Blood Characteristics of Broiler Chickens as Influenced by Dietary Inclusion of Neem (*Azadirachta indica*) Leaf Meal

Canadian Journal of Agriculture and Crops
Vol. 3, No. 2, 72-80, 2018
e-ISSN: 2518-6655

Ubua, J. A.[1]
Ozung, P.O.[2]
Inagu, P. G.[3]
Aboluja, B. A.[4]

[1,3,4]*Department of Animal Science, Taraba State University, Jalingo, Nigeria*
[2]*Department of Animal Science, University of Calabar, Calabar, Nigeria*

(*Corresponding Author*)

ABSTRACT

The blood characteristics of broiler chickens as influenced by dietary inclusion of Neem Leaf Meal (NLM) were investigated. The duration of feeding trial was eight weeks, with a total of 192 day - old broiler chicks. The chicks were randomly allotted to four (4) dietary treatments of 48 birds each and replicated three times with 16 birds per replicate in a Completely Randomized Design (CRD) experiment. The dietary treatments had inclusion levels of 0, 2.5, 5.0 and 7.5% NLM, respectively. A total of 36 blood samples (3 per replicate, i.e. 9 per treatment) were obtained with the aid of hypodermic needles and syringes via the prominent wing vein of the broilers at the 8th week. Blood for haematological assay was collected into EDTA bottles, while blood for serum biochemical determinations was collected into non – EDTA bottles. All blood parameters were assayed using standard laboratory procedures. Results were subjected to one – way ANOVA where haematological characteristics were not significantly ($P>0.05$) influenced by dietary treatments. The parameters ranged as follows: RBC (2.33 - 3.00 $\times 10^{12}$/L); WBC (6.90 - 8.90 $\times 10^{9}$/L), Hb (13.66 – 15.13 g/dl), MCHC (43.13 – 44.10%), MCH (58.67 – 61.67 pg), MCV (134.33 – 144.67 fl) and PCV (32.00 – 34.33%). In terms of serum biochemical indices only cholesterol content (3.77 – 6.03 mmol/L) was significantly ($P<0.05$) affected by dietary treatments, while albumin (4.31 – 5.58 g/dl), glucose(6.73 – 7.60 mmol/L), creatinine (44.40 – 47.50 µmol/L), total protein (6.57 – 8.62 g/dl), globulin (1.94 – 3.04 g/dl) and urea (1.77 – 2.33 mmol/L) showed no significant differences ($P>0.05$) between treatments. All haematological and serum biochemical parameters were within the normal ranges for apparently healthy broiler chickens. The study therefore concludes that including up to 7.5% neem leaf meal (NLM) in diets meant for broiler chickens will not adversely affect their blood characteristics.

Keywords: *Chickens, Haematology, Serum, Neem leaves.*

DOI: 10.20448/803.3.2.72.80
Citation | Ubua, J. A.; Ozung, P.O.; Inagu, P. G.; Aboluja, B. A. (2018). Blood Characteristics of Broiler Chickens as Influenced by Dietary Inclusion of Neem (Azadirachta indica) Leaf Meal. Canadian Journal of Agriculture and Crops, 3(2): 72-80.

Funding: This study received no specific financial support.
Competing Interests: The authors declare that they have no competing interests.
History: 18 September 2018/ Revised: 22 October 2018/ Accepted: 27 November 2018/ Published: 20 December 2018
Publisher: Online Science Publishing

1. INTRODUCTION

Natural leaf meals have been incorporated in the diets of poultry as a means of reducing the high cost of conventional protein sources as well as boosting growth and carcass characteristics [1-5]. Another way of reducing costs is by incorporating feed additives or growth promoters that will boost animal performance by increasing growth rate, better feed conversion efficiency, greater livability and lowered mortality in poultry birds [6]. Synthetic and semi-synthetic antibiotics as growth promoters are widely used in poultry production. Their use has generated some positive and negative effects over the years and this has directed research back to natural antimicrobial products. Kibria and Verna [7] reported that consumption of meat from birds that were fed with antibiotic growth promoters resulted in antibiotic residues entering the human body and thereby causing serious human health hazards. As a result of the negative outcome of antibiotics, there is considerable research interest in the possible use of natural products, such as essential oils, extracts of edible and medicinal plants, herbs and spices, for the development of new additives in animal feeding trials Mohammed, et al. [8]. Craig [9] stated that many herbs could help provide some protection against bacteria and stimulate the immune system and hence the overall growth and wellbeing of birds.

