

TECTONA GRANDIS LEAFMEAL IN BROILER CHICKENS DIET: EFFECT ON PERFORMANCE SERUM METABOLITES ANTIOXIDANT STATUS, CARCASS AND INTERNAL ORGANS AND SENSORY EVALUATION

ABSTRACT

This study assessed the effects of varying levels of *Tectonagrandis* leafmeal (TGLM) dietary supplementation on the performance of broiler chickens. A basal diet divided into four portions designated diet 1 (the control) and diets 2, 3 and 4 supplemented with 0.2, 0.4 and 0.6%, respectively. One hundred and forty four broiler chicks were randomly assigned to the four experimental diets (36 birds per diet; 9 birds per replicate) using a Completely Randomized Design. At the finisher phase, the body weight gain and feed conversion ratio of the birds fed 0.2 and 0.6% TGLM supplemented diets were was better ($P<0.05$) than those fed control diets and 0.4% TGLM supplemented diets. Serum cholesterol concentration was significantly ($P<0.05$) lower in the birds fed 0.4 and 0.6% TGLM supplemented diets compared to control diet.

The catalase concentration in the birds fed 0.4 and 0.6% TGLM supplemented diets were ($P<0.05$) higher than those birds fed control diet and 0.2% TGLM supplemented diets while the glutathione peroxidase concentration in the birds fed 0.4% TGLM supplemented diets was higher ($P<0.05$) than those fed control diet, 0.2 and 0.6% TGLM supplemented diet. The lipid peroxidation in the birds fed 0.6% TGLM supplemented diets was ($P<0.05$) significantly lower compared to the control diet, 0.2% and 0.4% TGLM supplemented diets. The live-weight of the birds fed 0.4 and 0.6% TGLM supplemented diet were was higher ($P<0.05$) than those fed control diet and 0.2% TGLM supplemented diets. The colour score of birds fed 0.4% TGLM supplemented diets was higher ($P<0.05$) than those fed other diets. The overall acceptability score thigh meat of birds fed 0.4% TGLM supplemented diet was significantly ($P<0.05$) higher compared to other experimental diets. The TGLM supplementation in this study has phytochemicals of health benefits and possess antioxidant properties.

INTRODUCTION

Poultry nutritionists have been interested in the use of phytochemical additives in chicken diets for a number of years now. The motivation for this interest is to improve health and performance while also perhaps lessening the negative effects of oxidative stress (1). This phenomena phenomenon could be explained by consumers' increased understanding of the consequences of using antibiotics, including the usage of novel chemical compounds in animal husbandry, as well as their increased awareness of the choices they make when it comes to their nutrition (2). Oloruntola et al. (3) have highlighted that poultry experts are forced to investigate alternative options, such as natural products, that can enhance the overall performance of poultry birds, in addition to the European Union's prohibition of synthetic antibiotics, as stated in the Official Journal of the European Union (4). One possible substitute for artificial antibiotic growth promoters could be the phytochemicals found in phytochemical supplements. Additionally, these

Comment [Vy1]: Make abstract comprehensive and add KEYWORDS (at least 5)

phytochemicals play important biological roles within cells, which boost the growth of chickens overall (5). The test components are commonly found in places like India, Nigeria, and Thailand. *Tectonagrandis* is a tree species that grows well in warm, humid settings (16). According to Tapsell et al. (7), *Tectonagrandis* leafmeal (TGLM) is a good substitute treatment for poultry birds since it has bioactive ingredients. Supplementing with TGLM has been shown to have medical benefits in multiple trials, suggesting that it can improve general health and treat a variety of illnesses (8).

One of the best sources of polyphenols, a group of substances frequently present in plants with a variety of antioxidant qualities, is TGLM (9). Research has shown that polyphenolic compounds exhibit a range of antioxidant and anticancer properties and may offer health advantages with regard to coronary heart disease. The main ways that phytochemical additions are expected to benefit humans and birds are: by improving their growth and well-being, strengthening their immune systems and triggering advantageous antibacterial activities (10). Several authors have reported that the use of phytochemical feed additives has been found to enhance the overall efficiency and physiological functioning of broiler chickens. The objective of this research was to examine the impact of varying levels of TGLM supplementation on the growth performance, serum metabolites, antioxidant activities, carcass and internal organs, and sensory evaluation of broiler chickens.

MATERIALS AND METHODS

Experimental site

The research was conducted within the poultry section of the Teaching and Research Farm in the department of Agricultural Technology, Federal Polytechnic, Ado, Ekiti State. Ekiti State is situated in the western region of the country and has a land area of 6353 square kilometres. As of 2005, the estimated population of Ekiti State was 2,737,186. The region experiences a tropical environment characterized by two distinct seasons. The two distinct seasons in this region are the rainy season, which spans from April to October, and the dry season, which occurs from November to March. Ado-Ekiti experiences a temperature range of 21°C to 28°C accompanied by high levels of humidity. The region is influenced by the southwestern wind during the rainy season and the north-east trade wind during the dry season.

