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Original Article

Crassocephalum crepidioides and *Chenopodium ambrosiodes* leaf meals supplementations in broiler chickens: Performance and health status

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Abstract

This study was carried out to determine the effect of leaf meals of *Crassocephalum crepidioides (CCLM)* and *Chenopodium ambrosiodes* (CALM) on the performance and health status of Cobb 500 broiler chickens. One hundred and twenty day-old Cobb 500 broiler chicks were randomly distributed to four dietary treatments of 30 birds per treatment (10 birds/replicate) during a six-week feeding experiment. The treatments were designated as Diet 1 (Basal/control diet), Diet 2 (Basal + 0.20g Vitamin C/kg), Diet 3 (Basal +. 0.25g CCLM/kg) and Diet 4 (Basal + 0.25g CALM/kg). The dietary supplementation of CCLM and CALM feed additives significantly ($p < 0.05$) increased the body weight gain and improved the feed conversion ratio of the broiler chickens compared to the control treatment. The broiler chickens fed diets supplemented with CCLM and CALM had higher ($p < 0.05$) packed cell volume, red blood cells, haemoglobin concentration and mean cell volume compared to the control group. No significant differences ($p > 0.05$) were observed in the dressing percentage and relative organ weights of the broiler chickens across the treatments. The dietary supplementations of CCLM and CALM additives in the diets significantly ($p < 0.05$) lowered the serum alanine aminotransferase, cholesterol and creatinine contents of the broiler chickens compared to the control group. This study has shown that CCLM and CALM could be used as potential feed supplements in a broiler diet to enhance growth performance and improve the health status of broiler chickens during production.

INTRODUCTION

The poultry industry, more specifically, the broiler chicken production, has advanced remarkably as one of the most rapid and cost-effective means of making high-quality animal protein available to humans across the world over the last two decades. In 2020, production of poultry meat reached 137 million tons, totalling 40.6% of global meat production and by 2030 this is expected to reach 41% above beef, pork and mutton production at 20, 34, and 5%, respectively (Bilgili, 2020, OECD/FAO 2021). This remarkable growth can mostly be attributed to advances in breeding genetics and the selection of fast-growing broilers to attain a body weight of at least 2.5kg within 6-weeks of intensive rearing

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(Kralik *et al*., 2018), improvements in nutrition with the addition of growth promoters as feed additives as well as improved management techniques (Tavárez and Solis de los Santos, 2016).

Since the use of antibiotic growth promoters in chicken farming has come under serious criticism for possibly contributing to the development of antimicrobial resistance in humans, health-conscious customers now prefer meat products with natural additives (Agyare *et al*., 2019). These have caused a paradigm shift in how antibiotics are used in animal agriculture. The withdrawal of antibiotics, however, can make pathogenic infections more common, which might negatively impact the health and productivity of livestock (Sutherland *et al*., 2013; Karavolias *et al*., 2018). Due to this circumstance, there has been a lot of research done on prospective antibiotic alternatives (Yang *et al*., 2009). There are a variety of phytochemicals found in medicinal plants that have antimicrobial and immune-modulating capabilities, which could be utilized as potential alternatives to antibiotics to promote livestock performance and health (Falowo, 2022).

The supplementation of these medicinal plants in poultry diets has been reported to stimulate endogenous antioxidants, facilitate nutrient metabolism, enhance carcass yield and organ weight and improve meat quality by inhibiting peroxidation and lowering cholesterol content (Oloruntola *et al*., 2018, Falowo, 2022). Interestingly, *Crassocephalum crepidioides* (Asteraceae) and *Chenopodium ambrosiodes* are among the medicinal plants with a lot of potential that can be utilized as natural additives in poultry production.