The neem tree (*Azadirachta indica*,) is native to South-East Asia especially India. It is a rapidly growing plant, drought resistant and is evergreen all year round. The neem tree belongs to the order Rutales; sub-order Rutinae, family Meliaceae, sub-family meloideae, tribe melicae, genus Azadirachta and species indica [10]. Various parts of the neem tree have been reported to have chemicals such as azadiractin, nimbin, nimbidin, quercetin, etc. [11-13] and these have antimicrobial, anthelminthic, antioxidant, antifungal, insecticidal, antiprotozoal and spermicidal activities [14, 15]. Neem is a tropical plant that is common in Nigeria and other parts of Africa. According to Elangovan, et al. [14] neem leaf yields querecin (flavonoid), nimbosterol (beta-sibosterol) as well as limonoids (nimbin and its derivatives). The main components of neem are terpenes and limonoids and the major active compounds of the limonoids are azadiractin, 3-deacetyl-3-annamoy azadirachtin, nimband, 3-tigloy-lizadirachtol, 3-acetyl-salanno and several others [16].

Neem leaf meal (NLM) can be incorporated in small quantities to animal diets so as to serve as natural growth promoter through its antimicrobial activity. The maximum tolerance level of NLM reported by Obikaonu, et al. [3] is 10% in starter broilers and Obun, et al. [4] recommended levels not exceeding 15% for optimal blood constituents in finishing broilers; however, Esonu, et al. [2] included up to 15% NLM in laying birds. Neem promotes growth because of its antibacterial and hepatoprotective properties [17]. Neem leaf meal has a proximate composition of 92.40% dry matter, 20.68% crude protein, 16.60% crude fibre, 4.13% ether extract, 7.10% ash and 43.91% Nitrogen Free Extract [18]. However, neem leaf meal has anti-nutritional factors which may affect nutrients utilization; hence its usage is drastically reduced in feeds to capture its beneficial effects with less adverse effects.

Neem diets fed to laying hens have been reported to significantly reduce the contents of haemoglobin, erythrocyte counts and packed cell volume in blood counts [19, 20]. Furthermore, neem has showed immune modulatory actions that induce cellular immune reaction [21]. Neem plays an important role in strengthening the immune system of the body. When neem is incorporated in poultry diets, it increases antibodies against Newcastle and Infectious Bursal Disease Viruses Durrani, et al. [22]. Bonsu, et al. [15] showed that the haematological indices of broilers fed neem leaf meal at different inclusion levels were not significantly affected. Serum protein, albumin and globulin increased with inclusion levels of groundnut cake with neem leaf suggesting that diets compounded with these ingredients were not nutritionally inferior to soybean control diet. However, the serum glucose was lower whilst the cholesterol level increased with increasing level of these ingredients [23] Considering

the unconventional nature of NLM, it is therefore further expedient to assay for the effect of this feeding material on the blood characteristics of animals. Haematological constituents reflect the physiological responsiveness of animals [24] and the influence of diet on haematological parameters can be very strong [25].

Extant studies have not shown satisfactory effects of neem compounds/extracts on the blood forming processes in animals. Hence, this study was designed to determine the influence of dietary neem inclusion on the blood characteristics (haematology and serum biochemistry) of broiler chickens.

2. MATERIALS AND METHODS

2.1. Location of the Study

The study was conducted at the Poultry Unit of the Teaching and Research Farm, Taraba State University, Jalingo, Ardo-Kola Local Government Area, Taraba State. It is located within the Guinea Savannah Zone. Jalingo lies between latitude 8°50′ North and longitude 11°31′ East of the equator [26]. The state is characterized by tropical climate marked by dry and rainy seasons. The rainy season usually commences in the month of March and ends in October. The dry season then starts in late October and ends in March. The annual rainfall is between 1000 and 1500 mm with an average minimum temperature of 30^{0}C and maximum temperature of 38^{0}C depending on the season [26].

2.2. Source And Processing Of Neem Leaf Meal (NLM)

Fresh neem leaves were harvested from neem trees within the premises of the University. The harvested leaves were air dried for 7 – 8 days until they become crispy retaining their greenish colouration. The dried neem leaves were milled using a hammer mill to 2mm sieve particle size to enable birds pick feed easily while feeding.