Experimental Management and Design

One hundred and forty four Cobb 500 breed, were used for this study. The initial body weight of these birds was 42.67 ± 0.22 . Four different basal diets were randomly assigned to them. Each treatment group comprised thirty-six birds, they were replicated four times. In this study, the Completely Randomised Design (CRD) was used. The wire-slatted floor system was employed to house the birds, each replicate having its own designated area. At the start of the experiment, the research pen's initial temperature was maintained within a range of 31 ± 20 °C. The pen's temperature was then gradually lowered by 20 °C per week until it finally reached a final temperature of 26 ± 20 °C. For the first phase (0–4 weeks) and the second phase (4–8 weeks), a feed was compounded to meet the requirements of the birds as recommended by the National Research Council (11). The basal diet was divided into four equal portions at the beginning of

each phase and allocated to treatments 1 to 4. Treatment 1 served as the control group while treatments 2, 3 and 4 were supplemented with TGLM at varying levels of 0.2%, 0.4% and 0.6%, respectively. The birds were fed *ad-libitum* throughout the study.

Table 1 Composition of Experimental Diets (g/100kg) for Broiler Starter

Ingredients	T1	T2	T3	T4
Maize	50.00	50.00	50.00	50.00
Soybean meal (SBM)	23.00	23.00	23.00	23.00
Groundnut cake (GNS)	17.00	17.00	17.00	17.00
Fish meal	2.00	2.00	2.00	2.00
Bone meal	4.00	4.00	4.00	4.00
Limestone	2.00	2.00	2.00	2.00
Premix	0.25	0.25	0.25	0.25
Methionine	0.25	0.25	0.25	0.25
Lysine	0.25	0.25	0.25	0.25
Salt	0.25	0.25	0.25	0.25
Oil	1.00	1.00	1.00	1.00
Total	100	100	100	100
Calculated composition				
Metabolizable energy	2938	2938	2938	2938
Crude protein	24.68	24.68	24.68	24.68
Average calcium	2.35	2.35	2.35	2.35
Average phosphorous	0.84	0.84	0.84	0.84
Lysine	1.33	1.33	1.33	1.33
Methionine	0.60	0.60	0.60	0.60

Table 2 Composition of Experimental Diets (g/100kg) for broiler finisher diets

Ingredients	T1	T2	T3	T4
Maize	58.00	58.00	58.00	58.00
Soybean meal (SBM)	17.00	17.00	17.00	17.00
Groundnut Cake (GNC)	16.00	16.00	16.00	16.00
Fish meal	2.00	2.00	2.00	2.00
Bone meal	3.00	3.00	3.00	3.00
Limestone	2.00	2.00	2.00	2.00
Premix	0.25	0.25	0.25	0.25
Methionine	0.25	0.25	0.25	0.25
Lysine	0.25	0.25	0.25	0.25
Salt	0.25	0.25	0.25	0.25
Oil	1.00	1.00	1.00.00	1.00
Total	100	100	100	100
Calculated composition				
Crude protein	19.50	19.50	19.50	19.50
Metabolizable Energy	3031	3031	3031	3031

Calcium	1.99	1.99	1.99	1.99
Average phosphorous	0.65	0.65	0.65	0.65
Lysine	1.27	1.27	1.27	1.27
Methionine	0.58	0.58	0.58	0.58

Data Collection

The growth performance, body weight, and feed intake of the experimental birds were weighed weekly. The calculation of the average body weight gain (BWG) involved determining the differences between the initial weights and final live weights of the birds in the experimental group. The feed conversion ratio was calculated by dividing the feed intake by the weight gain.

Collection of blood samples

On the 56th day of the studies, a random selection was made of four birds from each treatment group. The birds were appropriately identified, weighed, and euthanized using the method of severing the jugular veins located in the cervical area. Blood samples were collected and stored in a plain bottle to conduct serum biochemical analysis. The specific parameters to be measured include creatinine, cholesterol, aspartate amino transaminase, alanine amino transaminase, high-density lipoprotein, low-density lipoprotein, as well as antioxidant enzyme levels such as catalase, superoxide dismutase, glutathione peroxidase, and lipid peroxidation. Each blood sample included in the plain bottles was subjected to centrifugation, causing the separation of serum, which was subsequently transferred into another plain bottle. The serum was then subjected to deep freezing at a temperature of $-200\text{ }^{\circ}\text{C}$ in preparation for analysis. The serum catalase, superoxide dismutase and glutathione peroxidase was conducted using the methods outlined by Aebi (12), Misra and Fridovich (13), and Rotruck et al. (14), respectively.

A random selection of four birds from each treatment group was made to conduct carcass and internal organ analysis. Before the slaughtering process, the birds were weighed. The chickens undergone the slaughtering process by cutting the jugular vein. Subsequently, the carcasses were immersed in warm water for 30 seconds to facilitate scaling before the de-feathering procedure. Before the organs were dissected, the dressed chickens were eviscerated. The liver, spleen, kidney, gall bladder, proventriculus, gizzard, lung, heart, and intestine were weighed. The internal organs were expressed as a proportion of the live weight.

