



Haematology and Serum Biomarkers of Broiler Birds Fed Graded Levels of Clove (*Syzygium aromaticum* (L.) Buds Meal in Semi Arid Nigeria

H. B. Usman¹, K. M. Aljameel^{1*}, M. M. Mika'ilu², S. A. Maigandi²,
A. Z. Sanusi¹ and S. Ibrahim²

¹Department of Animal Science, Federal University Dutsin-ma, Katsina, Nigeria.
²Department of Animal Science, Usamanu Danfodiyo University Sokoto, Nigeria.

Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/ACRI/2021/v21i530246

Editor(s):

(1) Dr. Marco Muscettola, University of Bari, Italy.

Reviewers:

(1) Ghulam Abbas, Riphah International University, Pakistan.

(2) R J Padodara, Kamdhenu University, India.

Complete Peer review History: <https://www.sdiarticle4.com/review-history/75277>

Original Research Article

Received 29 July 2021
Accepted 01 October 2021
Published 04 October 2021

ABSTRACT

The study investigated the effect of graded levels of cloves bud powder on broiler birds. The study was conducted using a total of 288 broiler birds in a Completely Randomized Design Four treatments; four experimental diets were formulated and designated as T₁ (control), T₂ (0.5kg/100kg), T₃ (1kg/100kg) and T₄ (1.5kg/100kg). At the end of the feeding trial (eighth week), fifteen birds (5 per replicate) from each treatment group were randomly selected for blood collection. About 2ml of blood were collected per bird. Birds in treatment 4 (1.5kg/100kg) had the highest (P<0.05) concentration of haemoglobin and PCV compared to treatment 1 (control) and 3 (1kg/100kg). Significant difference (P<0.05) was observed in albumin, globulin, total protein, HDL, cholesterol, urea, AST and ALT. The result shows that birds fed 1.5/100kg clove buds meal have higher albumin, globulin and total protein. The study concludes that inclusion of clove buds meal up to 1.5/100kg in diet of broiler birds without adverse effect as indicated by haematological and serum biochemical of the birds in this study.

Keywords: Broiler; clove buds meal; haematology and serum biomarkers.

*Corresponding author: Email: muhdkjameel@gmail.com;

1. INTRODUCTION

One of the objectives of any poultry producer is to feed the chickens with balanced diet at least cost and also generate products that will attract premium prices in order to maximise profit. For many decades, farmers and feed manufacturers have been facing the challenge of effectively reducing the cost of poultry production and produce quality products. Several factors such as genotype, diet composition, digestible nutrient content, energy to protein ratio, feed form, feed processing, environment, and disease could affect the cost of production and poultry product quality through influencing feed intake, body weight gain and feed conversion ratio (FCR). In commercial poultry, the production of broiler feed contributes up to 70% of the total production cost. Due to increases in global feed prices, there is now a tendency in the poultry industry to move towards alternative or unconventional feed ingredients. This move is however limited by several issues: high and low fibre and protein contents and the presences of antinutritional factors (ANF) in unconventional feed ingredients that can reduce feed digestibility [1].

In Nigerian poultry farms, it is a common practice to add antibiotics in drinking water at the time of vaccinations. Antibiotics are also added in self formulated and commercial feeds. Antibiotics are also given as medications in prevention and control instances. Most farmers administer antibiotics at the time of vaccination without scientific knowledge of the effects on the immune response. The feed industry is facing the challenge of the awareness among the consumers of meat on the risk of bringing about antibiotic resistance in pathogenic microbiota through antibiotics used in animal and poultry feeds. It has directed them towards the non-antibiotic feed additives. Among them, the feed additives of plant origin, called as Phyto-genic Feed Additives (PFA) or Phytobiotics or Phyto-additives are considered to be a better alternative as non-antibiotic growth promoters, even though there are well established non antibiotic growth promoters such as organic acids and probiotics. The Phyto-genic feed additives also vary widely in their botanical origin, processing and composition. They have been used in solid, dried and ground forms or as extracts or essential oils [2]. Therefore, there is a need to exploit different natural plant products to achieve better production of farm animals [3].