2.3. Experimental Birds, Design and Management

One hundred and ninety two (192) unsexed day - old broiler chicks (Arbor Acre strain) were purchased from a reputable hatchery in Ibadan, Oyo State – Nigeria. The birds were randomly allotted to four dietary treatments with forty eight (48) birds per treatment where each treatment was further replicated three (3) times with sixteen (16) birds per replicate in a Completely Randomized Design (CRD). The birds were raised on deep litter system using 2.5 × 2.5m pen sizes in two phases; Starter (0 – 4 weeks) and Finisher (5 – 8 weeks). Routine vaccination practices were strictly adhered to and other management practices that ensured good health as recommended by Oluyemi and Roberts [27]. Feed and water were supplied *ad libitum*.

2.4. Experimental Diets

Four experimental diets were formulated with Neem Leaf Meal (NLM) incorporated at levels of 0 (control), 2.5, 5 and 7.50% designated as T_1, T_2, T_3 and T_4, respectively. The experimental diets were fed to the birds for eight weeks (56 days) comprising the starter (23% CP) and finisher (20% CP) phases of production. The composition of starter and finisher diets for all treatments are shown in Tables 1 and 2, respectively. The respective diets and test ingredient (NLM) were analysed for proximate composition using methods of AOAC [28].

2.5. Determination of Haematological and Serum Biochemical Indices

Twenty four blood samples (six per treatment) of 2ml each were collected from the prominent wing vein of chickens at the 8th week for haematological and serum biochemical assays, respectively. The blood samples for the hematological studies were placed into a sterilized glass tubes containing Ethylene Diamine - tetra - acetic acid

(EDTA) to determine the packed cell volume (PCV), Red Blood Cell (RBC), White Blood Cell (WBC), Haemoglobin (Hb), Mean Corpuscular Haemoglobin (MCH), Mean Corpuscular Volume (MCV) and Mean Corpuscular Haemoglobin Concentration (MCHC) using the methodology of Schalm, et al. [29]. For serum biochemical studies, the blood samples were placed into plain bottles, (without anticoagulant) to enhanced serum separation by centrifugation and the harvested sera were used to determine the serum total protein (STP) using the Kjeldahl method as describes by Kohn and Allen [30], serum albumin using BCG (Bromocresol green) method as described by Peter, et al. [31]; creatinine using a commercial kit (Creatinine Liquicolor, Germany) as well as serum glucose and urea using the Spectrophotometric method (Thermo Fisher Scientific Inc., Madison, Wisconsin, USA) and commercial reagent kits respectively.

2.6. Statistical Analysis

All data obtained were subjected to one – way analysis of variance (ANOVA) using statistical package for social sciences (SPSS 17.0, Microsoft, Window 2007 version) and significant means were separated using Duncan's Multiple Range Test (DMRT) [32].

3. RESULTS AND DISCUSSION

3.1. Haematological Indices of Broiler Chickens Fed Dietary Levels of Neem Leaf Meal (NLM)

The haematological indices of broilers chickens fed dietary levels of neem leaf meal are presented in Table 3.

The results showed no significant (P>0.05) differences between dietary treatments for all parameters measured. Values for each parameter ranged as follows: red blood cell counts (2.33 - 3.00 $\times 10^{12}$/L), white blood cell counts (6.90 – 8.90 $\times 10^{9}$/L), Haemoglobin concentration (Hb) (13.66 - 15.13 g/dl), Mean corpuscular haemoglobin (MCHC) (43.13 - 44.10 %), Mean Corpuscular Haemoglobin (MCH) (58.67 – 61.67 pg), Mean Corpuscular Volume (MCV) (134.33 – 141.67 fl) and Packed Cell Volume (PVC) (32.00 - 34.33 %). Values for red blood cell counts (RBC) were slightly lower than the normal ranges (4.21 – 4.84 $\times 10^{12}$/L) for apparently healthy broiler chickens reported by Wikivet [33] and (3.20 – 4.40 $\times 10^{12}$/L) reported by Obun, et al. [4] who fed sun - cured neem leaf meal to finisher broilers. The results were also similar to that of Bonsu, et al. [15] who reported a range of 1.91 – 2.65 $\times 10^{12}$/L. This suggests a fair oxygen carrying capacity of the blood in chickens fed NLM diets. Values for white blood cell counts (WBC) were slightly lower than the range (10.80 – 16.36 $\times 10^{9}$/L) reported by Obun, et al. [4] indicating that birds had good immunity to fight infection. The lower WBC values in this study further showed that NLM may not have increased the production of these blood components against the residual metabolites in the leaf meal. Hence, indicating that birds fed up to 7.5% NLM were not immunologically challenged. Haemoglobin (Hb) concentration was closed to the range (11.60 – 13.68 g/dl) reported by Wikivet [33] for apparently healthy birds and (10.50 – 12.00 g/dl) reported by Aderemi, et al. [34] for birds fed up to 7.5% cassava leaf meal, indicating a good oxygen carrying capacity of the blood that ensures vitality and general well-being of birds. Values for MCHC were slightly higher than 32.41 – 33.7% reported of Wikivet [33] which suggested a fairly good production of haemoglobin. Values of MCH and MCV were all fairly higher than that of Wikivet [33] which may be due to the different type of feed ingredients used and environmental factors in the tropics as against temperate areas. All haematological parameters of broiler chickens were not significantly (P>0.05) influenced by dietary neem; implying lower levels of residual anti – nutrients in neem leaf meal may not have deleterious effects on blood forming cells in broiler chickens. Blood has been shown to be an important index of physiological, pathological and nutritional status in animals Ewuola, et al. [35] ;Olorode, et al. [36]. Aletor [37]; Aletor and Egberongbe [38] have reported that the blood variables most consistently affected by dietary factors are RBC, PCV and plasma protein.