Samples of meat were obtained by cutting from the carcasses of birds that were selected randomly for sensory evaluation. The thigh portion of the birds were selected as the source of the samples. These samples were deboned and carefully weighed to ensure uniformity. Thereafter, they were exposed to an oven drying process at a temperature of 80°C for 20 minutes. A total of fifteen pre-trained taste panelists were established to conduct sensory evaluation.

Result

Statistical Analysis: Using SPSS software, a one-way analysis of variance was performed on the study's data. Duncan's Multiple Range Test was used to compare the means (DMRT).

Table 3 shows the growth performance of broiler chickens when subjected to varying levels of TGLM supplementation in the diet. The birds fed control diet exhibited considerably higher ($P < 0.05$) total feed intake and feed intake at 4942.97g and 88.29g, respectively compared to the birds fed TGLM supplementation. The total body weight and weight gain of birds on both the control diet and the 0.2% supplementation were similar although notably lower ($P < 0.05$) when compared to the birds fed 0.6% TGLM supplementation. Statistically significant effects ($P < 0.05$) were recorded concerning the feed conversion ratio (FCR). Birds fed with diet containing 0.6% TGLM supplementation exhibited the lowest feed conversion ratio (FCR) at 1.59 whereas the greatest FCR was recorded in birds fed with the control diet.

Table 3. Effect of varying levels of *Tectonagrandis* on growth performance of broiler chickens

Parameters	<i>Tectonagrandis</i> inclusion (%)				SEM	<i>p</i> -value
	T1	T2	T3	T4		
	0	0.2	0.4	0.6%		
Initial liveweight (g)	42.85	42.33	43.79	44.51	0.22	0.38
Final liveweight	2980.41 ^b	2935.01 ^c	2879.68 ^d	3025.94 ^a	16.34	0.02
Total feed intake (g)	4942.97 ^a	4762.18 ^c	4590.99 ^d	4832.54 ^b	38.54	0.01
Feed intake (g/bird)	88.29 ^a	85.08 ^c	81.73 ^d	86.30 ^b	0.72	0.02
Total bodyweight (g)	2930.42 ^b	2931.16 ^b	2817.19 ^c	3000.87 ^a	19.87	0.01
Body weight gain (g)	52.33 ^b	52.35 ^b	50.31 ^c	53.64 ^a	0.35	0.01
FCR	1.69 ^a	1.61 ^{bc}	1.62 ^b	1.59 ^c	0.01	0.02

a,b,c,; means in the same row with different superscript are significantly different ($p < 0.05$) FCR: feed conversion ratio

The effect of TGLM supplementation on serum metabolites of broiler chickens is shown in Table 4. The levels of serum creatinine, aspartate aminotransferase, alanine aminotransferase, high-density lipoprotein, and low-density lipoprotein did not show significant changes ($P > 0.05$) due to the dietary treatment given. However, the serum cholesterol concentration was significantly lower ($P < 0.05$) in birds fed diet supplemented with 0.4% and 0.6% TGLM supplementation compared to those fed a control diet and 0.2% TGLM supplementation.

Table 4. Effect of varying levels of *Tectonagrandis* on serum metabolites of broiler chickens

Parameters	<i>Tectonagrandis</i> inclusion (%)				SEM	<i>p</i> -value
	T1	T2	T3	T4		
	0	0.2	0.4	0.6		
Creatinine (mg/dl)	42.67	41.75	42.45	40.87	0.92	0.43
Cholesterol (mmol/l)	2.59 ^a	1.46 ^a	1.30 ^b	1.17 ^b	0.02	0.02
AST(IU/L)	72.81	70.75	73.92	73.01	0.13	0.54
ALT(IU/L)	29.74	30.45	32.75	27.63	0.75	0.31
HDL	0.39	0.40	0.42	0.42	0.36	0.22
LDL	0.54	0.54	0.53	0.51	0.12	0.34

a,b, means in the same row with different superscript are significantly different ($p < 0.05$). AST: Aspartate aminotransferase; ALT: Alanine amino transferase; HDL: High density lipoprotein; LDL : Low density lipoprotein.

Table 5 presents the effect of TGLM supplementation on the levels of serum antioxidant enzymes in broiler chickens. The serum catalase concentrations in birds fed 0.4% and 0.6% TGLM supplementation were similar ($P > 0.05$) however, these concentrations were significantly higher ($P < 0.05$) than those observed in the birds fed the control diet and 0.2% TGLM supplementation. Birds on 0.4% TGLM supplementation was significantly ($P < 0.05$) higher than birds on other experimental diets. The highest serum glutathione peroxidase was recorded for birds on 0.4% TGLM supplementation while the least was recorded for birds on control diet. The values of lipid peroxidation in birds fed control diet and 0.2% TGLM leafmeal were similar ($P > 0.05$) however, these values were significantly greater ($P < 0.05$) than the values recorded for birds fed 0.4% and 0.6% TGLM leafmeal, which were also similar ($P < 0.05$).