Crassocephalum crepidioides is an edible vegetable plant belonging to the Asteraceae family and locally known as "Ebolo" (Agbogidi, 2010). The plant has been proven to contain rich chemical nutrients (protein, crude fibre, fat, minerals, etc) and antioxidant properties (Falowo *et al*., 2023). The dietary inclusion of this plant has been reported to reduce oxidative stress in mice (Can and Thao, 2020) and improve the immune system of broiler chickens (Adeyemi *et al*., 2021). *Chenopodium ambrosioides* (Linn) plant belongs to the family *Chenopodiaceae*, genus *Chenopodium* and species *ambrosioides* (Farrag *et al.,* 2013). Its common names include Epazote, Mexican tea, American wormwood, while its local names in Hausa, Yoruba and Ikulu include Kafi-kashi-warri (i.e., your odour is worse than excreta), 'ewe imi' (i.e., leaf of excreta) and Anyinyung (i.e., something that smells), respectively (Lohdip *et al*., 2008). *Chenopodium ambrosioides plant* possesses a strong odour and has been used in the treatment of wounds and dysentery (Kasali et al., 20121). Report has also shown that *C. ambrosioides plant contains a relative amount of protein, crude fibres, fat, ash, minerals and phytochemicals with a strong ability to exhibit antioxidant, antimicrobial* and anti-inflammatory activities (Lohdip and Oyewale, 2009; Falowo *et al*., 2023).

Despite the aforementioned inherent medicinal properties of the plants, little or no work has been reported on the efficacy of their potential in poultry performance. Therefore, this study was designed to evaluate the effect of *C. crepidioides and C. ambrosioides* leaf meals on the growth performance and health status of broiler chickens

MATERIALS AND METHODS

Plant collection

Fresh leaves of *C. ambrosioides L.* and *C. crepidioides* were purchased from town markets in South-West, Nigeria. The leaves were identified and authenticated at the Department of Plant Science and Biotechnolgy, Adekunle Ajasin University Akungba- Akoko, Ondo State, Nigeria. The leaves were cleaned and air-dried in an open shade. The dried leaves were pulverized using an electric blending machine and the powdered samples were packed into a polyethene bag for further analysis.

Experimental site

The study was conducted at the Poultry Unit of the Teaching and Research Farm, Federal College of Agriculture, Akure, Nigeria. The experimental site is located at 7°25' N and 5°19' E with average annual temperature and annual rainfall of 25.3°C and 1455 mm, respectively. The entire study was carried out for six weeks following the research ethics and guidelines of the Animal Health and Prodution Technology Department of the Institution.

Experimental diets and animals

Two Basal diets (starter (0-21 days) and finisher phase (22-42 days)) were formulated to meet the broiler's nutritional requirement (Table 1). At each phase, the experimental diets were divided into four (4) treatments and designated as Diet 1 (Basal/control diet), Diet 2 (Basal + 0.20g Vitamin C/kg), Diet 3 (Basal +. 0.25g CCLM/kg) and Diet 4 (Basal + 0.25g CALM/kg). One hundred and twenty (120) 1-day-old Cobb 500 broiler chicks were randomly distributed to the four dietary treatments. Each treatment was replicated three times. Thirty (30) broiler chicks were assigned to each treatment (10 birds/replicate) in a completely randomized design (CRD). The chicks were housed in their respective pen (200 x 100 cm) with the floor covered with wood shavings. The house's temperature was kept within 31°C ± 2°C for the first seven days, then it was lowered by 2°C every subsequent week until it reached 26°C ± 2°C. During the six-week feeding experiment, the light was on for 23 hours each day while food and drink were available at all times.

Table 1. Composition of the Experimental Basal Diets

Ingredients	Starter feed	Finisher diet
Maize	52.33	59.32
Maize bran	7.02	0.00
Rice bran	0.00	6.03
Fish meal	3.00	3
Soybean meal	30	24
Premix	0.30	0.30
Bone meal	3.00	3.00
Soy oil	3.00	3.00
Methionine	0.30	0.30
Limestone	0.50	0.50
Salt	0.30	0.30
Lysine	0.25	0.25
Analyzed nutrients (%)		
Crude fibre	3.55	3.63
Crude fat	4.47	3.94
Crude protein	22.19	20.09
Calculated nutrients (%)		
Calcium	1.02	0.97
Available phosphorus	0.44	0.41
Methionine	0.68	0.65
Lysine	1.36	1.24
ME (Kcal/kg)	3018.93	3108.16

ME = Metabolizable energy

Experimental Procedures and Analyses

At the beginning of the experiment, birds in each replicate were weighed individually and subsequently on a weekly basis using a 10.1kg capacity precision weighing scale (model A and D weighing GF-10k industrial, balance, Japan). Also, data on feed intake, body weight (BW) gain and feed conversion ratio (FCR) were obtained from the experimental birds. Feed intake was calculated as the difference between feed given and feed not consumed. The final body weight was calculated as the maximum weight attained by the birds before slaughter. The FCR was calculated as grams of feed consumed divided by body weight. At 42 days of age, three birds per treatment were randomly selected and humanely slaughtered. Before slaughter, the feed was withdrawn from the birds overnight. After slaughtering, birds were allowed to bleed for 5 minutes, scalded and eviscerated. After that, the dressed percentage was estimated as a percentage of the slaughtered weight. The internal organs, which include the liver, heart, gizzard, spleen, bile and lungs were removed, cleaned, weighed and recorded.