3.2. Serum Biochemical Indices of Broiler Chickens Fed Dietary Levels of Neem Leaf Meal (NLM)

The serum biochemical indices of broiler chickens fed dietary levels of neem leaf meal are shown in Table 4. All parameters measured were not significantly (P>0.05) different except cholesterol. The ranges recorded for all parameters measured were as follows: total protein (6.57 – 8.62 g/dl); albumin (4.31 – 5.58 g/dl); globulin (1.94 – 3.09 g/dl), glucose (6.73 – 7.60 mmol/L), cholesterol (3.77 – 6.03 mmol/L), creatinine (44.40 – 77.50 mmol/L), urea (1.77 – 2.33 mmol/L). According to Wikivet [33] the normal range for total protein in broiler chickens is in the range 4.63 – 4.81 g/dl which is slightly lower than the result of the study. The total protein was however higher than the range (4.80 – 5.40 g/dl) reported by Aderemi, et al. [34] who fed cassava peel meal to broiler chickens. Odetola, et al. [39] has also reported the total protein of serum in broilers fed cooked kenaf seed meal to be in the range (2.20 – 2.93 g/dl). The slight disparity in results could be attributed to the effect of different test ingredients and associated anti – nutrients on the total protein content of the blood in the separate studies. The value for urea which was lower than 4.46 – 4.54 mmol/L reported by Wikivet [33] and might have indicated efficient removal of urea wastes from the body of birds. The values of albumin in this study were slightly higher than 3.28 – 3.45 g/dl reported by Wikivet [33]. Generally, the serum biochemical indices of birds in this study indicated a balanced in chemical constituents with increasing levels of neem leaf meal across all treatments; as there was no adverse effects on the birds. Birds fed up to 7.5% NLM inclusion compared well with birds fed the control diet in total protein, glucose, albumin, globulin, creatine and urea, indicating favourable protein and glucose digestion and metabolism [34] by birds in this study. The lower serum urea in this study could be due to the fact that amino acid composition of dietary treatments containing NLM was balanced. Imbalance in amino acids in diets could cause elevated blood urea concentration in animals [40]. Blood urea level especially in monogastric animals is influenced by quantity, quality and proximity of the proceeding meal, which increases mostly after consumption [34]. Blood urea is also an indication of protein quality, as increased urea value is an indication of poor protein quality [39] hence implying that the protein quality of the diets as well as kidney function in animals were optimum in this study. The serum glucose levels were not elevated in this study. Elevated glucose may be due to inhibition of glycolysis by the presence of glycoprotein and other anti- nutritional factors, which might adversely affect the regulation of insulin from islets of Langerhans [41]. The NLM diets recorded higher significant (P<0.05) levels of cholesterol compared to the control without NLM, implying that the constituents of neem leaf meal may boost cholesterol levels in the blood of birds. This finding disagrees with the reports of Ezeagu, et al. [42] and Aderemi, et al. [34] who reported decrease level of cholesterol in birds fed cassava leaf meal and neem leaf meal respectively. The difference in results could be attributed to processing methods of the leaf meals, varietal differences and inherent residual anti – nutrients.

4. CONCLUSION

In this study, all blood parameters were within the normal ranges for apparently healthy broiler chickens. It can be concluded that including up to 7.5% neem leaf meal (NLM) in diets meant for broiler chickens will not adversely affect their blood characteristics (haematology and serum biochemical indices), vis – a – vis haematopoietic and other life processes of birds.