Table 5. Effect of varying levels of *Tectonagrandis* on serum antioxidant enzymes of broiler chickens

Parameters	<i>Tectonagrandis</i> inclusion (%)				SEM	<i>p</i> -value
	T1	T2	T3	T4		
	0	0.2	0.4	0.6		
Catalase (mg/g)	0.31 ^c	0.54 ^b	0.92 ^a	0.84 ^a	0.02	0.03
SOD (%)	0.52	0.57	0.60	0.56	0.44	0.12
GPX (μ /mol)	29.25 ^c	35.27 ^{bc}	55.62 ^a	43.18 ^b	0.03	0.02
Lipid peroxidation	0.78 ^a	0.64 ^a	0.47 ^b	0.43 ^b	0.01	0.01

a,b,c means in the same row with different superscript are significantly different ($p < 0.05$). SOD: superoxidismutase; GPx: glutathionine peroxidase

Table 6 presents the effect of varying levels of TGLM supplementation on carcass and internal organs of broiler chickens. The liveweight of birds fed 0.4% and 0.6% TGLM supplementation were similar ($P > 0.05$) but significantly different from birds on control diet and 0.2% TGLM supplementation. The TGLM did not have any influence on the slaughtered weight, eviscerated weight and the internal organs.

Table 6. Effect of varying levels of *Tectonagrandis* on carcass and internal organs of broiler chickens

Parameters	<i>Tectonagrandis</i> inclusion (%)				SEM	<i>p</i> -value
	T1	T2	T3	T4		
	0	0.2	0.4	0.6		
Liveweight (g)	2896.10 ^b	2788.75 ^c	2949.81 ^a	2899.11 ^a	16.37	0.01
Slaughtered wt (g)	2673.57	2650.13	2676.75	2668.00	5.51	0.35
Dressed wt (g)	2358.00	2351.75	2360.75	2358.50	1.60	0.24
Eviscerated wt (g)	2063.50	2061.75	2068.00	2064.10	1.12	0.26
Liver (%)	1.84	1.85	1.85	1.84	0.06	0.16
Spleen (%)	0.11	0.11	0.11	0.10	0.02	0.12
Kidney (%)	0.50	0.50	0.49	0.51	0.01	0.10
Gall bladder (%)	0.14	0.15	0.15	0.14	0.01	0.10
Proventriculus (%)	0.47	0.47	0.48	0.47	0.02	0.25
Gizzard (%)	1.82	1.81	1.81	1.80	0.02	0.29
Lungs (%)	0.53	0.53	0.51	0.53	0.01	0.35

Heart (%)	0.52	0.51	0.53	0.53	0.01	0.33
Intestine (%)	3.75	3.79	3.75	3.76	0.07	0.15

a,b,c means in the same row with different superscript are significantly different ($p < 0.05$). wt : weight

Table 7 presents the impact of using *Tectonagrandis* leaf meal supplements on the sensory evaluation thigh meat of broiler chicken. The colour score exhibited statistically significant difference ($P < 0.05$) across the birds on the experimental diet. The overall acceptability score of thigh meat of broiler chicken fed control diet and 0.2% *Tectonagrandis* were similar ($P > 0.05$) but significantly lower ($P < 0.05$) than birds on 0.4% *Tectonagrandis*. The flavour, taste and tenderness were not significantly ($P > 0.05$) affected by the test ingredients.

Table 7. Effect of varying levels of *Tectonagrandis* on sensory evaluation of broiler chickens

Parameters	<i>Tectonagrandis</i> inclusion (%)				SEM	<i>p</i> -value
	T1	T2	T3	T4		
	0	0.2	0.4	0.6		
Colour	5.47 ^b	4.28 ^c	6.25 ^a	5.80 ^b	0.32	0.04
Flavour	4.20	4.20	4.21	4.19	0.06	0.21
Taste	4.35	4.32	4.33	4.33	0.13	0.23
Tenderness	3.78	3.67	3.70	3.67	0.06	0.20
Overall acceptability	6.53 ^b	6.50 ^b	7.46 ^a	5.54 ^c	0.24	0.03

a,b,c means in the same row with different superscript are significantly different ($p < 0.05$).

Discussion

In this study, supplementation of diets with varying levels of TGLM in broiler chicken enhanced better performance and improve the health status, this could be as a result of secondary bioactive compounds like flavonoid, phenol present in TGLM.

The results of Allison et al. (15), who established the beneficial effects of herbs on chicken performance, are consistent with the observed significant increase in weight gain per bird and the noteworthy decrease in feed conversion ratio (FCR) among birds on diet 4. It was discovered that these herbs increased the feed conversion ratio, which significantly decreased the number of bacteria and oocysts. It has been demonstrated that TGLM supplementation contains bioactive substances that affect the health and productivity of chickens. Without causing any contact between the host and microorganisms, these chemicals mainly function by preserving the equilibrium of normal gut microbiota (16). They have also been observed to improve the functionality and increase the secretion of digestive enzymes (17).