During slaughtering, blood samples were obtained from jugular veins into EDTA bottles for the determination of haematological parameters and also into a plain blood sample bottles for serum biochemistry (creatinine, alanine aminotransaminase, and cholesterol) assesement. The blood sample in each of the plain bottles was centrifuged for 10 min at 3000 rpm to obtain clean supernatant serum. The harvested supernatant sera were kept at -20 °C before analysis. The haematological indices were determined by the method described by Shastry (1983). The content of serum biochemistry was determined on a Reflectron ®Plus 8C79 (Roche Diagnostic, GombH Mannheim, Germany), using kits.

Statistical analysis

All data obtained on growth performance, organ weight, haematological indices and serum biochemistry were subjected to a one-way analysis of variance (ANOVA) using SPSS version 20 for a Completely Randomised Design. The differences between treatment means were examined by Duncan's Multiple Range Test of the same package. For all statistical tests, significance was determined at $p < 0.05$.

RESULTS

Table 2 shows the body weight gain (BWG), feed intake (FI) and FCR of the broiler chickens fed diet supplemented with leaf meals of *C. crepidioides* (CCLM) and *C. ambrosioides* (CALM). No significant difference ($p > 0.05$) in BWG, FI and FCR of the broiler chickens was observed during the starter phase. However, at the finisher phase, broiler chickens fed the diets supplemented with CCLM, CALM and Vitamin C had higher BWG and lower FCR compared to the Control group. At overall period (1-42d), the result did not show a significant difference ($p > 0.05$) in BWG and FI across all the treatments but broiler chickens receiving CCLM, CALM and Vitamin C supplemented diets had lower ($p < 0.05$) FCR compared to the Control group.

Table 3 shows the result of dressing percentage (%) and relative internal organ weights of broiler chickens fed the diets supplemented with CCLM and CALM. The result revealed that only the relative weights of the liver and gizzard of the broiler chickens were significantly ($p < 0.05$) influenced by the dietary treatments. The relative weights of the liver of birds fed the control diet (2.36%) were significantly higher ($p < 0.05$) than those fed diets supplemented with CCLM (2.25%), CALM (2.13%) and Vitamin C (2.24%). The relative weights of the gizzard of birds fed CCLM (2.41%) and CALM (2.29%) supplemented diets were statistically similar but significantly ($p < 0.05$) higher than those fed the control (2.12%) and Vitamin C supplemented diets (1.19%).

Table 2. Growth performance of broiler chickens fed diets supplemented with *Crassocephalum crepidioides* and *Chenopodium ambrosioides* leaf meals

Parameters	Diet 1 Control	Diet 2 0.20g Vit C/kg	Diet 3 0.25g CCLM/kg	Diet 4 0.25g CALM/kg	SEM	*P value*
Starter phase (1-21days)						
IBW (g)	40.48	40.23	40.48	40.63	0.69	0.66
BWG (g)	792.38	822.62	841.89	831.13	25.09	0.58
FI (g)	1174.87	1158.75	1146.01	1156.28	16.42	0.56
FCR	1.48	1.41	1.36	1.39	0.08	0.58
Finisher phase (21- 42d)						
BWG (g)	1921.09^{b}	2013.87^{a}	2035.79^{a}	2055.89^{a}	20.96	0.01
FI (g)	3481.96	3377.09	3332.63	3225.87	92.55	0.61
FCR	1.81^{a}	1.67^{ab}	1.63^{ab}	1.57^{b}	0.04	0.05
Overall period (0 - 42days)						
BWG (g)	2713.46	2836.49	2877.68	2887.03	40.52	0.07
FI (g)	4656.83	4535.84	4478.65	4382.16	96.02	0.56
FCR	1.72^{a}	1.60^{ab}	1.56^{ab}	1.51^{b}	0.04	0.04

IBW: initial body weight; BWG: body weight gain; FI: feed intake; FCR: feed conversion ratio; Means within a row with different letters and significantly different ($p < 0.05$). SEM = Standard error. CCLM = *Crassocephalum crepidioides* leaf meal, CALM = *Chenopodium ambrosioides* leaf meal.