REFERENCES

[1] F. C. Nworgu, G. N. Egbunike, C. E. Onanogbu, J. B. Fapokunda, and J. U. Ogbonna, "Effect of mimosa (Mimosa invisa) leaf meal supplements on broiler finisher performance," in *Proceedings of the 8th Annual Conference of the Animal Science Association of Nigeria (ASAN)*, 2003, pp. 36-38.

[2] O. Esonu, C. Okoli, M. Opara, O. Obikaonu, C. Udedibie, and M. Iheshiulor, "Physiological response of laying birds to Neem (Azadirachta indica) leaf meal-based diets," *Online Journal of Health and Allied Sciences*, vol. 5, p. 4, 2006.

[3] H. Obikaonu, I. Okoli, M. Opara, V. Okoro, I. Ogbuewu, E. Etuk, and A. Udedibie, "Haematological and serum biochemical indices of starter broilers fed leaf meal of neem (Azadirachta indica)," *J. Agric. Technol*, vol. 8, pp. 71-79, 2012.

[4] C. O. Obun, A. S. Kehinde, and P. O. Osaguona, "Effects of feeding broiler chicks fed graded levels of sun – cured neem (Azadirachta indica A. Juss) leaf meal on blood chemistry. ," in *Proceeding of 38th Annual Conference of the Nigerian Society for Animal Production (NSAP), Held Rivers State University of Science and Technology*, Port Harcourt, 2013, pp. 128 -130.

[5] P. O. Ozung, O. O. K. Oko, V. N. Ebegbulem, E. I. Evans, L. E. Asuquo, and J. O. Ekpo, "Utilization of neem leaf meal in broiler diets: Effect on growth, carcass characteristics and internal organ weights," in *Proceedings of the 18th Annual Conference of Animal Science Association of Nigeria, Held in Abuja*, Nigeria, 2013, pp. 264 – 267.

[6] R. Feltwell and S. Fox, *Practical poultry feeding*. Great Britain: English Language Book Society, 1979.

[7] K. R. C. Kibria and S. V. S. Verna, *Feed additives, poultry nutrition*. Delhi: Kalyani Publication, 2009.

[8] A. R. Mohammed, A. Ashraf, K. S. Biddut, A. Abdallah – Al –Hassan, and M. Mahbub, "Use of neem leaf and ginger extracts for cost effective broiler production," *International Journal of Natural and Social Sciences*, vol. 212, pp. 11 – 16, 2015.

[9] W. J. Craig, "Health-promoting properties of common herbs," *The American Journal of Clinical Nutrition*, vol. 70, pp. 491s-499s, 1999.Available at: https://doi.org/10.1093/ajcn/70.3.491s.

[10] K. Girish and S. Shankara Bhat, "Neem–a green treasure," *Electronic Journal of Biology*, vol. 4, pp. 201-111, 2008.

[11] W. Blaney, M. Simmonds, S. Ley, J. Anderson, and P. Toogood, "Antifeedant effects of azadirachtin and structurally related compounds on lepidopterous larvae," *Experimental and Applied Entomologia*, vol. 55, pp. 149-160, 1990.Available at: https://doi.org/10.1111/j.1570-7458.1990.tb01358.x.

[12] M. Gandhi, R. Lal, A. Sankaranarayanan, C. K. Banerjee, and P. L. Sharma, "Acute toxicity study of the oil from Azadirachta indica seed (neem oil)," *Journal of Ethnopharmacology*, vol. 23, pp. 39-51, 1988.Available at: https://doi.org/10.1016/0378-8741(88)90113-4.

[13] H. Makeri, V. Maikai, and J. Nok, "Effect of topical application of neem seed (Azadiracta indica) extract on sheep infested with Amblyomma variegatum," *African Journal of Biotechnology*, vol. 6, pp. 2324 - 2327, 2007.Available at: https://doi.org/10.5897/ajb2007.000-2364.

[14] A. V. Elangovan, S. V. S. Verna, and V. R. B. Sastry, *Effect of feeding neem leaf meal on growth and nutrient utilization of animals*. Welling Fort, UK: CAB International, 2000.

[15] F. Bonsu, J. Kagya-Agyemang, W. Kwenin, and H. Zanu, "Medicinal Response of broiler chickens to diets containing neem (Azadirachta indica) leaf meal, haematology and meat sensory analysis," *World Applied Sciences Journal*, vol. 19, pp. 800-805, 2012.

