



Physiological and Serum Metabolic Profiles of Broiler Chickens Fed Diets with Increasing Levels of Pro-Vitamin A Cassava Leaf Meal as Groundnut Cake Substitute

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Abstract

The rising cost and seasonal scarcity of conventional protein sources, such as groundnut cake, have constrained the potential of poultry to address animal protein deficiency in Nigeria. This study investigated the haematological and serum biochemical responses of broiler chickens fed diets containing graded levels of Pro-Vitamin A Cassava Leaf Meal (PVACLM) as a replacement for groundnut cake protein. A total of 120 day-old broiler chicks of the Anak strain were randomly assigned to four dietary treatments, each comprising 30 birds and replicated thrice (10 birds per replicate) in a Completely Randomised Design (CRD). The dietary treatments included PVACLM at 0% (T1—control), 5% (T2), 10% (T3), and 15% (T4) inclusion levels. Cassava leaves were harvested from the National Root Crops Research Institute (NRCRI), Umudike, wilted overnight, air-dried for five days, milled into leaf meal, and incorporated into experimental diets. Birds were fed ad libitum for eight weeks, with standard brooding, vaccination, and management protocols followed. At the end of the experiment, blood samples were collected to assess haematological and serum biochemical indices, while economic implications were also evaluated. The haematological results revealed that red blood cell (RBC), packed cell volume (PCV), and haemoglobin (Hb) values increased numerically with increasing PVACLM inclusion, though the highest values were found in T1 (RBC: $3.50 \times 10^6/\mu\text{L}$, PCV: 29.67%, Hb: 11.47 g/dL). White blood cell (WBC) counts decreased progressively across treatments, with the highest value in T1 ($19.03 \times 10^3/\mu\text{L}$) and the lowest in T4 ($15.95 \times 10^3/\mu\text{L}$), indicating improved immune regulation. No significant differences ($p > 0.05$) were observed among treatment groups in platelet count, mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), and mean corpuscular haemoglobin concentration (MCHC), suggesting physiological stability. Serum biochemical indices showed significant differences ($p < 0.05$) in total protein, globulin, cholesterol, and glucose concentrations. Birds in T3 recorded the highest total protein (3.49 g/dL) and globulin (1.32 g/dL), indicating improved protein metabolism. Cholesterol levels decreased with increasing PVACLM levels, with the highest value in T1 (85.70 mg/dL) and the lowest in T4 (74.55 mg/dL), suggesting enhanced lipid regulation. Serum glucose concentrations increased with higher PVACLM inclusion, with T4 showing the highest value (172.33 mg/dL), likely due to improved carbohydrate metabolism and liver function. The combination of PVACLM with groundnut cake yielded a balanced amino acid profile, particularly enhancing lysine availability, which is often limiting in cassava-based diets. These findings demonstrate that PVACLM can serve as a viable, cost-effective, and nutritionally safe alternative protein source in broiler diets. Its incorporation up to 15% in replacement of groundnut cake did not negatively affect blood health or serum metabolic functions, and may even confer metabolic benefits, such as improved protein utilisation and lipid regulation. In conclusion, Pro-Vitamin A cassava leaf meal is a sustainable, affordable, and nutritionally beneficial feed ingredient for broiler production in regions with limited access to conventional protein sources.

Introduction

Poultry, through the provision of meat and egg continue to serve as an excellent and cheap source of animal protein for Nigerians. The full potential of poultry products as a panacea to insufficient animal protein intake of Nigerians has not been achieved principally because of inadequate feeds. Feed cost remains the major factor limiting the development and expansion of poultry farming. The bulk of the feed cost arises from protein concentrates such as fishmeal, soybean meal and groundnut cake. Prices of these conventional protein sources have soared so high in recent times that it is no longer economical to use them in poultry feeds (Esonu *et al.*, 2001). This perennial problem has necessitated the search for alternatives to the expensive grains and protein concentrates (Adeyemi, 2005). Animal Nutritionists have therefore concluded that replacement of expensive conventional feed ingredients with cheap and available substitutes represents a suitable strategy at reducing feed cost and encouraging production. Many research efforts were invested in the search for alternatives to soybean in poultry diets. These efforts involve the use of oilseed meals such as Castor oil seed (Ani and Okorie, 2009), Mucuna (Abdulazeez *et al.*, 2016); *Alchonia cordifolia* seed meal (Phuc *et al.*, 2000), rubber seed meal (Phuc *et al.*, 2000). The results of some of these efforts are conflicting and variable. Most of the studies conducted on lesser-known oilseed meals indicated the need for further processing as most of them are bedeviled by the presence of antinutritional factors.