Herbs and spices (such as cinnamon, oregano, thyme, ginger, garlic, cloves etc) are known to

have health benefits (such as appetite and digestion stimulants [4] anti-microbial action [5] anti-oxidative action and immune-stimulant function [3] on animals when used as feed additives in animal nutrition. For effective use of herbs and spices, they can be added to feed as dried plants or as extracts.

2. MATERIALS AND METHODS

2.1 Experimental Site

The study was conducted at the Teaching and Research Farm of the Department of Animal Science of Usmanu Danfodiyo University, Sokoto, Nigeria. The farm lies at longitude 5° 27" E and latitude 13° 08"N and at altitude of 266m above sea level, the readings were obtained from GNSS viewer software for androids. The average annual environmental temperature is 28.3°C (82.9° F). However, the maximum daytime temperature for most of the year are generally under 40°C (104.0°F). The low humidity of Sokoto state makes the heat bearable. Heat is more severe in the state in March and April, but the weather in the state is always cold in the mornings and hot in the afternoons except during the harmattan period [6]. The rainy season starts from late May to October. Rainfall starts late and ends early with annual rainfall ranging between 500mm to 1,200mm [6]. There are two major seasons in the state namely: wet and dry seasons. The dry season starts from October and last up to April and may extend to May or June. The wet season on the other hand begins in most part of the state in May and last up to September or October [6].

2.2 Experimental Design

The study was conducted as follows; using a Complete Randomised Design, a total of 288 broiler birds were used, 4 treatments, (72 birds per treatment) replicated 6 times, with 12 birds in each replicate. Four (4) graded levels of cloves was used as additives in this experiment. The graded levels are 0, 0.5, 1 and 1.5kg/100kg dose, the treatments was added after the diet was formulated and compounded, the cloves powder was then mixed thoroughly with the feed

2.3 Experimental Birds and their Management

Day old boiler chicks for this study were obtained from reputable farm in Nigeria. The birds were

transported to Sokoto under the cool hours of the evening through the night and arrived in the morning hours. The house was cleaned, washed and disinfected a week to the arrival of the birds. The birds were raised on deep litter in tropical house type, with open side walls and concrete floor. Litter materials (wood shavings and old newspaper) were spread on the floor, feeding trays and small drinkers were used for the first 0-4 weeks (Starter phase), while conical feeders and plastic containers with wire guard were used at finishing phase. Feed was given to the birds at free choice on tray feeders for the first 10 days and the tray feeders were replaced with small conical feeders at second week of their age for proper feed management and efficiency. Fresh water was given to the birds every morning in small drinkers. Their health care was ensured by giving them routine vaccination and medication as at when due, proper sanitation and hygiene was ensured. The floor spacing was maintained at (4/9 ft) per replicate [7].

2.4 Experimental Diet Formulation

Maize, wheat offal, bone meal, Fish meal and salt were obtained from Sokoto central market. Soya bean meal, Groundnut cake, limestone and micro ingredients such as Premix, Lysine, and Methionine were sourced from a vendor called Alkanchi farm Ltd in the Sokoto Metropolis. The

cloves was also purchased and ground into powder form.

Feed ingredients that were used for this experiment, such as Maize, Groundnut cake (GNC), Soya bean meal and Bone meal required crushing so that the particle size will suit the group of birds the feed are to be meant for. Feed ingredients that were in powdery form were weighed and mixed with the crushed ones. The feed compounding was done on a clean concrete floor, and thoroughly mixed with shovel to a uniform mix.

