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Performance Characteristics and Blood Indices of Broilers Fed Varying Levels of Cassava (*Manihot esculenta* Crantz) Leaf Meal

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Abstract: Cassava leaves are a by-product of cassava root after harvest, and are highly nutritious. The potential of cassava leaves as protein substitute to conventional protein sources was investigated in broiler diet. One hundred and eighty "$Cobb_{500}$" unsexed two weeks old broilers were used to assess the partial substitution of groundnut cake with cassava leaf meal (CLM) on growth characteristics and blood indices in the research unit of the University of Ngaoundere. The birds were randomly alloted to four treatments groups of fourty five birds each in which CLM was incorporated at 0, 4%, 6% and 8% for treatments ML 0%, ML 4%, ML 6% and ML 8% respectively. Each treatment was replicated three times with fifteen birds per replicate in a complete randomize design. Treatment effect was evaluated on 09 birds / treatment at the end of the trial. FI (147.88 g), TG (2042.71 g), ADG (68.09 g), LW (2757.33 g), CW (2162.00 g), CY (96.04%), heart (0.55 g) and gizzard (1.91 g) as well as Plt ($3.56*10^9$/L) and TRIG (55.67 mg/dL) contents were higher ($P < 0.05$) in birds fed ML 6% diet. FCR (2.37) and PEC (2.51) were higher ($P < 0.05$) in birds fed ML 8% and Control diets. WBC ($125.40*10^9$/L), RBC ($2.51*10^{12}$/L), Hb (12.74 g/dL) and Ht (34.00%) contents and also serum levels in TP (2.64 g/dL), TOT CHOL (78.01 mg/dL), HDL (44.02 mg/dL), ASAT (209.14 IU), ALAT (19.22 UI) and in mineral [P (5.75 mEq/L), Ca (9.68 mEq/L and Mg (2.10 mEq/L)] were higher ($P < 0.05$) in birds consuming ML 4% diet. These results suggest that inclusion of *M. esculenta* leaf meal up to 6% supports optimum performances and improves blood indices of broilers.

Keywords: Broiler, Cassava Leaves, Growth Parameters, Blood Indices, Ngaoundere

1. Introduction

Poultry production remains the most wide spread of all livestock enterprises. Broiler production in particular represents a good source of protein, of income, and quick returns on investment [1]. Indeed, Broiler breeding is a short-cycle breeding that can produce good quality protein in 45 days at a lower cost. Unfortunately, feed cost remains the major factor limiting the development and expansion of broiler farming. Most of the feed cost comes from protein concentrates such as fish meal, soybean meal and peanut meal. Prices of these conventional protein sources have risen so high in recent times that it is no longer economical to use them in poultry feeds [2]. Replacement of expensive conventional feed ingredients with cheap and available substitutes represents a suitable strategy at reducing feed cost and encouraging production. One possible source of cheap protein is the leaf meals of some tropical legume browse plants. Leaf meals do not only serve as protein source but also provide some necessary vitamins, minerals and oxycaretenoids which cause yellow colour of broiler skin, shank and egg yolk [3].

Cassava leaves considered as a byproduct of cassava root harvest is (depending on the varieties) rich in protein (14 - 40% Dry Matter), minerals, Vitamin B1, B2, C and carotenes [4, 5]. Cassava leaves can yield more than 6 tons of crude protein per hectare a year with the proper agronomic practices directed towards foliage harvesting [6]. The nutritive value of cassava leaves trough available literature, clearly suggests that, apart from lower methionine, lysine and perhaps isoleucine content, the amino acid profile of cassava leaf protein compares favourably with those of milk, cheese, soyabean, fish and egg [7]. In spite of these good qualities, the nutritional potentials of cassava leaf meal and cassava protein concentrates remain currently under-researched [7]. The major drawback to the widespread use of cassava leaves as food is "cyanide scare" as its content of cyanogenic glucosides could, depending on the variety, be 6 times higher than in the roots. Apart from cyanide, tannin and possibly phytin [8] may limit the nutritional value of cassava leaves. Studies on the use of leaves and other vegetative parts of plants in monogastrics have shown that pretreatment through sun drying improves palatability, ingestion, growth and renders antinutritional factors inactive [9, 10].

The use of cassava (*Manihot esculenta*) as a protein source for poultry farming has been recently of high interest [3, 11]. Cassava leaves have quality attributes that makes it a potential replacement for soyabean meal, fish meal or groundnut cake in non-ruminant diets which could help cut down dependence on traditional protein sources use in poultry keeping [10, 12, 13]. Hence, the need therefore, to explore the use of non-conventional feed sources that have the capacity to yield the same output as conventional feeds, and perhaps at cheaper cost is the aim of this study.