Table 4 illustrates the effect of TGLM supplementation on the blood metabolites of broiler chickens. The dietary intervention did not significantly alter the levels of serum creatinine, low-density lipoprotein, aspartate aminotransferase, alanine aminotransferase, or high-density lipoprotein ($P > 0.05$). On the other hand, compared to birds fed a control diet and 0.2% TGLM supplementation, birds fed a food supplemented with 0.4% and 0.6% TGLM had considerably lower serum cholesterol concentrations ($P < 0.05$).

Given that the birds on the control diet and those given TGLM supplementation had similar levels of creatinine concentration, it appears that the supplements used in this study did not endanger the birds' ability to function as kidneys (18). As markers of cellular injury and inflammation, aspartate aminotransferase and alanine aminotransferase levels are widely used (3). Peter and Susan's (18) study shows that the herbal supplements studied in experimental birds efficiently inhibits the development of non-specific tissue injury, cardiac illness, and liver and biliary system disease. The birds' constant levels of aspartate and alanine aminotransferase concentrations during the course of the investigation provide evidence for this. A higher level of serum cholesterol causes more cholesterol to deposit on the artery walls, which leads to the development of plaques. According to Oloruntola et al. (3), these plaques aid in the arterial lumen's narrowing, which lowers the heart's blood flow rate. The investigation's findings about the reduction in serum cholesterol concentration in the birds fed diets enhanced with phytogetic substances may have positive health effects. Olkowski et al. (19) have established a correlation between the development of arteriosclerosis and sudden death syndrome in grill chickens and elevated serum cholesterol values. The TGLM leaf meal showed signs of hypocholesterolemia.

According to research by (20), enzymes such as catalase (CAT), superoxide dismutase (SOD), and glutathione peroxidase (GPx) provide protection against oxidative stress. The results of this study showed that the birds' levels of catalase and glutathione peroxidase activities were significantly higher when their diets were supplemented with Tectonagrandisleafmeal. This discovery offers additional proof of the phytogetics' antistress and antioxidant properties. The presence of polyphenolic compounds, such as flavonoids or phenolic acids, within the TGLM may be responsible for the observed increase in antioxidant activities among the avian subjects that were administered TGLM supplementation at concentrations of 0.2%, 0.4%, and 0.6% in this investigation (21). Plants have powerful antioxidant chemicals that can effectively neutralise free radicals or boost the activity of enzymes such as glutathione peroxidase, superoxide dismutase, and catalase. This was reported by Dhama et al. (22). The results of this investigation show that lipid peroxidation was decreased in broiler chickens when TGLM supplementation was added to their diet. This reduction in lipid peroxidation points to a boost in TGLM supplementation's antioxidant activity. As a result, broiler chickens' serum levels of antioxidant enzymes seem to be positively impacted by the addition of TGLM supplementation to their diet. Its beneficial antioxidant effect has been ascribed to the bioactive components found in TGLM supplementation, such as tectoquinone, lapachol, and deoxilapacol. Tectonagrandis contains a number of important metabolites and phenolic compounds with anti-oxidant properties.

The present study's results demonstrate that the live weight of birds that were fed diets supplemented with 0.4% and 0.6% TGLM supplementation is in line with earlier studies that have demonstrated the ability of phytogetic supplements to increase both live weight and dressed weight in broiler chicken diets (23) (1). Based on this conclusion, the phytogetic supplements used in this study have bioactive components that can control animal metabolism similarly to a chemical that acts as a β -adrenergic agonist (1). The study's conclusions show that the internal organs of these animals responded to phytogetic supplementation in a manner that was comparable. This indicates that the supplementation of TGLM might have an effect on the internal organs of the bird operating at optimal efficiency.

The higher score for the thigh meat of grill chicken fed 0.4% on overall acceptability may have resulted from the overall contribution of other eating factors, such as colour, which in turn contributed to the meat's relatively high flavour, juiciness, and texture as well as its relatively high tenderness, which may have been caused by the meat's high protein, water-holding capacity,

and moisture. Additionally, the colour and appearance of meat have a significant impact on its acceptability because a red, appealing colour actually beckons people to buy the meat, while a dull, unattractive tint makes them decide not to (24)

CONCLUSION

The phytochemicals in TGLM supplements are antioxidants and have positive health effects. Broiler's performance: live-weight, body weight gain, and feed conversion ratio during the finisher phase were all improved by a 0.6% TGLM nutritional supplement. Improved immunomodulatory and hypocholesterolemic effects were also observed in the broiler chickens supplemented with phytochemicals. The broiler chicken's cholesterol level decreased, its glutathione concentration increased, and its lipid peroxidation decreased when varying levels of TGLM were supplemented.