2.5 Blood Collection and Evaluation of Blood Parameters

At the end of the feeding trial (eighth week), fifteen birds (5 per replicate) from each treatment group were randomly selected for blood collection. About 2ml of blood were collected per bird. The collection was done by puncturing the brachial vein with a 5ml scalp vein needle and syringe, two separate blood samples were collected immediately, one sample into a set of sterile plastic bottles, containing ethylene diamine tetra acetic acid (EDTA) as the anticoagulant for determination of haematological parameters and the other into un-heparinised tubes for the determination of serum biochemical indices.

Table 1. Gross and analysed chemical composition of experimental starter and finisher Diets

INGREDIENT (kg)	STARTER	FINISHER
Maize	50.0	55.5
Soya beans meal	19.0	13.0
Groundnut cake	15.5	12.0
Fish meal	2.5	1.50
Wheat offal	8.0	11.0
Limestone	2.0	4.0
Bone meal	2.0	4.0
Premix	0.25	0.25
Salt	0.25	0.25
Methionine	0.25	0.25
Lysine	0.25	0.25
TOTAL	100kg	100kg
Analysed value of feed	Starter %	Finisher%
Crude protein	22	20
Energy kcal/kg	3001	3450
Methionine	0.5	0.5
Lysine	1.0	0.9
Calcium	1.4	2.6
Phosphorous	0.6	0.8
Fibre	5.4	5.1

2.6 Haematological Indices Determination

The haematological parameters analysed include packed cell volume (PCV), red blood cells (RBC) count, total white blood cells (WBC) count, leucocytes differential count and haemoglobin concentration (Hb) in accordance with the methods outlined by Bush [8].

Erythrocyte indices which include the mean corpuscular volume (MCV) and mean corpuscular haemoglobin concentration (MCHC) was computed in accordance with the standard formula indicated by Jain [9] and [10] as shown below:

$$\text{MCV (fl)} = \frac{\text{PCV}}{\text{RBC Count In } 10^6/\text{Mm}^3} \times \frac{10}{1}$$

$$\text{MCH (pg)} = \frac{\text{Hb (G/Dl)}}{\text{RBC (In } 10^6/\text{Mm}^3)} \times \frac{10}{1}$$

$$\text{MCHC (g/dl)} = \frac{\text{Hb (G/Dl)}}{\text{PCV (\%)}} \times \frac{100}{1}$$

Where: MCV= mean corpuscular volume, PCV= packed cell volume, RBC= red blood cell, MCH = mean corpuscular haemoglobin, Hb = haemoglobin, MCHC = mean corpuscular haemoglobin concentration

2.7 Serum Chemistry

The plasma total protein was measured using biuret reaction according to the procedure of [11] while albumin was measured by colorimetric estimation using sigma diagnostic kit according to the method described by [12]. Globulin was obtained by calculating the difference of total protein and albumin. The serum enzyme, Aspartate aminotransferase (AST), Alanine aminotransferase (ALT) and alkaline phosphatase (ALP) were determined using photoelectric colorimeter as described by [13]. Blood urea, nitrogen and creatinine levels were determined using photoelectric colorimeter as described by [14]. Total cholesterol was evaluated as described by [15].

2.8 Statistical Analysis

Analysis of variance (ANOVA) was used to compare the haematology and serum biomarkers of broiler birds administered fed graded levels of cloves powder, where significant differences

($P < 0.05$) occur, means were separated using Duncan New Multiple Range Test (DNMRT).

