

Haematological and Serum Biochemistry of Broiler Chickens Fed Diets Containing Enzyme Supplemented Cocoa Bean Shell as Partial Replacement for Maize

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ABSTRACT

Citation

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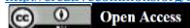
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A study was conducted to determine the haematological and serum biochemical parameters of broiler chickens fed diets containing enzyme supplemented Cocoa Bean Shell (CBS) as partial replacement for maize. A total of 150 day-old broiler chicks (Abor Acre Plus) were randomly assigned into five dietary treatments: T1 (0% CBS), T2 (5% CBS), T3 (10% CBS), T4 (15% CBS), and T5 (20% CBS), with enzyme inclusion in T2–T5 at 10g/kg. The experiment lasted for eight weeks and blood samples were collected via wing vein for haematology and serum biochemistry. Data collected for haematology were White blood cells, eosinophil, basophil, monocytes, lymphocytes, red blood cells, haemoglobin, mean corpuscular volume, mean corpuscular hemoglobin, mean corpuscular haemoglobin concentration and for the serum: Total protein, glucose and cholesterol) were subjected to Analysis of Variance (ANOVA) using SAS and means with significant differences separated using Duncan's Multiple Range Test. Results obtained indicated that the inclusion of enzyme-supplemented CBS significantly ($P<0.05$) influenced the blood parameters. Haematological parameters showed that red blood cell count and haemoglobin were significantly ($P<0.05$) higher in T3 ($2.57 \times 10^6/\mu\text{l}$ and 11.47 g/dl, respectively), while white blood cell count was lowest in T5 ($64.35 \times 10^3/\mu\text{l}$). Serum total protein was highest ($P<0.05$) in T5 (5.68 g/dl), while cholesterol was lowest in T5 (141.30 mg/dl) compared to T1 (203.33 mg/dl). In conclusion, including Enzyme-Supplemented Cocoa Bean Shell (CBS) as a partial maize replacement significantly influenced the blood and serum parameters of broiler chickens. Feeding 10% CBS improved red blood cell count and haemoglobin, indicating better health, while 20% CBS reduced white blood cells but increased total protein and lowered cholesterol, suggesting possible immune suppression despite some metabolic benefits. Therefore, based on the findings, a moderate 10% inclusion is recommended for enhancing health without compromising immunity.

Keywords: Haematology, Serum, Broiler chickens, Enzyme, Cocoa bean shell, Maize, replacement

Introduction

Agro-industrial by-products (AIBPs) are increasingly being used in broiler chicken diets as a sustainable and cost-effective alternative to traditional feedstuffs. While they offer several nutritional benefits, their impact on the haematological and serum characteristics of broiler chickens is an area of ongoing research. Haematological and serum parameters provide valuable insights into the overall health status of birds. They can be affected by various factors, including nutrition, environment, and disease. The inclusion of AIBPs in broiler diets can influence these parameters in several ways (Georganas *et al.*, 2023). The CBS possesses a nutritional profile encompassing fibre, polyphenols, and minerals, which theoretically contribute to enhanced gastrointestinal health and antioxidant capacity, potentially indirectly supporting robust haematopoiesis and erythrocyte functionality (Olumide *et al.*, 2017).

Empirical investigations into the effects of CBS on broiler haematology and serum biochemistry have yielded heterogeneous outcomes. Certain studies have demonstrated no statistically significant adverse effects on key haematological indices, including erythrocyte and leukocyte counts, haemoglobin concentration, and packed cell volume (PCV), at moderate inclusion levels, with some instances

reporting improvements potentially attributable to the antioxidant activity of CBS constituents (Lakurbe *et al.*, 2018). Conversely, other investigations have documented negative impacts, particularly at higher inclusion rates. Olumide *et al.* (2017) observed no significant differences in red blood cell count, packed cell volume, and haemoglobin values across treatments. White blood cell counts, and globulin levels increased with higher CBS inclusion, indicating a potential immune response. On serum biochemistry, the total protein, creatinine, and glucose levels were within acceptable ranges across all treatments, while cholesterol levels decreased as CBS inclusion increased, possibly due to anti-nutritional factors like saponins in CBS (Olumide *et al.*, 2017).