[16] I. P. Ogbuewu, "Physiological response of rabbits fed graded levels of neem (Azadirachta indica) leaf meal," MSc. Thesis, Dept. of Animal Science and Technology, Federal University of Technology, Owerri, Nigeria, 2008.

[17] R. V. Padalwar, "M.Sc. Thesis on growth promoters in broiler production," *Biotechnology in Animal Husbandry*, vol. 25, pp. 387- 397, 1994.

[18] B. O. Esonu and M. N. Opara, "Response of laying birds to neem (Azadirachta indica) leaf meal based diets, body weights, organ characteristics and haematology," *Online Journal of Health and Allied Sciences*, vol. 5, pp. 22- 28, 2006.

[19] N. L. Sadre, V. Y. Deshpande, K. N. Mendulkar, and D. H. Nandal, *Male anti-fertility activity of Azadirachta indica, A. Juss (Neem) in different species*. Eschborn, Germany: Duetsche Gesellschaft fiir Technische zusamenenar belt (GTZ), 1984.

[20] S. Gowda, "Neem (Azadirachta indica) kernel meal in the diet of white Leghorn layers," *British Poultry Science*, vol. 39, pp. 648-652, 1998.Available at: https://doi.org/10.1080/00071669888520.

[21] C. Devakumar and D. V. Sukh, "Neem research and development," *Indian Journal of Poultry Science*, vol. 3, pp. 63 – 66, 1993.

[22] F. Durrani, N. C. Abidullah, Z. Durrani, and S. Akhtar, "Hematological, biochemical, immunomodulatory and growth promoting effect of feed added wild mint (Mentha longifolia) in broiler chicks," *Sarhad Journal of Agriculture*, vol. 24, pp. 661-665, 2008.

[23] P. G. Aguihe, A. S. Kehinde, and C. J. Iwu, "Haematological and serum biochemical indices of broilers fed local groundnut cake meal as replacement for soybean meal," in *Proceedings of the 17th Annual Conference of Animal Science Association of Nigeria*, 2012, pp. 12-15.

[24] B. Esonu, O. Emenalom, and A. Udedibie, "Performance and blood chemistry of weaner pigs fed raw mucuna bean (velvet bean) meal," *Tropical Animal Production Investigation*, vol. 4, pp. 49–54, 2001.

[25] G. M. Babatunde, W. O. Pond, L. Krook, L. Dvan, E. R. Walker, and D. Chapman, "Effect of dietary safflower oil or hydrogenated coconut oil on growth rate and on swine blood and tissue components of pigs fed fat free diets," *Journal of Nutrition*, vol. 92, p. 1903, 1987.

[26] Taraba State Diary, *Taraba State Diary*. Jalingo, Nigeria: Yearly Publication by the Government of Taraba State, 2018.

[27] J. A. Oluyemi and F. A. Roberts, *Poultry production in warm climates: Spectrum books limited*, 2nd ed. London: MacMillan Publishers Limited, 2000.

[28] AOAC, *Association of official analytical chemists, official methods of analysis*, 19th ed. Washington DC, USA: AOAC, 2010.

[29] O. W. Schalm, N. C. Jain, and E. J. Caroll, *Veterinary Haematology*, 3rd ed. Philadelphia: Lea and Fabiger, 1975.

[30] R. Kohn and M. Allen, "Enrichment of proteolytic activity relative to nitrogen in preparations from the rumen for in vitro studies," *Animal Feed Science and Technology*, vol. 52, pp. 1-14, 1995.Available at: https://doi.org/10.1016/0377-8401(94)00711-h.

[31] T. Peter, C. T. Biomont, and B. T. Doumas, *Protein in serum, urine and cerebrospinal fluid, albumin in serum: In Selected Methods of Clinical Chemistry, W. R. Faullkner and S. Meites (eds.)* vol. 9. Washington D.C: American Association of Clinical Chemists, 1982.

[32] D. B. Duncan, "Multiple range and multiple F tests," *Biometrics*, vol. 11, pp. 1-42, 1955.Available at: https://doi.org/10.2307/3001478.

[33] Wikivet, "Haematology: Available at en.wikipedia/wiki/haematology," 2013.

[34] F. A. Aderemi, T. O. Faniyi, and O. A. Jimoh, "Haematology and serum biochemistry of broiler chickens fed cassava leaf meal based diets," in *Proceeding of 38th Annual Conference of the Nigerian Society for Animal Production (NSAP), Held Rivers State University of Science and Technology*, Port Harcourt, 2013, pp. 114–116.