2. Materials and Methods

2.1. Study Site

The study was conducted at the University of Ngaoundere, capital of the Adamawa region in Cameroon. This town is located between the 6th and 8th degrees of North latitude and between the 11^{th} and 15^{th} degree of East longitude on the Adamawa ridge. Ngaoundere is a transition zone between the northern lowlands and the southern Cameroon plateau. This position gives it a Sudano-Guinean climate with a rainy season of 8 months, from April to November and a dry season of 4 months, from December to March. The plant cover consists of Sudano-Guinean shrub savannah. The annual rainfall varies between 900 and 1500 mm. Average temperatures vary between 23 and 25°C. The region of Adamawa, thanks to its climate and its vegetation cover, is a zone of strong potentialities.

2.2. Sampling and Production of Cassava Leaf Meal

The processing technique of cassava (*Manihot esculenta* Crantz) was as earlier reported [10]. Briefly, the leaves of cassava harvested at the pre-flowering stage were separated from stems, pooled together and sun-dried until the leaves become crispy while still retaining the greenish coloration. The dried cassava leaves were then milled using a hammer grinding machine to produce cassava leaf meal (CLM) and stored in airtight condition until utilization.

2.3. Experimental Diets and Animal Management

Four iso-nitrogenous diets were formulated to meet the nutritional requirements of broilers during the growth-finishing [14] phase with partial substitution of peanut cake such that Diet 1 (ML 0%) contained no CLM and served as the Control, Diets 2 (ML 4%), 3 (ML 6%) and 4 (ML 8%) contained 4, 6 and 8% CLM respectively (Table 1). Each of the diets representing a treatment was analyzed for proximate composition [15]. One hundred and eighty (180) two 2 weeks old broilers of the "$Cobb_{500}$" strain obtained from the starter phase were balanced for weight and randomly assigned to the four dietary treatments in a complete randomized design. Each treatment group of 45 birds was further subdivided into three replicates of 15 chicks each and kept in a cage (size 2.2 x 1.9 x 2.45 m). Cages were all mounted in a house (9 m long and 4 m wide) built in hard, the upper third being made of wire mesh to allow good ventilation and sufficient natural light. The chicks were raised on deep litter (wood shavings) of good absorbent quality and artificial light (electric bulb) provided to encourage the birds to eat at night. Feed and water were provided *ad libitum* on a daily basis and the birds were subjected to standard management procedure. Feed intake and body weights were recorded weekly. The birds received proper vaccination care.

Table 1. *Chemical composition of the experimental diets.*

Feed Characteristics	**Calculated chemical composition**			
	ML 0%	**ML 4%**	**ML 6%**	**ML 8%**
ME (Kcal/Kg DM)	2926,71	2794,15	2661,2	2596,33
Crude protein (% DM)	20,75	20,69	20,23	20,36
Energy/Protein	128,62	134,40	132,86	128,77
Fat (% DM)	5, 34	4,19	4,06	4,30
Crude Fiber (% DM)	5,01	4,98	4,89	5,79

ME= Metabolisable Energy; DM= Dry Matter; ML 0% = control ration (with no *M. esculenta* leaf meal); ML 4% = ration containing 4% *M. esculenta* leaf meal; ML 6% = ration containing 6% *M. esculenta* leaf meal; ML 8% = ration containing 8% *M. esculenta* leaf meal.

2.4. Growth Performances Evaluation

Weekly weights were recorded to establish growth curve. The weight of the feed given, feed leftover and also weight of birds were recorded weekly and then the feed intake was determined by subtracting the leftover feed from total feed offered. The feed intake, average daily gain, feed conversion ratio [16] and the protein efficiency coefficient were calculated using the following formula [17].

$$Feed\ Intake\ (FI) = \frac{Total\ feed\ given\ (g) - Feed\ leftover\ (g)}{Experimental\ period\ (Number\ of\ days)}$$

$$Average\ daily\ gain\ (ADG) = \frac{Weight\ gain\ of\ the\ animal\ during\ a\ period\ (g)}{Duration\ of\ the\ period\ (in\ days)}$$

$$Feed\ conversion\ ratio\ (FCR) = \frac{Feed\ intake\ (g)}{Body\ weight\ gain\ (g)}$$

$$Protein\ efficiency\ coefficient\ (\text{PEC}) = \frac{Body\ weight\ gain\ (g)}{Protein\ intake\ (g)}$$

2.5. Slaughtering of Birds and Sample Collection

Carcass and organs characteristics were conducted according to the method described by Mweugang et al. [18]. Briefly At the end of the 30 days feeding trial, 9 birds per treatment (3 per replicates) were randomly selected, fasted overnight, then slaughtered, dressed and eviscerated. Weights of the visceral organs and carcass were recorded and expressed as percentage of live weight.