Enzyme supplementation offers a promising approach to improving the nutritional quality of CBS by degrading complex fibers and reducing anti-nutritional compounds, thereby enhancing nutrient availability. Evaluating haematological and serum biochemical parameters in broiler chickens fed enzyme-supplemented CBS is essential to assess the physiological and health impacts of such dietary inclusion. Monitoring these blood parameters provides vital information on the birds' immune status, metabolic function, and overall well-being, beyond mere growth performance.

Therefore, investigating the haematological and serum biochemical responses to enzyme-supplemented CBS inclusion is justified to support sustainable poultry nutrition, optimize feed formulations, and promote the utilization of agro-industrial by-products in animal feeding systems. Therefore, the objective of this study is to evaluate the effect of enzyme supplemented cocoa bean shell as partial replacement for maize on the blood profile of broiler chickens.

Methodology

Study location

This study was carried out at the Poultry Unit of the Teaching and Research farm of Animal Science Department, Faculty of Agriculture, University of Abuja, in Gwagwalada Area Council of the Federal Capital Territory, Abuja. The project site lies between latitude 8.917⁰N, 7.181⁰E at an average elevation of 476m above sea level. The mean annual rainfall in the area is 1267mm lasting from March to October. The annual mean temperature is 27.2⁰C with maximum and minimum daily temperatures of 32⁰C and 24⁰C respectively, with relative humidity of about 68.7% (Balogun, 2001).

Collection/sun-drying of cocoa bean shell

Cocoa bean shell which is the test ingredient was collected from Cocoa Bean processors at Premium Cocoa Products, Ile Oluji, in Ondo State, spread out thinly on polythene sheets to remove foreign particles. The CBS was turned at regular intervals to prevent fermentation and to allow even drying until constant weight was obtained as described by Adeyina *et al.* (2012). The dried cocoa bean shell was packed and well-stored for use in experimental diets.

Preparation of experimental diets

The sun-dried Cocoa Bean Shell was used along with other feed materials to formulate the experimental diets and fed to the experimental birds (broilers). Other materials for the feed were purchased from reputable sources. Five isonitrogenous and isocaloric diets (NRC, 1994) were formulated with dried cocoa bean shell included in the diets at 0, 5, 10, 15 and 20%, respectively for the starter mash and finisher mash. The sun-dried cocoa bean shell which is the experimental test material was used to formulate the experimental diet at a supplementation level of 0% (T1), 5% (T2), 10% (T3), 15% (T4) and 20% (T5), respectively as seen in Tables 1 and 2. Smartchoice ®phytase enzyme was purchased from commercial feed input distributors and added at 10g/kg to treatment diets excluding the control diet T1 (0% CBS).

Table 1: Composition of broiler starter diet

Ingredients	T1(0%CBS)	T2(5%CBS)	T3(10%CBS)	T4((15%CBS)	T5(20% CBS)
Maize	47.50	45.25	42.75	40.38	38.00
CBS	0.00	2.38	4.75	7.13	9.50
Wheat bran	7.00	7.00	7.00	7.00	7.00
Fish Meal	2.50	2.50	2.50	2.50	2.50
Soya Bean cake	31.00	31.00	31.00	31.00	31.00
Groundnut cake	8.50	8.50	8.50	8.50	8.50
Bone meal	2.70	2.50	2.50	2.50	2.50
Vitamin Premix	0.25	0.25	0.25	0.25	0.25
Salt	0.25	0.25	0.25	0.25	0.25
L-Lysine	0.60	0.60	0.60	0.60	0.60
Methionine	0.30	0.30	0.30	0.30	0.30
Total	100.00	100.00	100.00	100.00	100.00
®Phytase (FTU/kg)	0.00	0.006	0.012	0.018	0.024

Calculated Analysis

Crude Protein (%)	23.03	23.02	23.01	23.01	23.00
Crude Fibre (%)	4.68	4.89	4.90	4.91	4.94
ME (Kcal/kg)	2,807.00	2,805.00	2,804.00	2,803.00	2,801.00

