects on growth performance, feed intake, and feed conversion ratio in broilers from 7 to 42 days of age. Although ZCLM did not significantly improve growth performance or feed efficiency, the study provides baseline evidence that inclusion up to 1.0% was not associated with apparent growth depression under the conditions evaluated. These findings establish an empirical basis for further investigation of *Z. chalybeum* as a locally available phyto-genic feed resource, particularly through studies incorporating chemical and phytochemical characterization, greater experimental replication, nutrient utilization, gut health, and safety assessments

Authors Contribution

FK conceived and designed the study. RP executed the experiment and collected data, SJH, FK and SL analyzed and interpreted the data. All authors critically revised the manuscript for important intellectual content and approved the final version.

Generative AI and AI assisted technology statement

The authors declare that no generative AI and AI assisted technology was used in the creation of this manuscript.

Conflict of interest

The authors have declared no conflict of interest.

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