The results of the haematological indices of broiler chickens fed the supplemented diets are presented in Table 4. Broiler chickens fed diets supplemented with CCLM, CALM, and Vitamin C had higher packed cell volume (PCV), red blood cells (RBC), haemoglobin concentration (HC) and white blood cells (WBC) ($p < 0.05$) compared to Control, while the mean cell haemoglobin concentration and lymphocyte values of the broiler chickens were not significantly influenced ($p > 0.05$) by CCLM and CALM

Table 3. Dressing percentage and relative internal organ weights (% body weight) of broiler chickens fed diets supplemented with *Crassocephalum crepidioides and Chenopodium ambrosioides* leaf meals

Parameters	Diet 1 Control	Diet 2 0.20g Vit C/kg	Diet 3 0.25g CCLM/kg	Diet 4 0.25g CALM/kg	SEM	*P value*
Dressing (%)	73.72	73.50	74.72	74.72	0.73	0.70
Heart (%)	0.46	0.43	0.43	0.41	0.02	0.73
Liver (%)	2.36^{a}	2.24^{ab}	2.25^{ab}	2.13^{b}	0.03	0.04
Pancreas (%)	0.23	0.21	0.24	0.22	0.02	0.78
Gizzard (%)	2.12^{ab}	1.91^{b}	2.41^{a}	2.29^{a}	0.10	0.03
Lung (%)	0.51	0.59	0.67	0.64	0.05	0.29
Spleen (%)	0.18	0.17	0.15	0.13	0.01	0.50

Means within a row with different letters and significantly different ($p < 0.05$). SEM Standard error. CCLM = *Crassocephalum crepidioides* leaf meal, CALM = *Chenopodium ambrosioides* leaf meal. % = percentage

Table 4. Haematology of broiler chickens fed diets supplemented with *Crassocephalum crepidioides* and *Chenopodium ambrosioides* leaf meals

Parameters	Diet 1 Control	Diet 2 0.20g Vit C/kg	Diet 3 0.25g CCLM/kg	Diet 4 0.25g CALM/kg	SEM	*P value*
PCV (%)	28.50^{b}	31.50^{ab}	34.00^{a}	32.50^{a}	0.80	0.01
RBC ($x10^{12}$/l)	2.40^{b}	3.05^{a}	2.65^{b}	3.85^{b}	0.17	0.01
Haemoglobin (g/dl)	9.50^{b}	10.50^{ab}	11.33^{a}	11.50^{a}	0.27	0.01
MCHC (g/dl)	33.29	33.28	33.22	33.27	0.05	0.83
MCH (pg)	39.49^{ab}	34.43^{bc}	42.76^{a}	31.38^{c}	1.40	0.01
WBC ($x10^{9}$/l)	6.00	7.80	6.00	6.80	0.60	0.22

Means within a row with different letters and significantly different ($p < 0.05$).
SEM = Standard error. CCLM = *Crassocephalum crepidioides* leaf meal,
CALM = *Chenopodium ambrosioides* leaf meal.PCV= Packed cell volume,
RBC= Red blood cells, MCHC= Mean cell haemoglobin concentration,
MCV = Mean cell volume, MCH = Mean cell haemoglobin, WBC= White blood cells.