CBS = Cocoa bean shell

Table 2: Composition of Broiler Finisher Diets

Ingredients	T1(0%CBS)	T2 (5%CBS)	T3(10%CBS)	T4(15%CBS)	T5(20%CBS)
Maize	56.50	53.68	50.85	48.03	45.20
CBS	0.00	2.83	5.65	8.48	11.30
Wheat bran	3.00	3.00	3.00	3.00	3.00
Fish meal	2.50	2.50	2.50	2.50	2.50
Soybean Meal	26.00	26.00	26.00	26.00	26.00
Groundnut cake	8.50	8.50	8.50	8.50	8.50
Bone meal	3.00	3.00	3.00	3.00	3.00
Vitamin Premix	0.25	0.25	0.25	0.25	0.25
Salt	0.30	0.30	0.30	0.30	0.30
L-Lysine	0.40	0.40	0.40	0.40	0.40
Methionine	0.06	0.06	0.06	0.06	0.06
Total	100.00	100.00	100.00	100.00	100.00
®Phytase (FTU/kg)	0.00	0.006	0.022	0.018	0.024
Calculated Analysis					
Crude Protein (%)	21.09	21.07	21.05	21.03	21.01
Crude Fibre (%)	4.04	5.33	6.18	6.75	7.48
ME (Kcal/kg)	3,010.00	3,007.00	3,005.00	3,003.00	3,001.00

CBS = Cocoa bean shell

Experimental animal, design, and management

Experimental pens, equipment and metabolic cages were swept, cleaned, and disinfected prior to the arrival of the experimental birds. A total of one hundred- and fifty-(150) day-old broilers (Abore Acre Plus) were purchased from a reputable hatchery Farm (Vertex Agrie Solution Ltd), Ibadan, Oyo State, Nigeria and used for the experiment. The birds were weighed and randomly allotted to five treatment groups with thirty (30) birds per treatment and three replicates of ten birds each in a completely randomized design (CRD). The chicks were brooded for two (2) weeks and necessary routine

vaccination and medication were administered. Feed and water were supplied *ad-libitum* and the experiment lasted for eight weeks. Other daily management operations were carried out as outlined according to Oluyemi and Robert (1979) in Olumide and Hamzat (2021).

Collection of blood and blood analysis

At the end of the 8 weeks, blood samples were collected from the birds for haematological and serum biochemical studies by the technique of John and Lewis (1991). The birds were bled by the wing vein using hypodermic needle with syringe. One into a container with Ethylene Diamine Tetra Acetic Acid (EDTA) as an anti-coagulant while the other for serum collection had no anti-coagulant. The haematological parameters measured include white blood cells, eosinophil, basophil, monocytes, lymphocytes, red blood cells, haemoglobin, mean corpuscular volume, mean corpuscular hemoglobin, mean corpuscular haemoglobin concentration. Serum biochemical indices investigated include total protein, glucose and cholesterol

Statistical Analysis

Data obtained on the blood profile were subjected to Analysis of Variance (ANOVA) using SAS (SAS, 2004) and means were separated using Duncan's Multiple Range Test.

Results

Haematology of Broiler Chickens Fed Enzyme-Supplemented Cocoa Bean Shell as Partial Replacement for Maize

Table 3 shows the haematology of broiler chickens fed enzyme-supplemented cocoa bean shells as a partial replacement for maize. The white blood cells indicated that T3 had significantly ($P<0.05$) higher values ($114.70 \times 10^3/\text{ul}$) though similar to T4 ($109.57 \times 10^3/\text{ul}$) and the least was found in T5. However, the highest ($P<0.05$) Eosinophil and Basophil were obtained in T1 and T2, respectively while the highest ($P<0.05$) monocytes, lymphocytes and red blood cells were found in T4, T5, T3, respectively. The T3, T5, T2 and T1 had significantly ($P<0.05$) higher Haemoglobin, mean corpuscular volume, mean corpuscular haemoglobin and mean corpuscular haemoglobin concentration, respectively. Mean corpuscular haemoglobin concentration was the lowest ($P<0.05$) in T5 (30.70) than other treatments.