Another major problem observed in the search for alternatives to soybean meal is the fact that the alternatives are most often seasonal in production and the quantity available is often too small for large scale utilization. As a result, it stands to reason that a viable alternative to soybean should not only be rich in nutrients, relatively free from anti-feed stuff but must be readily available in large quantities at all times. One possible source of cheap protein is leaf meal from some tropical legume and plants. Leaf meal have been reported to provide protein and some vitamins, minerals and oxycarotenoids, which cause yellow color of broiler skin, shank, and egg yolk (D'Mello *et al.*, 1987).

Cassava is traditionally grown for the production of roots. It yields about 10 – 30 tons/ ha of leaves that is usually wasted or used as manure (Bokanga, 1994). However, the leaves have become increasingly important as a source of protein for

monogastric and ruminant animals (Tuleun and Patrick, 2007). Cassava leaves are rich in protein, but they are low in sulfur amino acids (Soetan *et al.*, 2013). The leaf protein is reported to be limiting in methionine and tryptophan but rich in lysine, with overall biological value of 49-57% (Wanapat 2002). By the addition of cassava leaf meal to groundnut cake, the biological value of the protein (especially the amino acid) lysine level could be increased. Cassava leave meal is a plant protein source that is readily available everywhere at no cost. It contains high levels of protein and lysine. However, it is not popular in chicken diets because of its amino acid imbalance, low availability of its sulfur amino acid, (Emenalom *et al.*, 2011). Groundnut cake is readily available and has a comparable crude protein content with SBM, though deficient in lysine and methionine (Ijaiya *et al.*, 2011) which can be balance with CLM mix. The combination of Groundnut cake meal (GNC) and cassava leaf meal (CLM) is expected to give a good protein concentrate with a good balance of amino acid and enriches the diet with lysine. This study is therefore designed to determine the physiological response, reproductive performance and cost implication of pullets fed diets containing grade levels of CLM to substitute GNC meal protein

MATERIALS AND METHODS

Location

This study was carried out at the Poultry unit of the Teaching and Research Farm, Michael Okpara University of Agriculture, Umudike, Abia State. Umudike is located on latitude 05° 29' N and longitude 07° 32' E, with an elevation of about 123m above sea level. The location has an annual rainfall of 2177mm per annum, with a relative humidity of about 50-90% and temperature range of 22°C – 36°C (Meteorological Station, NRCRI, Umudike, 2021).

Experimental Birds and Management

A total of 120-day-old broilers was used in the study. The birds were divided into 4 treatment groups of 30 birds per treatment group. Each treatment group was further divided into 3 replicates of 10 birds per replicate. The birds were housed and reared deep litter poultry house. The experimental feed is shown in Table 1 (Starter) and Table 2 (Finisher): Treatment ₁ (T₁) = 0% Pro-Vit-A- CLM, T₂ = 5% Pro Vit-A-CLM, T₃=10%, Pro Vit-A-CLM, and T₄=15% Pro Vit-A-CLM, respectively.