[35] E. Ewuola, O. Folayan, F. Gbore, A. Adebunmi, R. Akanji, J. Ogunlade, and J. Adeneye, "Physiological response of growing west-African dwarf goats fed groundnut shell-based diets as the concentrate supplements," *Bowen Journal of Agriculture*, vol. 1, pp. 61-66, 2004.

[36] B. R. Olorode, E. B. Ibitoye, and J. O. Abiola, "Evaluation of Moringa oleifera seed meal as replacement for groundnut cake in broiler diets," *Tropical Journal of Animal Science*, vol. 10, pp. 59–62, 2007.

[37] V. Aletor, "Effect of varying levels of fishmeal substitution with soybean meal on certain serum metabolites," *Niger. J. Technol. Res*, vol. 1, pp. 111-114, 1989.

[38] V. Aletor and O. Egberongbe, "Feeding differently processed soya bean part 2. An assessment of haematological responses in the chicken," *Food/Nahrung*, vol. 36, pp. 364-369, 1992.Available at: https://doi.org/10.1002/food.19920360406.

[39] O. M. Odetola, A. O. Owosibo, R. O. Sobayo, O. A. Adeyemi, O. O. Adejinmi, and T. I. Ijaduola, "Performance characteristics and serum biochemistry of broiler chickens fed graded levels of cooked kenaf (Hibiscus cannabinus L.) seed meal as a replacement for full fat soybean meal," in *Proceedings of the 18th Annual Conference of Animal Science Association of Nigeria, Held in Abuja*, Nigeria, 2013, pp. 333–336.

[40] B. O. Eggum, *Blood urea measurements as a technique for assessing protein quality, in Eggum, B.A. 1973, A study of certain factors influencing protein utilization in rats and pigs*. Copenhagen: National Institute of Animal Science, Publication 406, 1970.

[41] B. Mandal, S. G. Majumdar, and C. R. Maity, "Nutritional evaluation of the protein isolate obtained from seed of Eucalyptus kirtoniana in albino rats- A comparative study," *Proceedings of Indian Veterinary Science Academy*, vol. 48, pp. 596–602, 1982.

[42] I. E. Ezeagu, K. J. Petzke, A. D. Ologhobo, C. C. Metges, and A. O. Akinsoyinu, "Compositional evaluation and feed potential of the African kudzu (Pueraria phaseoloides, Roxb. Benth) seed meal," *Journal of Food Science and Technology-Mysore*, vol. 37, pp. 191-195, 2000.

[43] U. Pauzenga, "Feeding parent stock," *Zootecnica International*, vol. 17, pp. 22-24, 1985.

Table-1. Composition of broiler starter diets (0-4weeks

Ingredient	T_1 (0%)	T_2 (2.5%)	T_3 (5.0%)	T_4 (7.5%)
Maize	42.64	41.64	40.64	39.64
Maize bran	11.50	12.50	13.50	14.50
Soybean (Full fat)	39.16	36.66	34.16	31.66
NLM	0.00	2.50	5.00	7.50
Fish meal	2.50	2.50	2.50	2.50
Limestone	1.50	1.50	1.50	1.50
Bone meal	2.00	2.00	2.00	2.00
Premix	0.25	0.25	0.25	0.25
Salt	0.25	0.25	0.25	0.25
Lysine	0.10	0.10	0.10	0.10
Methionine	0.10	0.10	0.10	0.10
Total	**100**	**100**	**100**	**100**
Calculated analysis				
Crude Protein%	23.00	23.00	23.02	23.03
ME (Kcal/kg)*	3,011.94	2,961.00	2,939.12	2,916.92
Crude Fibre %	4.45	4.61	4.80	4.92

Determined analysis				
Crude protein (%)	24.10	23.50	22.90	23.00
Crude fibre (%)	3.00	3.50	4.00	4.50
ME (Kcal/kg)*	3,142.65	3,020.00	3,055.58	3,020.95

ME (Kcal/kg)* calculated from Pauzenga [43] equation

Table-2. Composition of broiler finisher diets (5-8weeks)