The study revealed variations in the haematological profiles of broiler chickens fed enzyme-supplemented cocoa bean shells (CBS) as a partial replacement for maize. The treatment with 20% inclusion level of CBS, exhibited the lowest concentration of white blood cells (WBCs) compared to all other treatments. This observation suggests a potential immune-modulatory effect of the enzyme-treated CBS at this concentration. This aligns with the report of Mamun *et al.* (2024) who indicated that certain dietary fibres and polyphenols, components present in CBS, can influence the activity of immune cells. It's also plausible that the reduced WBC count in T5 could be attributed to decreased exposure to antigens or stressors within that treatment (Mamun *et al.*, 2024). The WBC differentials provided a more nuanced understanding of the immune response. Eosinophil counts, associated with allergic reactions and parasitic infections, were highest in the control treatment and lowest in both T3 and T5. These variations likely reflect differences in immune responses to specific dietary components or environmental influences. Basophil counts, involved in inflammation and allergic reactions, peaked in treatment 2 (5% inclusion of ETCBS), suggesting a distinct response to the CBS inclusion level in this treatment. Monocyte levels, crucial for immune surveillance and inflammation, were lowest in T5, potentially linked to the overall reduction in WBCs or a specific effect of the enzyme-treated CBS on monocyte production or migration. Interestingly, while the total WBCs were lowest in T5, this treatment exhibited the highest lymphocyte count. Lymphocytes are central to adaptive immunity, and this elevation in T5, despite the lower total WBC count, may indicate a shift towards cell-mediated immunity or a compensatory mechanism (Fabre *et al.*, 2009; Olumide and Hamzat, 2021). Meanwhile, the red

blood cell parameters, treatment 3 (10% inclusion of ESCBS) demonstrated the highest red blood cell (RBC) counts and haemoglobin (Hb) concentrations. This finding suggests that the enzyme-treated CBS in the T3 diet may have enhanced iron availability or stimulated erythropoiesis, the process of RBC production. This aligns with studies demonstrating the capacity of certain dietary components to improve iron absorption and promote RBC production (Kim and Nemeth, 2015; Olumide and Hamzat, 2021).

The analysis of red blood cell indices showed notable differences among the treatments. The mean corpuscular volume (MCV), which measures the average size of RBCs, was greatest in T5 and smallest in T3. Mean corpuscular hemoglobin (MCH), reflecting the average hemoglobin content per RBC, was highest in T2 and T4. These variations in MCV and MCH might be associated with differences in iron levels, nutrient supply, or rates of red blood cell production across the dietary groups. Additionally, mean corpuscular hemoglobin concentration (MCHC), indicating the average hemoglobin concentration inside RBCs, was lowest in T5, potentially pointing to changes in hemoglobin synthesis or RBC development, as noted by Olumide and Hamzat (2021) and Manar *et al.* (2024).

Serum Biochemistry of Broiler Chickens Fed Enzyme-Supplemented Cocoa Bean Shell as a Partial Replacement for Maize

Table 4 shows the serum biochemistry of broiler chickens fed enzyme-supplemented cocoa bean shell as a partial replacement for maize. The results revealed significant ($P < 0.05$) effects in all the serum biochemical parameters. The T5 had significant ($P < 0.05$) higher value (5.68 g/dl) for total protein followed by T3 (4.91 g/dl) and the least was found in T4. The T3 had significantly ($P < 0.05$) higher glucose (403.67 mg/dl) and the least was found in T1 while higher ($P < 0.05$) cholesterol (203.33 mg/dl) and the least cholesterol was observed in T5 (141.30 mg/dl).