Table 5: Serum metabolites of broiler chickens fed diets supplemented with *Crassocephalum crepidioides* and *Chenopodium ambrosioides* leaf meals

Parameters	Diet 1 Control	Diet 2 0.20g Vit C/kg	Diet 3 0.25g CCLM/kg	Diet 4 0.25g CALM/kg	SEM	*P value*
AST (U/L)	89.76	84.60	82.80	85.30	2.81	0.50
ALT (U/L)	47.18^{a}	46.30^{a}	37.95^{b}	37.90^{b}	1.45	0.01
Cholesterol (mmol/l)	5.01a	5.15a	3.60c	4.22^{b}	0.09	0.01
Creatinine (mmol/l)	43.98^{a}	38.10^{b}	31.85^{c}	39.40^{b}	0.66	0.01
Total Protein (g/l)	44.8	49.80	45.65	51.00	3.07	0.54
Albumin (g/l)	21.85^{b}	25.50^{a}	22.65^{b}	27.30^{a}	0.78	0.01
Globulin (g/l)	22.95	24.30	23.00	23.70	2.48	0.97
Glucose	17.65	17.10	17.25	16.85	0.35	0.80

Means within a row with different letters and significantly different ($p < 0.05$).
SEM = Standard error. CCLM = *Crassocephalum crepidioides* leaf meal,
CALM = *Chenopodium ambrosioides* leaf meal, AST = Aspartate aminotransferase,
ALT= Alanine aminotransferase

supplementation compared to Control group.

The results of serum metabolites of broiler chickens fed the supplemented diets are presented in Table 5. Supplementation of CCLM, CALM and Vitamin C significantly lowered ($p < 0.05$) the alanine aminotransferase, cholesterol and creatinine contents compared to the Control group, while the values of total protein, albumin and glucose were not significantly influenced ($p > 0.05$) by CCLM, CALM and Vitamin C supplementation compared to Control group.

DISCUSSION

The dietary supplementation of plant materials as alternative non-antibiotic growth promoters in poultry production has increased substantially because of increasing consumers' preference for products that are healthy and void of antimicrobial residues (Falowo *et al*., 2014). The results from this study showed that dietary supplementation of CCLM and CALM significantly enhanced the body weight gain (BWG) of the broiler chickens at the finisher phase compared to the control group during the feeding experiment. The ability of CCLM and CALM to increase the BWG of the birds could be attributed to the presence of bioactive and phytochemical compounds inherent in both plants (Falowo *et al*., 2023). Many studies have reported that plant meal high in phytochemicals can increase feed intake and enhance nutrient digestion and absorption to promote BWG of broiler chickens (Mohiti-Asli and Ghanaatparast-Rashti 2017; Oloruntola *et al.,* 2018). This result is in contrast with the findings of Adeyemi *et al*. (2021) and Manyelo *et al*. (2022) that broiler chicken fed diets supplemented with *C. crepidioides* and amaranth leaf meals, respectively, had no significant influence of BWG compared to Control group. Conversely, at the finisher and overall phases, broiler chickens fed diets containing CCLM and CALM had better FCR than the Control group. This result indicated that CCLM and CALM had the capacity to improve the efficiency of feed utilization during production. The efficient utilization of the feed could also be the reason why broiler chickens fed diets containing CCLM and CALM had the highest body weight gain compared to the Control group. This is similar to the findings of Egu (2019) and Akintomide *et al*. (2021) that broiler chickens fed diets supplemented with graded levels of Moringa leaf meal and sweet potato leaf meal, respectively, had better feed conversion ratio compared to the Control group. As expected, supplementation with Vitamin C in this study significantly increased the BWG and improve FCR compared to the Control group. Vitamin C is an antioxidant which is commonly used as a therapeutic feed additive to improve animal health, boost immunity and growth performance of broiler chickens under heat stress environments (Hieu *et al*., 2022).

Furthermore, the result of the carcass trait and organ weights of the broiler chickens showed no significant difference in dressing percentage, relative weight of heart, pancreas, lung and spleen across experimental treatments, while supplementation of CCLM and CALM significantly lowered the relative weight of liver and increased the relative weight of gizzard compared to the Control group. This finding showed that the bioactive compounds inherent in CCLM and CALM produce a variable effect on the organs of the gastrointestinal tract of broiler chickens. This finding is similar to the report of Oloruntola *et al*. (2018) that supplementation with Neem, pawpaw and bamboo leaf meals produced different effects on the relative weights of the internal organs of broiler chickens compared to the Control group.