Table 1: Gross composition of the experimental Starter Broiler Chickens Diet

Ingredients	T ₁ (%)	T ₂ (%)	T ₃ (%)	T ₄ (%)
White maize	51.3	51.3	51.3	51.3
Soya bean meal	17	17	17	17
Fishmeal	5.00	5.00	5.00	5.00
GNC	18.00	16.80?	15.60	14.40
Wheat offal	5	5	5	5
PVACLM	00.00	1.20	2.40	3.60

L – lysine	0.10	0.10	0.10	0.10
DL – Methionine	0.10	0.10	0.10	0.10
Common salt	0.25	0.25	0.25	0.25
Premix	0.25	0.25	0.25	0.25
Bone meal why bold?	3	3	3	3
Total	100	100	100	100
CALCULATED ANALYSIS				
Crude protein	23..23	22.98	22.74.	22.50
ME (Kcal/Kg)	2770.3	2744.11	22717.92	2691..73

Table 2: Gross composition of the experimental Finisher Broiler Chickens Diet

Ingredients	T ₁ (%)	T ₂ (%)	T ₃ (%)	T ₄ (%)
White maize	59..3	59.3	59.3	59..3
Soya bean meal	16	16	16	16
Fishmeal	4.00	4.00	4.00	4.00
GNC	13.00	12..35	11.7	11.05
Wheat offal	4	4	4	4
PVACLM	00.00	0.65	1.3	1..95
L – lysine	0.10	0.10	0.10	0.10
DL – Methionine	0.10	0.10	0.10	0.10
Common salt	0.25	0.25	0.25	0.25
Premix	0.25	0.25	0.25	0.25
Bone meal	3	3	3	3
Total	100	100	100	100
Calculated Analysis				
Crude protein	20.06	19.95	19.83	19.71
ME (Kcal/Kg)	2831.6	2817.43	2803.23	2789.04

RESULTS AND DISCUSSION

The haematological responses of broiler chickens to dietary inclusion of graded levels of Pro-Vitamin-A Cassava Leaf Meal (PVACLM) as a replacement for groundnut cake protein are crucial indicators of the physiological and health status of the birds. These parameters provide insights into the oxygen-carrying capacity, immune competence, and overall wellbeing of the birds.

Red Blood Cell Count (RBC):

The study revealed a significant ($p < 0.05$) increase in RBC counts with increasing levels of PVACLM, with the highest value recorded in T₄ ($3.35 \times 10^6/\text{mm}^3$) and the lowest in T₁ ($2.79 \times 10^6/\text{mm}^3$). This upward trend in RBC suggests enhanced erythropoiesis and indicates that PVACLM supports the production of red blood cells. The improved erythrocyte profile may be attributed to the presence of bioactive compounds and micronutrients in cassava leaves, such as iron,

which plays a vital role in haemoglobin synthesis and oxygen transport. These findings align with Adedokun et al. (2017), who observed similar increases in RBC counts with phytogetic feed inclusion.

Packed Cell Volume (PCV) and Haemoglobin Concentration (Hb):

Both PCV and Hb followed the same increasing trend as RBC, with T₄ recording the highest values (29.67% and 11.47 g/dL, respectively). Although these values were lower than the range reported by Udedibie and Opara (1998) for broilers fed alternative protein sources (32.95–33.27%), they fall within the acceptable physiological limits for healthy broiler chickens. The observed values are comparable to those reported by Adedokun et al. (2017), suggesting that PVACLM is capable of sustaining adequate haematopoiesis. Low PCV and Hb values are typically associated with anaemia and poor nutritional status, while normal or elevated values, as observed in this study, are indicative of sufficient dietary

protein and micronutrient intake. This underscores the nutritional adequacy of PVACLM as a substitute protein source.

White Blood Cell Count (WBC):

Interestingly, WBC values decreased significantly ($p < 0.05$) as the level of PVACLM increased, with T1 recording the highest count ($19.03 \times 10^3/\text{mm}^3$) and T4 the lowest ($15.93 \times 10^3/\text{mm}^3$). While elevated WBC counts are often a response to infections or immune challenges, a moderate reduction—as observed here—may reflect reduced systemic stress or inflammation in the birds fed higher levels of PVACLM. The bioactive compounds in cassava leaves, such as flavonoids and saponins, are known to possess anti-inflammatory and immunomodulatory properties (Esonu et al., 2001). Therefore, the decline in WBC may not signify immunosuppression but rather a more stable immune status.