Total serum protein concentrations showed significant variations, with the highest value observed in T5 (20% ESCBS) at 5.68 g/dl, closely followed by T3 (10% ESCBS) at 4.91 g/dl. The lowest total protein concentration was recorded in T4 (15% ESCBS). Elevated total protein levels, particularly in T5 and T3, could indicate an improvement in protein synthesis and utilization. This might suggest that enzyme supplementation at these levels effectively released more amino acids from the CBS, making them available for protein synthesis, including immune proteins (Olumide and Hamzat, 2021). Alternatively, a robust immune response to dietary components could also elevate total protein, though this would need to be corroborated with other immune markers. The lower value in T4 might suggest an imbalance or reduced digestibility at that specific inclusion level, potentially due to an anti-nutritional effect outweighing the enzyme benefit. The significantly higher glucose in T3 suggests that the combination of 10% CBS and enzyme supplementation led to more efficient carbohydrate digestion and absorption, providing a readily available energy source. Enzymes likely improved the breakdown of complex carbohydrates in CBS, enhancing glucose availability for metabolic processes. This finding aligns with the role of exogenous enzymes in improving nutrient digestibility and energy utilization in poultry diets (Adeyemo *et al.*, 2014). The higher glucose level could also reflect a sustained energy supply beneficial for growth. A striking finding was the significant cholesterol-lowering effect observed in T5 (20% ESCBS), which recorded the lowest cholesterol concentration at 141.30 mg/dl, compared to the highest in T1 (control) at 203.33 mg/dl. This reduction in serum cholesterol is a beneficial outcome, potentially improving the overall health profile of the birds and the nutritional value of their meat. This cholesterol-lowering effect in T5 can be primarily attributed to the inherent fiber content of CBS. Dietary fiber is known to bind bile acids in the intestine, promoting their excretion and consequently reducing cholesterol reabsorption and stimulating new bile acid synthesis from cholesterol. Furthermore, CBS is rich in polyphenols, which are bioactive compounds that have been shown to possess hypocholesterolemic properties by inhibiting cholesterol synthesis or increasing its excretion (Marcel *et al.*, 2011). The enzyme treatment might have enhanced the release or activity of these beneficial

components from CBS, contributing significantly to the observed reduction in cholesterol levels in the T5 group.

Table 3: Haematology of broiler chickens fed enzyme-supplemented cocoa bean shells as a partial replacement for maize

	T1	T2	T3	T4	T5	SEM
White blood cells (x 10³/ul)	113.77 ^a	93.63 ^b	114.70 ^a	109.57 ^a	64.35 ^c	9.50
Eosinophil (%)	3.00 ^a	2.27 ^c	1.90 ^d	2.73 ^b	1.90 ^d	0.22
Basophil (%)	1.33 ^c	2.63 ^a	1.57 ^b	1.13 ^c	1.50 ^b	0.26
Monocytes (%)	5.00 ^c	5.77 ^{ab}	5.43 ^b	6.00 ^a	3.55 ^d	0.43
Lymphocytes (%)	86.37 ^b	85.37 ^c	86.93 ^b	85.80 ^c	89.50 ^a	0.73
Red blood cells (x 10⁶/ul)	2.12 ^c	2.32 ^b	2.57 ^a	2.33 ^b	2.31 ^b	0.07
Haemoglobin (g/dL)	9.73 ^d	10.60 ^b	11.47 ^a	10.77 ^b	10.30 ^c	0.28
MCV (fl)	138.67 ^c	139.37 ^{bc}	137.50 ^d	140.70 ^b	146.25 ^a	1.53
MCH (pg)	45.90 ^b	46.60 ^a	44.90 ^c	46.40 ^a	44.85 ^c	0.37
MCHC (g/dL)	33.10 ^a	33.40 ^a	32.63 ^b	32.97 ^b	30.70 ^c	0.48

Means with different superscripts differ ($P < 0.05$) significantly; ESCBS = Enzyme supplemented cocoa bean shell; T1=0%ESCBS, T2 =5%ESCBS, T3=10%ESCBS, T4=15%ESCBS, T5=20%ESCBS; SEM=standard error of mean

Table 4: Serum biochemistry of broiler chickens fed enzyme-supplemented cocoa bean shell as a partial replacement for maize

Treatment	T1	T2	T3	T4	T5	SEM
Total protein (g/dl)	4.73 ^c	4.50 ^d	4.91 ^b	4.36 ^e	5.68 ^a	0.19
Glucose (mg/dl)	329.33 ^c	335.33 ^c	403.67 ^a	371.15 ^b	338.07 ^c	21.62
Cholesterol (mg/dl)	203.33 ^a	186.67 ^b	175.10 ^c	179.32 ^c	141.30 ^d	6.91

Means with different superscripts differ ($P < 0.05$) significantly; ESCBS = Enzyme supplemented cocoa bean shell; T1=0%ESCBS, T2 =5%ESCBS, T3=10%ESCBS, T4=15%ESCBS, T5=20%ESCBS; SEM=standard error of mean