REFERENCES

- ~~Valenzuela Grijalva NV, Pinelli Saavedra A, Muhlia-Almazan A, Dominguez Diaz D, Gonzalez Rios H. Dietary inclusion effects of phytochemicals as growth promoters in animal production. Journal of Animal Science and Technology. 2017;59:8. Doi: <http://doi.org/10.1186/s40781-071-0133-9>.~~
~~Gonzalez RM, Angeles, HJC. Antibiotic and synthetic growth promoters in animal diets: review of impact and analytical methods. Food Control. 2017; 72: 255-267.~~
~~Oloruntola, OD, Agbade JO, Ayodele SO and Oloruntola DA. Neem, pawpaw and bamboo leaf meal dietary supplementation in broiler chickens: Effect on performance and health status. Journal of Food Biochemistry. 2018; e12723. <https://doi.org/10.1111/jfbc.12723>.~~
~~OJEU. Regulation (EC) No.1831/2003 of European parliament and the council of 22 September 2003 on additives for use in animal nutrition. Official Journal of the European Union, 2003. Page L268/36 in OJEU. Brussels: OJEU.~~
~~Gonzalez Rios H, Davila Ramirez JL, Pena Ramos EA, Valenzuela Melendres M, Zamorano Garcia L, Islava Lagarda TY, Valenzuela Grijalva NV. Dietary supplementation of ferulic acid to steers under commercial feedlot feeding conditions improves meat quality and shelf life. Animal Feed Science and Technology. 2016; 222: 111-121.~~
~~Mahesh S, Vaishnav V, Kumar P, Mohammed N, Ansari SA. Characterization and validation of teak plus trees ramets of national teak germ plasm bank, Chandrapur, Maharashtra through microsatellites. Tropical Plant Research 2016;3(1):213-220.~~
~~Tapsell LC, Hemphill I, Coblae L. Health benefits of herbs and spices. 'The past, the~~

Formatted: Indent: Left: 0.38", No bullets or numbering

- present and future". Medical Journal of Australia 185 (4 suppl.). 2006; 24-54.
- Shruthi DP, Sunith, KE, Harith, Kumari E, Govindappa M, Siddalindeshwara, KG. Phytochemical screening, antioxidant and anti-inflammatory activity of different extracts from leaf, stem and bark of *Tectona grandis*. International Journal of Research in Pharmacology and pharmacotherapeutics. 2012;1(2)140-146.
- Gladine C, Morand C, Rock E, Bauchart D, Durand D. Plant extracts rich in polyphenols (PERP) are efficient antioxidants to prevent lipoperoxidation in plasma lipids from animals fed n-3 PUFA supplemented diets. Journal of Animal Feed Science and Technology. 2007; 136: 3-4, 281-296.
- Windisch W, Schedle K, Plitzner C, Kroismayr A. (2008). Use of phytogetic products as feed additives for swine and poultry. Journal of Animal Science. 2008; 86, 140-147.
- NRC. Nutrient requirements of poultry (9th Revised Ed.). 1994; Washington, DC: National Academy Press.
- Aebi H. Catalase estimation. In: Bergmeyer, H.V (ed.). Methods of enzymatic analysis. 1974, Verlag Chemical, New York Academic Press, New York.
- Misra HP, Fridovich I. The univalent reduction of oxygen by flavins and quinines. Journal of Biological Chemistry 1972; 247 (1), 188-192.
- Rotruck JT, Pope AL, Ganther HE, Hafeman DG, Hoekstra WG. Selenium: Biochemical role as a component of glutathione peroxidase. Science. 1973; 179, 588-590. <https://doi.org/10.1126/science.179.4073.588>.
- Allinson IB, Ekunseitan DA, Ayoola AA, Ogunade IM, Njoku CP. Effect of herbal supplement on growth response and faecal egg counts of cockerel. Online Journal Animal Feed Research. 2013; 3: 68-73.
- Tekeli, A, Celik L, Kutlu, HR, Gorgulu, M. Effect of dietary supplemental plant on performance, carcass characteristics, digestive system development, intestinal microflora and some blood parameters of broiler chicks. Proceedings of 12th European Poultry Conference, September 10-14, 2006, Verona, Italy. Pp.307-308.
- Lee KW, Everts H, Beynen AC. Essential oils in broiler nutrition. International Journal Poultry Science. 2004; 3: 738-752.
- Peter ML, Susan CEF. Interpretation of Laboratory results. Australian Veterinarian practitioner. 1999; 21(4); 188-193.
- Oloruntola OD, Agbede JO, Ayodele SO, Oloruntola DA. Neem, pawpaw and bamboo leafmeal dietary supplementation in broiler chickens: Effect on performance and health status. Journal of Food Biochemistry. 2018 e12723. <https://doi.org/10.1111/jfbc.12723>.
- Olkowski AA, Wojnarowicz C, Nain S., Ling, B., Alcorn, JM and Laarveld, B. A study on pathogenesis of sudden death syndrome in broiler chickens. Research in Veterinary Science. 2007; 85, 131-140.
- Young IS, Woodside JV. Antioxidants in health and disease. Journal of Clinical Pathology. 2001; 54 (Suppl 3): 176-186.
- Goyal AK, Brahma BK. Antioxidant and nutraceutical potential of bamboo: An overview. International Journal of Fundamental and Applied Sciences; 3: 2-10.