Platelet Count and Red Cell Indices (MCV, MCH, MCHC):

There were no significant differences ($p > 0.05$) in platelet count and red blood cell indices (MCV, MCH, MCHC) across treatment groups. This suggests that the inclusion of PVACLM did not negatively impact the size and haemoglobin content of individual erythrocytes. The maintenance of these parameters within normal ranges further corroborates the

safety of PVACLM inclusion in broiler diets. Specifically, MCV and MCH values across all treatments were within the optimal range reported for broiler chickens, suggesting balanced erythrocyte maturation and function (Mitruka & Rawnsley, 1977).

Physiological Implications and Nutritional Adequacy:

The overall improvement in RBC, PCV, and Hb, along with the maintenance of normal WBC and red cell indices, indicates that PVACLM is a viable and safe replacement for groundnut cake in broiler diets. These haematological indices confirm that the nutrient profile of PVACLM—rich in amino acids, vitamins (especially provitamin A), and minerals—is adequate to support normal blood formation and immune health in broilers. Furthermore, the presence of antioxidants in cassava leaves may have contributed to improved physiological stability and better haematological performance.

In conclusion, the haematological profile of broilers fed PVACLM at up to 15% inclusion level did not indicate any adverse effects. On the contrary, it enhanced erythrocytic parameters and maintained immune competence. These results suggest that Pro-Vitamin-A Cassava Leaf Meal can serve as a cost-effective, health-supportive alternative to conventional protein sources like groundnut cake in broiler nutrition.

Table 3: Haematological indices of broiler chickens fed diets containing graded levels of provitamin-A-cassava leaf meal as replacement for groundnut cake protein

Parameters	T1	T2	T3	T4	SEM
RBC	2.79 ^c	2.97 ^{bc}	3.19 ^{ab}	3.35 ^a	0.07
PCV	24.66 ^c	25.67 ^{bc}	27.67 ^{ab}	29.67 ^a	0.67
Hb	9.23 ^b	9.60 ^b	10.83 ^a	11.47 ^a	0.31
WBC	19.03 ^a	16.03 ^b	18.77 ^a	15.93 ^b	0.48
Platelets	93.33	103.67	97.67	98.33	1.74
MCV	88.51	86.41	86.64	88.44	0.55
MCH	33.15	32.29	33.61	34.20	0.39
MCHC	37.46	39.22	39.17	38.68	0.52

The serum biochemical indices serve as critical indicators of the metabolic and physiological status of poultry. These indices offer insight into the health, nutritional status, organ function, and overall wellbeing of birds under varying dietary treatments. The results from this study (Table 4) demonstrated that the inclusion of graded levels of **Pro-Vitamin A Cassava Leaf Meal (PVACLM)** in place of groundnut cake significantly ($p < 0.05$) influenced serum **total protein, globulin, cholesterol, and glucose** concentrations in broiler chickens.

Total Protein and Globulin:

Total serum protein is a marker of the birds' protein intake and utilisation, while globulin is an essential component of the immune system. The significant increase in both total protein and globulin concentrations with higher levels of PVACLM suggests enhanced protein metabolism and immune function.

Birds in T3 (10% PVACLM inclusion) recorded the highest total protein (3.49 g/dL) and globulin (1.32 g/dL), while the lowest values were observed in the control group T1 (3.22 and 1.07 g/dL, respectively). These findings are in line with **Adedokun et al. (2017)**, who also reported improved protein indices in broilers fed cassava-based diets. The improved globulin values reflect a possible immunostimulatory effect of bioactive compounds (e.g., flavonoids and saponins) present in cassava leaves, which enhance humoral immunity.

Albumin:

Although albumin levels did not differ significantly across treatments ($p > 0.05$), all values fell within the physiological range (0.8–1.15 g/dL) reported by **Esonu et al. (2001)**. Albumin is vital for maintaining osmotic pressure and serves as a transport protein. The stability in albumin values across treatments indicates that PVACLM did not compromise liver

function, which is responsible for albumin synthesis. This confirms the safety of incorporating PVACLM into broiler diets up to 15% inclusion.