Conclusion

The study demonstrated that dietary inclusion of enzyme-supplemented cocoa bean shell (CBS) as a partial replacement for maize significantly affected haematological and serum biochemical parameters in broiler chickens. Broilers fed 10% CBS with enzyme supplementation (T3) exhibited improved red blood cell count and haemoglobin concentration, indicating enhanced oxygen-carrying capacity and potentially better health status. Conversely, the highest inclusion level (20% CBS, T5) resulted in the lowest white blood cell count but the highest serum total protein and lowest cholesterol, suggesting metabolic adaptations that may influence immune function and lipid metabolism. Overall, moderate inclusion levels of enzyme-supplemented CBS can positively influence broiler health parameters, while higher inclusion warrants careful consideration. Incorporating enzyme-supplemented CBS at around 10% of the diet is recommended to improve haematological health indicators without compromising immune parameters while 20% CBS inclusion altered some serum parameters favorably (reduced cholesterol), the lowered white blood cell count suggests potential immune suppression, necessitating further investigation before recommending such high levels in practical feeding.

References

- Adeyemo, G. O., Ajayi, A. O. and Olubamiwa, O. O. (2014). Performance of broilers fed graded levels of bio-detoxified cocoa bean shell based diets. *Journal of Experimental Agriculture International*, 4(2):123-130.
- Adeyina, A.O., Apata, D.F., Annongu, A.A., Olatunde, O.A., Alli, O.I. and Okupke, K.M. (2012). Performance and physiological response of weaner rabbits fed hot water treated cocoa bean shell-based diet. *Research Journal, Animal Veterinary Science*, 5: 53–57.



- Balogun, O. (2001). *The Federal Capital Territory of Nigeria: A geography of its development*. Ibadan University Press.
- Fabre, V., Beiting, D. P., Bliss, S. K., Gebreselassie, N. G., Gagliardo, L. F., Lee, N. A., Lee, J. J. and Appleton, J. A. (2009). Eosinophil deficiency compromises parasite survival in chronic nematode infection. *Journal of Immunology* 182(3): 1577–1583. <https://doi.org/10.4049/jimmunol.182.3.1577>
- Georganas, A., Giamouri, E., Pappas, A. C., Zoidis, E., Goliomytis, M. and Simitzis, P. (2023). Utilization of Agro-Industrial By-Products for Sustainable Poultry Production. *Sustainability*, 15(4): 3679-3685. <https://doi.org/10.3390/su15043679>.
- Kim, A. and Nemeth, E. (2015). New insights into iron regulation and erythropoiesis. *Current opinion in haematology*, 22(3): 199–205.
- Lakurbe, O.A., Doma, U.D., Bello, K.M. and Abubakr, M. (2018). Haematological and serum biochemical indices of broiler chickens fed sorghum SK5912 (*Sorghum bicolor* L. Moench) variety as a replacement for maize. *Nigerian Journal of Animal Production*, 45(3): 242-247.
- Marcel, B.G., Andre, K.B., Theodore, D. and Seraphin, K.C. (2011). Waste and by-products of cocoa in breeding: Research synthesis. *International Journal of Agronomy and Agricultural Research*, 1: 9 – 19.
- NRC (1994). *Nutrient Requirement of poultry* (8th revised edition). National Academy Press. Washington D.C., USA. 112Pp.
- Olumide, M.D., Aknisoyinu, A.O. and Hamzat, R.A. (2017). Evaluation of performance, carcass characteristics, serum biochemistry and hematological parameters of broilers fed graded levels of raw cocoa bean shell-based diet. *Nigerian Journal of Animal Production*, 44(3): 210-221.
- Olumide, O. A. and Hamzat, O. A. (2021). Hematological and serum biochemical profile of broilers fed diets containing processed plant by-products. *Journal of Animal Physiology and Animal Nutrition*, 105(4): 678-688.
- Oluyemi, J.A. and Robert, F.A (1979). *Poultry production in warm wet climates*. MacMillan London.
- SAS (2004). *SAS/STAT User's Guide*, version 8, Cary, NC: SAS Institute Inc