~~Dhama K, Latheef SK, Mani S, Samad HA, Karthik K, Tiwari R, Khan RU, Alagawany M, Farag MR, Alam GM, Laudadio V, Tufarelli V. Multiple beneficial applications and modes of action of herbs in poultry health and production—A review. International Journal of Pharmacology. 2015; 11:152-176.~~

~~Kanduri, A.B., Munde, V.K., Khan, M.A., Thakur, P.N., Saxena, M.J., Ravikanth, K., Thakur, A. and Maini, S. Study on the comparative efficacy of natural growth promoter (AV/AGP/10) with antibiotic supplements on overall growth performance and intestinal micrometry of broiler birds. British Microbiology Research Journal. 2013; 3, 623-634.~~

~~Apata ES. Quality attributes of Red Sokoto buck meat as influenced by post-slaughter processing methods. Ph.D. 2011; Thesis in the Department of Animal Science, University of Ibadan, Ibadan Oyo State, Nigeria.~~

1. Valenzuela-Grijalva NV, Pinelli- Saavedra A, Muhlia-Almazan A, Dominguez-Diaz D,
2. Gonzalez-Rios H. Dietary inclusion effects of phytochemicals as growth promoters in animal production. Journal of Animal Science and Technology. 2017;59:8.Doi: <http://doi.org/10.1186/s40781-071-0133-9>.
3. Gonzalez RM, Angeles, HJC. Antibiotic and synthetic growth promoters in animal diets :
4. review of impact and analytical methods. Food Control. 2017; 72: 255-267.
5. Oloruntola, OD, Agbede JO, Ayodele SO and Oloruntola DA .Neem, pawpaw and bamboo
6. leaf meal dietary supplementation in broiler chickens: Effect on performance and health status. Journal of Food Biochemistry. 2018, e12723. <https://doi.org/10.1111/jfbc.12723>.
7. OJEU. Regulation (EC) No.1831/2003 of European parliament and the council of 22
8. September 2003 on additives for use in animal nutrition. Official Journal of the European Union, 2003. Page L268/36 in OJEU. Brussels: OJEU.
 - a. Gonzalez-Rios H, Davila-Ramirez JL, Pena-Ramos EA, Valenzuela- Melendres M,
9. Zamorano-Garcia L, Islava-Lagarda TY Valenzuela-Grijalva NV. Dietary supplementation of ferulic acid to steers under commercial feedlot feeding conditions improves meat quality and shell life. Animal Feed Science and Technology.2016; 222: 111-121.
10. Mahesh S, Vaishnav V, Kumar P, Mohammed N, Ansari SA. Characterization and
11. validation of teak plus trees ramets of national teak germ plasm bank, Chandrapur,
12. Maharashtra through microsatellites. Tropical Plant Research 2016;3(1):213-220.
13. Tapsell LC, Hemphill I, Coblac L. Health benefits of herbs and spices. " The past, the
14. present and future". Medical Journal of Australia 185 (4 suppl.). 2006; 24-54.
15. Shruthi DP, Sunith, KE, Harith, Kumari E, Govindappa M, Siddalindeshwara, KG.
16. Phytochemical screening, antioxidant and anti inflammatory activity of different extracts from leaf, stem and bark of Tectonagrandis. International Journal of Research in Pharmacology and pharmacotherapeutics. 2012; 1(2)140-146.
17. Gladine C, Morand C, Rock E, Bauchart D, Durand D. Plant extracts rich in polyphenols
18. (PERP) are efficient antioxidants to prevent lipoperoxidation in plasma lipids
19. from animals fed n-3 PUFA supplemented diets. Journal of Animal Feed Science and Technology.2007; 136: 3-4, 281-296.
20. Windisch W, Schedle K, Pletzner C, Kroismayr A. (2008). Use of phytogenic
21. products as feed additives for swine and poultry. Journal of Animal Science.
22. 2008; 86, 140-147.