Ingredient	T_1 (0%)	T_2 (2.5%)	T_3 (5.0%)	T_4 (7.5%)
Maize	52.33	51.33	50.33	49.00
Maize bran	10.00	10.00	10.00	10.00
Soybean (Full fat)	29.47	26.97	24.47	21.97
NLM	0.00	2.50	5.00	7.50
Fish meal	4.00	5.00	6.00	7.00
Limestone	1.50	1.50	1.50	1.50
Bone meal	2.00	2.00	2.00	2.00
Premix	0.25	0.25	0.25	0.25
Salt	0.25	0.25	0.25	0.25
Lysine	0.10	0.10	0.10	0.10
Methionine	0.10	0.10	0.10	0.10
Total	**100**	**100**	**100**	**100**
Calculated Analysis				
Crude protein (%)	20.00	20.01	20.02	20.03
ME (Kcal/kg)	2,996.99	2,989.99	2,967.99	2,945.99
Crude fibre (%)	5.13	5.22	5.31	5.41
Determined analysis				
Crude protein (%)	20.90	20.60	19.90	20.20
Crude fibre (%)	5.10	5.50	5.80	6.00
ME (Kcal/kg)*	2,933.50	2,874.10	2,878.55	2,990.08

Premix: Vit. A=10,000,000IU, Vit. D3=2,000,000UI, Vit. C=20,000mg, Vit. K=2,000mg, Vit.B1=3,000mg, Vit. B2=5,000mg, Vit. B6=45,000mg, Vit. B12=10,000mg, Niacin=4,000mg, Pantothenic Acid=20mg, Folic Acid=300,000mg, Biotin=1,000mg, Choline Chloride=50mg, Manganese=300,000mg, Zinc=120,000mg, Iron=80,000mg, Copper=3,500mg, Iodine=1,500mg, Selenium=300mg, Cobalt=120mg, Antioxidant=120,000mg
NLM=Neem Leaf Meal
ME = Metabolizable Energy
ME (Kcal/kg)* calculated from Pauzenga [43] equation: ME= (37 × %CP + 81.8 × % EE + 35.5 × %NFE)

Table-3. Haematological indices of broiler chickens fed dietary levels of Neem Leaf Meal

Parameter	T_1 (0%)	T_2 (2.5%)	T_3 (5%)	T_4 (7.5%)	SEM
RBC (× 10^{12}/L)	3.00	2.67	2.33	3.00	0.24
WBC (× 10^9/L)	7.60	6.90	8.90	8.30	7.26
Hb (g/dl)	14.80	15.13	13.66	14.83	5.55
MCHC (%)	43.73	44.10	43.13	43.63	7.57
MCH (pg)	58.67	61.67	61.67	60.67	1.27
MCV (fl)	134.33	139.33	141.67	139.00	3.42
PCV (%)	34.00	34.33	32.00	34.33	1.55

T_1 =Treatment 1 (Control – without Neem Leaf Meal)
T_2 =Treatment 2 (2.5% Neem Leaf Meal Inclusion)
T_3 =Treatment 3 (5% Neem Leaf Meal Inclusion)
T_4 =Treatment 4 (7.5% Neem Leaf Meal Inclusion)
SEM = Standard Error of Means
RBC = Red Blood Cell counts
WBC = White Blood Cell counts
Hb = Haemoglobin concentration
MCHC = Mean Corpuscular Haemoglobin Concentration
MCH = Mean Corpuscular Haemoglobin
MCV = Mean Corpuscular Volume
PCV = Packed Cell Volume

Table-4. Serum biochemical indices of broiler chickens fed dietary levels of Neem Leaf Meal

Parameter	T_1 (0%)	T_2 (2.5%)	T_3 (5%)	T_4 (7.5%)	SEM
Total protein (g/dl)	6.77	8.62	6.57	7.47	5.06
Albumin (g/dl)	4.31	5.58	4.49	5.53	3.09
Globulin (g/dl)	2.46	3.04	2.08	1.94	2.29
Glucose (mmol/L)	7.60	7.30	7.20	6.73	1.09
Cholesterol (mmol/L)	3.77^b	4.23^b	6.03^a	5.33^a	0.32
Creatinine (µmol/L)	44.60	44.40	47.50	45.43	0.80
Urea (mmol/L)	1.77	2.33	2.13	2.00	0.18

Means within the same row bearing different superscripts differ significantly (P<0.05).
SEM = Standard Error of Means
T_1 =Treatment 1 (Control – without Neem Leaf Meal)
T_2 =Treatment 2 (2.5% Neem Leaf Meal Inclusion)
T_3 =Treatment 3 (5% Neem Leaf Meal Inclusion)
T_4 =Treatment 4 (7.5% Neem Leaf Meal Inclusion)