The reduction in the relative weights of liver broiler chickens fed CCLM and CALM compared to the Control group could mean that the bioactive compounds inherent in both leaf meals possessed hepatoprotective and anti-inflammatory properties, as reported by Oloruntola *et al.* (2018). Extracts of CCLM and CALM have been reported to exhibit anti-inflammatory and hepatoprotective actions in biological systems (Aniya *et al*., 2002; Ibironke and Ajiboye, 2007). Also, the observed increase in relative heart weights of birds fed diets supplemented with CCLM and CALM in this study could be due to the activity of the organs to efficiently digest the nutrients of the diets thereby leading to better growth and development of the BWG (Table 2). Gizzard is an important organ in chicken that aids in digestion by reducing particle size, regulating feed flow and degrading chemical nutrients (Svihus, 2011).

The result of the haematological indices of the broiler chickens also revealed that supplementation with CCLM and CALM significantly increased packed cell volume, red blood cells, and haemoglobin concentration compared to those fed the Control diet. The mean cell volume and granulocytes were significantly higher in broiler chickens fed diets supplemented with CCLM and CALM, respectively, compared to the Control group. The PVC, which is the proportion of blood occupied by red blood cells, is important for diagnosing anaemia while the red blood cells are responsible for the transportation of oxygen and carbon dioxide in the blood as well as the manufacture of haemoglobin (Nouala *et al*., 2006; Elbashier and Ahmed, 2013). Therefore, the observed higher values of PCV, RBC and Hb in this study indicated a greater potential for their functions and a better state of health for the broiler chickens.

The result of this study indicated that both CCLM and CALM possessed the potential to boost the health status of broiler chickens because of their inherent bioactive compounds and nutritional content (protein and minerals) (Falowo *et al*., 2023). Earlier studies have shown that dietary utilization of leaf meals that contain rich chemical nutrients and bioactive compounds such as carotenoids, flavonoids, chlorophyll, phenolics, xanthins, cytokines, alkaloids, etc. could improve the health status of broiler chickens during production (Mahfuz and Piao, 2019; Omatsuli *et al*., 2021). The result of this study is in line with the finding of Elbashier and Ahmed (2013) that dietary inclusion of *Moringa olefera* significantly increased red blood cell (RBC), packed cell volume (PCV), mean cell volume, and haemoglobin (HB) values of broiler chickens compared to the Control group.

The results of the serum metabolites of the broiler chickens showed that alanine aminotransferase (ALT), cholesterol and creatinine contents were significantly lower compared to the Control group. Alanine aminotransferase is an important enzyme commonly used to identify the presence of liver cell inflammation or damage (Yang *et al*., 2008; Oloruntola *et al*., 2018). The observed decrease in concentration of ALT in broiler chickens fed diets supplemented with CCLM and CALM in this study confirmed that these leaf meals had hepato-protective properties. This result is in agreement with the report of Oloruntola *et al*. (2018) who found that broiler chicken fed diets supplemented with Neem, pawpaw and bamboo leaf meals had lower ALT content compared to those fed Control diets. Similarly, the observed reduction in the cholesterol level of broiler chickens fed diets supplemented with CCLM and CALM in comparison with the Control group, suggested that these leaf meals possessed hypocholesterolemic properties, which could be due to their ability to delay intestinal absorption of dietary fat by inhibiting pancreatic lipase activity in diets (Adu *et al*., 2020; Falowo, 2022). Also, the reduced creatinine level of broiler chicken fed supplemented diets could be suggesting that CCLM and CALM possess the properties to confer and maintain the integrity of the kidney and their normal functioning during production. High serum cholesterol and creatinine production has been implicated in causing cardiovascular disease and severe kidney dysfunction/damage, respectively, in the body (Naseem *et al*., 2018; Oloruntola *et al*., 2018; Adu *et al*., 2020). This study agreed with the finding of Oloruntola *et al.* (2018) that broiler chickens fed diets supplemented with Neem, pawpaw and bamboo leaf meals had lower cholesterol and creatinine content compared to those fed the Control diet. In addition, the observed similarity in the values of AST, total protein, albumin and globulin further confirmed the potential of CCLM and CALM to maintain liver function and health status of broiler chickens during production.

CONCLUSION

This study revealed that *C. crepidioides* and *C. ambrosiodes* leaf meals could be used as natural feed additives at 0.25% to improve the body weight gain of broiler chickens during production. It also revealed that the inclusion of CCLM and CALM at 0.25% will help reduce the cholesterol content and improve the health status of broiler chicken during production. Further study should be conducted to ascertain the efficacy of these leaf meals as an alternative to antibiotic growth performance on other species of livestock.

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