2.6. Blood Sample Collection

Blood samples (2.5 ml) were collected into labeled Ethylene-deamine-tetra-acetic acid (EDTA) treated tubes from three birds per replicate for a total of nine per treatment at the end of the 30 days trial for haematological analysis evaluation. Evaluations were performed on blood samples using an automated hematology analyzer (Humacount; Human, Weisbaden, Germany). The recorded parameters were white blood cells, red blood cells, hemoglobin, hematocrit and platelets.

Another blood sample (2.5 ml) collected in the sterile glass test tubes was allowed to coagulate at room temperature for 30 min and were subsequently centrifuged at 3000 g for 10 min. Serum was removed and stored frozen at (−4°C) until required analysis. Estimation of biochemical parameters in serum such as Total cholesterol [19], Triglycerides [20], high-density lipoprotein (HDL) [21] and Total proteins [22] were measured. The activity of transaminases, Aspartate aminotransferase (ASAT) and Alanine Aminotransferase (ALAT) [23] was determined.

Serum mineral content were determined as recommended by Hochleithner and Schwendenwein [24].

2.7. Statistical Analysis

Data collected were subjected to analysis of variance as described for completely randomized design [25], and differences between treatment means were separated using Duncan's New Multiple Range Test [26].

3. Results

3.1. Effect of Cassava Leaf Meal on Growth Performances of Broilers During Growth-Finishing Phase

3.1.1. Live Weight Evolution of Broilers

The weight evolution of chicks during the growth-finishing phase is a sigmoid curve similar in all batches (Figure 1). During weeks 2, 3 and 4, all animals presented a comparable evolution ($p>0.05$) whatever the treatment. However, at the end of the experiment (5th week), animals receiving Control, ML 4% and ML 6% diets had comparable and statistically ($P<0.05$) higher weight evolution than those receiving ML 8% diet.

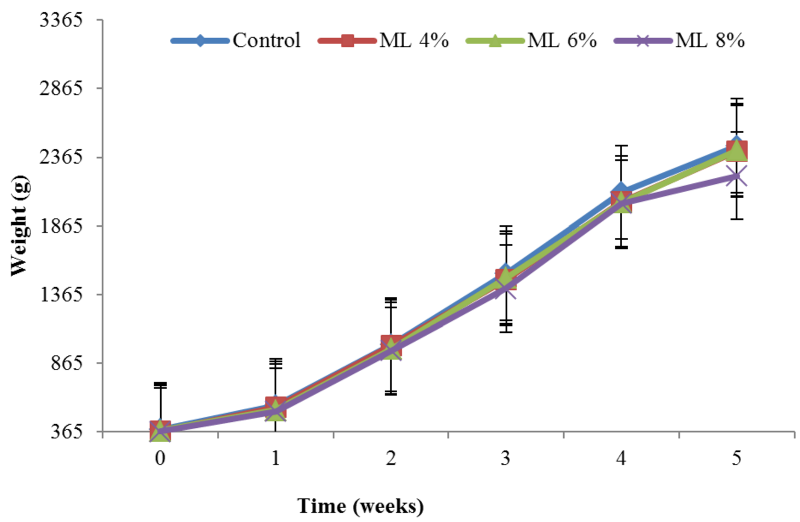

Figure 1. *Weight evolution of broilers during growth-finishing phase according to experimental diets.*

ML 0% = control ration (with no *M. esculenta* leaf meal); ML 4% = ration containing 4% *M. esculenta* leaf meal; ML 6% = ration containing 6% *M. esculenta* leaf meal; ML 8% = ration containing 8% *M. esculenta* leaf meal.