3. RESULTS AND DISCUSSION

The result of haematology of broiler birds fed graded levels of clove buds meal was presented in Table 2. Significant variation ($P < 0.05$) was observed in haemoglobin concentration, PCV, MCH, MCV, MCHC and lymphocytes. The blood parameters were within the normal ranges for broiler chickens and indicated that the nutrients were adequately utilized by the broilers and posed no problem to the birds. Birds in treatment 4 (1.5kg/100kg) had the highest ($P < 0.05$) concentration of haemoglobin and PCV compared to treatment 1 (control) and 3 (1kg/100kg), which indicate that PCV increased the availability of protein, energy and the degradation of antinutritional factors as a result of the addition of clove buds meal. There is no difference ($P > 0.05$) between treatment 4 (1.5kg/100kg) and 2 (0.5kg/100kg) so also 1 and 2 in terms of haemoglobin concentration. Birds in treatment 1(Control) and 3(1kg/100kg) have similar ($P > 0.5$) PCV. MCH and MCV are statistically higher ($P < 0.05$) in treatment 1 (control) compared to the other treatments. The value obtained in this study for MCV is lower than the range of 92.44-122.09 reported by Akinola and Etuk [16] but MCHC was within the range of 30.78-33.39 by the same author. MCHC levels are lower ($P < 0.05$) in birds fed 0.5kg/100kg clove buds meal while lymphocytes are higher compared to the other treatments. Birds in control had lower lymphocytes ($P < 0.05$). haematological traits, especially PCV and Hb were correlated with the nutritional status of the animal [17] and agreed with Oyawoye and Ogunkunle, [18] who stated that PCV is an index of toxicity in the blood and high-level usually suggest presence of toxic factors which has adverse effect on blood formation. The absent of Monocyte in the result is an indication that birds fed cloves bud meal were not stressed during the experiment by nutritional or environmental factors, since leucocytes responses are considered as better indicators of chronic stress [19].

The result of serum biomarkers of broiler birds fed graded levels of clove buds meal was presented in Table 3. Significant difference ($P < 0.05$) was observed in albumin, globulin, total protein, HDL, cholesterol, urea, AST and ALT. The result shows that birds fed 1.5/100kg clove buds meal have higher albumin, globulin and

total protein this indicate that clove buds meal have significant influence on the dietary treatment. The values obtained in this study were slightly higher the normal range of serum protein (4.55-6.46g/dl) reported by Udoyong et al. (2010). Higher value indicates that there is enzyme hydrolysis of dietary proteins and explained that the blood pool serves as a major source of amino acids needed for the synthesis of protein [20,21]. High density lipoprotein (HDL) decreases with increase in clove buds meal. Cholesterol levels are significantly higher for birds fed 0.5kg/100kg clove buds meal diet. The values were lower than 3.10-3.64 mg/dl reported by Duwa et al. [22] and within 2.77-3.90mol/l reported by Akinola and Etuk [16]. Urea and blood glucose increases with increasing levels of the test ingredient (cloves bud meal).

The values where than the normal range of 9.9-11.1 mmol/l stated by Banerjee [23] for urea, and 8.17-9.77 mmol/l reported by Akinola and Etuk [16] for glucose. AST and ALT are significantly higher in Treatment 4 (1.5/100kg) compared to the control, AST values obtained in this study were higher than the range of 13.72-15.65 lu/c reported by Akinola and Etuk [16] while The values obtained for this study were lower than the range of 17.36-29.41 i.u/l Duwa et al. [22]. The similarity in the levels of AST and ALT showed that there was no liver damage by the diets. This was in line with the finding of Ekpenyong and Biobaku [24] who stated that the values of AST and ALT are normally low in blood but become high when there is occurrence of liver damage by toxic substances.

Table 2. Heamatologic of parameter of Broiler birds fed graded levels of clove bud meal

Parameter	Treatments				SEM
	Control	0.5kg/100kg	1kg/100kg	1.5/100kg	
Haemoglobin (g/dL)	6.93 ^b	7.21 ^{ab}	6.11 ^c	7.67 ^a	0.23
PCV (%)	20.32 ^{bc}	21.50 ^{ab}	18.33 ^c	23.25 ^a	0.71
RBC (x10 ⁶ /ul)	5.31	6.13	6.10	6.67	0.51
MCH (pg)	13.05 ^a	11.76 ^b	10.01 ^c	11.50 ^b	0.31
MCV (fl)	38.27 ^a	35.07 ^b	30.05 ^c	34.86 ^b	0.98
MCHC (g/dL)	34.10 ^a	28.27 ^b	33.33 ^a	33.00 ^a	1.21
WBC (x 10 ⁹ /L)	3.21	3.45	3.32	4.11	0.35
Neutrophils (%)	21.5	20.00	21.00	21.5	0.09
Eosinophil (%)	3.5	3.0	3.0	2.5	0.20
Lymphocytes (%)	75.00 ^c	77.00 ^a	76.00 ^b	76.00 ^b	0.31