Formatted: List Paragraph, Indent: Left: 0", Numbered + Level: 1 + Numbering Style: 1, 2, 3, ... + Start at: 1 + Alignment: Left + Aligned at: 1.25" + Indent at: 1.5"

Formatted: Indent: Left: 0"

Formatted: List Paragraph, Indent: Left: 0", Numbered + Level: 1 + Numbering Style: 1, 2, 3, ... + Start at: 1 + Alignment: Left + Aligned at: 1.25" + Indent at: 1.5"

Formatted: Indent: Left: 0"

Formatted: List Paragraph, Indent: Left: 0", Numbered + Level: 1 + Numbering Style: 1, 2, 3, ... + Start at: 1 + Alignment: Left + Aligned at: 1.25" + Indent at: 1.5"

Formatted: Indent: Left: 0"

Formatted: List Paragraph, Indent: Left: 0", Numbered + Level: 1 + Numbering Style: 1, 2, 3, ... + Start at: 1 + Alignment: Left + Aligned at: 1.25" + Indent at: 1.5"

Formatted: Indent: Left: 0"

Formatted: List Paragraph, Indent: Left: 0.5", Numbered + Level: 2 + Numbering Style: a, b, c, ... + Start at: 1 + Alignment: Left + Aligned at: 1.75" + Indent at: 2"

Formatted: List Paragraph, Indent: Left: 0", Numbered + Level: 1 + Numbering Style: 1, 2, 3, ... + Start at: 1 + Alignment: Left + Aligned at: 1.25" + Indent at: 1.5"

23. [NRC. Nutrient requirements of poultry \(9th Revised Ed.\). 1994; Washington, DC: National Academy Press.](#)
24. [Aebi H. Catalase estimation. In: Bergmeyer, H.V \(ed.\). Methods of enzymatic i. analysis. 1974, Verlag Chemical, New York Academic Press, New York.](#)
26. [Misra HP, Fridovich I. The univalent reduction of oxygen by flavins and quinines. Journal of Biological Chemistry 1972; 247 \(1\), 188-192.](#)
27. [Rotruck JT, Pope AL, Ganther HE, Hafeman DG, Hoekstra WG. Selenium: Biochemical role as a component of glutathione peroxidase. Science. 1973; 179, 588-590. <https://doi.org/10.1126/science.179.4073.588>.](#)
30. [Allinson IB, Ekunseitan DA, Ayoola AA, Ogunade IM, NjokuCP. Effect of herbal supplement on growth response and faecal egg counts of cockerel. Online Journal Animal Feed Research. 2013; 3: 68-73.](#)
32. [Tekeli, A, Celik L, Kutlu, HR Gorgulu, M. Effect of dietary supplemental plant on performance, carcass characteristics, digestive system development, intestinal microflora and some blood parameters of broiler chicks. Proceedings of 12th European Poultry Conference, September 10-14, 2006, Verona, Italy. Pp.307-308.](#)
34. [Lee KW, Everts H, Beynen AC. Essential oils in broiler nutrition. International Journal Poultry Science. 2004 ; 3: 738-752.](#)
36. [Peter ML, Susan CEF. Interpretation of Laboratory results. Australian Veterinarian practitioner. 1999; 21\(4\); 188-193.](#)
38. [Oloruntola OD, Agbede JO, Ayodele SO, Oloruntola DA. Neem, pawpaw and bambooleafmeal dietary supplementation in broiler chickens: Effect on performance and health status. Journal of Food Biochemistry. 2018 e12723. <https://doi.org/10.1111/jfbc.12723>.](#)
40. [Olkowski AA, Wojnarowicz C, Nain S., Ling, B., Alcorn, JM and Laarveld, B. A study on pathogenesis of sudden death syndrome in broiler chickens. Research in Veterinary Science. 2007; 85, 131-140.](#)
42. [Young IS, Woodside JV. Antioxidants in health and disease. Journal of Clinical Pathology. 2001; 54 \(Suppl 3\): 176-186.](#)
44. [Goyal AK, Brahma BK. Antioxidant and nutraceutical potential of bamboo: An overview. International Journal of Fundamental and Applied Sciences; 3: 2-10.](#)
46. [Dhama K, Latheef SK, Mani S, Samad HA, Karthik K, Tiwari R, Khan RU,](#)
47. [Alagawany M, Faraq MR, Alam GM, Laudadio V, Tufarelli V. Multiple beneficial applications and modes of action of herbs in poultry health and production- A review. International Journal of Pharmacology. 2015; 11:152-176.](#)
48. [Kanduri, A.B., Munde, V.K., Khan, M.A., Thakur, P.N., Saxena, M.J., Ravikanth, K.,](#)
49. [Thakur, A. and Maini, S. Study on the comparative efficacy of natural growth promoter \(AV/AGP/10\) with antibiotic supplements on overall growth performance and intestinal micrometry of broiler birds. British Microbiology Research Journal. 2013; 3, 623-634.](#)
50. [Apata ES. Quality attributes of Red-Sokoto buck meat as influenced by post-slaughter processing methods. Ph..D .2011; Thesis in the Department of Animal Science, University of Ibadan, Ibadan Oyo-State, Nigeria.](#)

Formatted: List Paragraph, Indent: Left: 1.13", Numbered + Level: 3 + Numbering Style: i, ii, iii, ... + Start at: 1 + Alignment: Right + Aligned at: 2.38" + Indent at: 2.5"

Formatted: List Paragraph, Indent: Left: 0", Numbered + Level: 1 + Numbering Style: 1, 2, 3, ... + Start at: 1 + Alignment: Left + Aligned at: 1.25" + Indent at: 1.5"