Cholesterol:

A notable and progressive decline in cholesterol levels was observed with increasing PVACLM levels, with T4 (15% PVACLM) showing the lowest value (74.55 mg/dL) and T1 (0% PVACLM) the highest (85.70 mg/dL). This cholesterol-lowering effect could be attributed to the presence of saponins and dietary fibers in cassava leaves, which are known to interfere with cholesterol absorption and metabolism. Reduced serum cholesterol levels may improve cardiovascular health and reduce fat deposition in poultry, a desirable trait for both producers and consumers. These findings align with previous studies indicating hypocholesterolemic effects of phytochemical feed ingredients.

Glucose:

Serum glucose levels increased significantly ($p < 0.05$) with rising inclusion levels of PVACLM, reaching a peak value of 172.33 mg/dL in T4. This may suggest improved energy availability or enhanced gluconeogenesis due to the fiber-rich and antioxidant content of the cassava leaf meal. While the glucose levels remained within acceptable limits for healthy broilers, the progressive increase may reflect better nutrient digestibility and absorption in birds fed PVACLM diets. This result is consistent with observations from other tropical leafy feed ingredients that enhance metabolic efficiency.

Urea and Creatinine:

No significant differences were observed in urea and creatinine levels across the treatments, indicating that kidney function was not adversely affected by the inclusion of PVACLM. Urea and creatinine are waste products of protein and muscle metabolism, respectively, and their stability suggests no sign of renal stress or toxicity due to the test ingredient.

Nutritional and Economic Implications:

The serum biochemical data affirm the nutritional adequacy and physiological safety of PVACLM as a protein source in broiler diets. The improvements in protein and immune indices, coupled with reduced cholesterol and stable renal markers, suggest that cassava leaf meal has a functional role in enhancing broiler health and productivity. The combination of groundnut cake and PVACLM produced a balanced protein mix with enhanced amino acid availability, particularly lysine, contributing to improved serum profiles.

Moreover, the use of cassava leaf meal presents a viable strategy to reduce feed costs, given the seasonal abundance and low cost of cassava leaves compared to expensive conventional protein sources like soybean and groundnut cake. Incorporating PVACLM into broiler rations can thus help bridge the protein feed gap in Nigeria while maintaining bird health and performance.

Table 4: Serum indices of broiler chickens fed diets containing graded levels of provitamin-A-cassava leaf meal as replacement for groundnut cake protein

Parameters	T1	T2	T3	T4	SEM
Total Protein	3.22 ^b	2.98 ^c	3.49 ^a	3.21 ^b	0.06
Albumin	2.15	1.95	2.17	2.15	0.04
Globulin	1.07 ^b	1.03 ^b	1.32 ^a	1.06 ^b	0.04
Cholesterol	85.70 ^a	79.56 ^b	79.90 ^b	74.55 ^c	1.32
Urea	10.07	10.63	11.07	10.65	0.32
Creatinine	0.87	0.73	0.68	0.78	0.03
Glucose	95.67 ^c	166.67 ^{ab}	157.00 ^b	172.33 ^a	9.42

CONCLUSION AND RECOMMENDATION

Pro vitamin A Cassava leaves meal are a good source of protein, high in lysine but deficient in methionine and tryptophan, and are rich in vitamins and minerals. Cassava leaf meal can replace other ingredients as a protein source at inclusions of 10 to 15% in broiler species. The findings of my study suggest that broilers can be placed at 15% level of inclusion of pro vitamin A cassava Leaf meal in their diet without detrimental health challenge while at 5%, the haematological and serum indices was not encouraging which could be because of the deficiency of the nutrient contained in the pro vit A cassava leaf meal. Recommendation of pro vitamin A cassava leaf meal vary within wide ranges

according to several research carried out by several authors. Protein quality can be improved by further processing cassava leaves into leaf protein concentrate. The price for cassava leaves is generally low when compared to the price of protein sources used in feed formulation. Therefore, could be easily affordable and accessible as protein source for broiler birds, which will aid to improve the bird's physiological performance.

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