a,b,c means in the same row with different superscripts are significant (P<0.05) different. RBC- Red blood cells, MCV- Mean corpuscular volume, MCH- mean corpuscular haemoglobin, MCHC- mean corpuscular haemoglobin concentration, WBC-white blood cells

Table 3. Serum Biomarkers of Broiler birds fed graded levels of clove bud meal

Parameters	Treatments				SEM
	Control	0.5kg/100kg	1kg/100kg	1.5/100kg	
Albumin (g/dl)	2.67 ^b	2.70 ^b	3.00 ^a	2.97 ^a	0.03
Globulin (g/dl)	3.90 ^a	3.90 ^a	3.51 ^b	3.92 ^a	0.04
Total protein (g/dl)	6.57 ^b	6.60 ^b	6.51 ^b	6.89 ^a	0.06
HDL (Mmol/L)	0.76 ^a	0.76 ^a	0.69 ^b	0.65 ^b	0.02
LDL (Mmol/L)	1.32	1.37	1.58	1.73	0.05
Triglycerides (Mmol/L)	0.98	0.93	0.96	0.89	0.06
Chloride (Mmol/L)	100.61	101.75	102.67	101.33	0.75
Cholesterol (mmol/L)	2.17 ^b	2.88 ^a	2.35 ^b	2.33 ^b	0.13
Urea (Mmol/L)	3.97 ^c	4.07 ^{bc}	4.97 ^{ab}	4.22 ^a	0.30
Glucose (Mmol/L)	2.38 ^c	2.99 ^b	3.02 ^b	3.11 ^a	0.20
AST (u/L)	24.85 ^b	31.50 ^a	27.50 ^{ab}	34.00 ^a	2.31
ALT (u/L)	19.61 ^b	27.31 ^a	24.65 ^{ab}	25.11 ^a	1.67

a,b,c means in the same row with different superscripts are significant (P<0.05) different

4. CONCLUSION

The study conclude that inclusion of clove buds meal up to 1.5kg/100kg in diet of broiler birds without adverse effect as in indicated by haematological and serum biochemical of the birds in this study.

DISCLAIMER

The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

ETHICAL APPROVAL

As per international standard or university standard written ethical approval has been collected and preserved by the authors.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Ferket PR, Gernat AG. Factors that affect feed intake of meat birds: A review. *International Journal of Poultry Science*. 2006;5(10):905-911.
2. Guo FC, HFJ. Savelkoul RP, Kwakkel BA. Williams, MWA Verstegen. Immuno-active, medicinal properties of mushroom and herb polysaccharides and their potential use in chicken diets. *World's Poult. Sci. J*. 2003;59:427-440.
3. Cruz OTB, Valero MV, Zawadzki F, Rivaroli DC, Prado RM, Lima BS, Prado IN. Effect of glycerine and essential oils (*Anacardium occidentale* and *Ricinus communis*) on animal performance, feed efficiency and carcass characteristics of crossbred bulls finished in a feedlot system. *Italian Journal of Animal Science*, 2014;13:790-797.
4. Windisch W, Schedle K, Plitzner C, Kroismayr A. Use of phytogenic products as feed additives for swine and poultry. *Journal of Animal Science*. 2008;86:E140-E148.
5. Fasseas MK, Mountzouris KC, Tarantilis PA, Polissiou M, Zervas G. Antioxidant activity in meat treated with oregano and sage essential oils. *Food Chemistry*. 2008;106:1188-1194.
6. SSMIYSC. Sokoto State Government Dairy. Ministry of Information and Youth, Sport and Culture. 2010;1-33.
7. Oluyemi JA, Roberts SA. Poultry production in warm wet climates. 2nd edition spectrum book ltd, Ibadan, Nigeria. 2000;244.
8. Bush BM. Interpretation of laboratory results from small animal clinician. United Kindom: Black Well Scientific Publication; 1991.
9. Jain NC. Essentials of Veterinary haematology (1st ed.).Philadelphia: Lea and Febiger publishers; 1993;1-18.
10. Schalm OW, Jain NC, Carrol EJ. Veterinary Haematology (pp. 15-218). USA: Lea and Febiger publishers, Philadelphia; 1975.
11. Savory JPH, Sunderman FW. A Buiret Method for the Determination of Protein in normal Urine. *Clinical Chemist*. 1968;14:1160-1171.
12. Reinhold JA. Manual Determination of Serum Protein, Albumin and Globulin Fractions by Buirete Method in Standard Method of Clinical Chemistry New York Academic Press. 1953;67.
13. Duncan, JR, Prasse KW, Mahaffey EA. Veterinary laboratory, medicine (clinical Pathology). Iowa State University Press: Ames. 1994;94-96.
14. Gbore FA, Ogunlade JT, Ewuola EO. Effects of Dietary Fumonisin on Organ Characteristics and some Serum Biochemical Parameters of Bucks. *Moor Journal of Agricultural. Research*. 2006;2:28-34.
15. Baker JF, Silverton RE, Cain JP. Introduction to Medical Laboratory Techniques. (5th ed., pp. 540-621). London Butterworth and Co-publishers Ltd.; 2007.
16. Akinola LAF, Etuk MO. Haematological and Serum Biochemical Responses of Broilers Fed Varying Levels of Indomie Waste-Based Diets. *Journal of Agriculture and Veterinary Science*. 2015;8(1):66-70 www.iosrjournals.org
17. Adejumo DO. Performance, organ development and haematological indices

- of rats fed sole diets of graded levels of cassava flour and soybean flour (Soyabean meal) as substitutes for energy and protein concentrates. *Tropical Journal Animal Science* 2004;7:57-63.
18. Oyawoye EO, Ogunkunle M. Chemical analysis and biochemical effects of raw beans on broiler. *Proceeding of Nigerian Society for Animal Production*. 1998;141-142.
 19. Siegel HS. Stress, strain and resistance. *British Poultry Science*. 1995;36(1):3-22.
 20. Scott A. Absorption of carbohydrate and protein metabolism. In: *Duke's Physiology of Domestic Animals*. 18 edn. (Swenson, M. J. eds). Cornell University Press Limited, London; 1970.
 21. Njidda AA, Igwebike JU, Ngoshe AA, Tijjani, AO. Effect of substituting maize with graded levels of cane molasses on the performance of broiler finisher birds in the semi- arid region of Nigeria, *J. Sustainable Agric. Environ*, 2006;8(1):1-13.
 22. Duwa HE, Oyawoye O, Njidda AA. Haematological Responses and Serum Biochemical Indices of broilers Fed Differently Processed Sorrel Seed (*Hibiscus Sabdariffia*) Meal in Semi-Arid Region of Nigeria. *British Journal of Poultry Sciences*. 2012;1(1):05-10.
 23. Banerjee GCA. *Textbook of Animal Husbandry*, 8th ed., Oxford and IBH Publishing Co. PVT. Ltd., New Delhi, India. 2009;118-139.
 24. Ekpenyong TE, Biobaku WO. Growth response of rabbits fed activated sewage sludge and dry poultry waste. *Journal of Rabbit Research*. 1986;9(1):14-16.

© 2021 Usman et al.; This is an Open Access article distributed under the terms of the Creative Commons Attribution License (<http://creativecommons.org/licenses/by/4.0>), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

Peer-review history:
The peer review history for this paper can be accessed here:
<https://www.sdiarticle4.com/review-history/75277>