Table 2. *Growth characteristics of broilers during growing-finishing phase according to experimental diets.*

Growth parameters	Control 0%	Rations with Manihot leaf meal			Sem	P
		ML 4%	ML 6%	ML 8%		
FI	132.18	141.81	147.88	146.71	1.34	0.00
TG	2065.90	2038.14	2042.71	1861.09	20.63	0.00
ADG	68.86	67.93	68.09	62.04	0.69	0.00
FCR	1.92	2.09	2.17	2.37	0.02	0.00
PEC	2.51	2.30	2.30	2.10	0.02	0.00

FI=feed intake; TG=total gain; ADG=average daily gain; FCR=feed conversion ratio; PEC=protein efficiency coefficient; Sem= standard error of mean; P= probability. ML 0% = control ration (with no *M. esculenta* leaf meal); ML 4% = ration containing 4% *M. esculenta* leaf meal; ML 6% = ration containing 6% *M. esculenta* leaf meal; ML 8% = ration containing 8% *M. esculenta* leaf meal.

3.1.2. Growth Characteristics of Broilers

The substitution of peanut meal by *M. esculenta* leaf meal showed that birds receiving 6% and 8% ML diets had a comparable and statistically (P<0.05) higher feed intake (FI) and those in Control lower value (P<0.05) (Table 2). Animals fed ML 4% and ML 6% had total gains (TG) comparable to those of the Control and significantly (P<0.05) higher than those of ML 8% group. The same trend was observed in ADG. The feed conversion ratio (FCR) was decreasing as follows ML 8%> ML 6%> ML 4%> Control. The protein efficiency coefficient (PEC) of animals in the control batch showed a significantly (P<0.05) higher value and those receiving ML 8% showed significantly (P<0.05) lower value.

3.1.3. Characteristics of Carcass and Organs of Broilers

Birds fed Control and ML 6% diets presented a comparable and significantly (P<0.05) higher live weight (LW), in contrast to birds fed ML 8% which presented lower value (P<0.05) (Table 3). The same trend was observed for carcass weight (CW) and carcass yield (CY). Heart and gizzard of birds fed 6% and 8% ML diets were comparable and statistically (P<0.05) higher than those fed ML 4% diet. Heart and spleen did not differ significantly (P>0.05) whatever the ration.

Table 3. *Carcass and organ characteristics of broilers during growth-finishing phase according to experimental diets.*

Carcass parameters	Control ML 0%	Rations with Manihot leaf meal			Sem	P
		ML 4%	ML 6%	ML 8%		
LW (g)	2759.66	2646.17	2757.33	2595.00	51.14	0.08
CW (g)	2167.50	2116.33	2162.00	2018.50	37.95	0.04
CY (%)	86.54	85.04	86.04	84.79	28.45	0.02
Liver (g)	1.65	1.63	1.62	1.64	0.5	0.99
Heart (g)	0.42	0.43	0.55	0.54	1.12	0.01
Spleen (g)	0.10	0.09	0.09	0.10	0.1	0.64
Gizzard (g)	1.76	1.62	1.91	1.94	1.77	0.04

LW=live weight; CW=carcass weight; CY=carcass yield; Sem= standard error of mean; P=probability; ML 0% = control ration (with no *M. esculenta* leaf meal); ML 4% = ration containing 4% *M. esculenta* leaf meal; ML 6% = ration containing 6% *M. esculenta* leaf meal; ML 8% = ration containing 8% *M. esculenta* leaf meal.

3.2. Effect of Cassava Leaf Meal on Hematological Parameters of Broilers During Growth-Finishing Phase

White blood cells (WBC) of animals fed ML 4% was significantly (P<0.05) higher than that of animals of Control and ML 8% batches who presented comparable and significantly lower (P<0.05) levels (Table 4). Birds of ML 4% batch recorded significantly (P<0.05) higher RBC, Hb and Ht levels compared to birds of other batches (Control, ML 6%, ML 8%) which recorded lower values (P<0.05) (RBC) as well as birds of ML 8% batch (Hb and Ht). Birds receiving 6% ML presented significantly higher (P<0.05) Plt content while those receiving 4% ML presented lower (P<0.05) value.

Table 4. *Hematological parameters of broilers during growth-finishing phase according to experimental rations.*

Hematological parameters	Control ML 0%	Rations with Manihot leaf meal			Sem	P
		ML 4%	ML 6%	ML 8%		
WBC ($*10^9$/L)	117.27	125.40	122.17	117.29	2.22	0.05
RBC ($*10^{12}$/L)	2.26	2.51	2.30	2.21	0.04	0.00
Hb (g/dL)	68.86	12.74	12.13	11.40	0.2	0.00
Ht (%)	1.92	34.00	32.36	29.86	0.4	0.00
Plt ($*10^9$/L)	2.51	1.50	3.56	2.26	0.5	0.04

WBC=white blood cell; RBC=red blood cells; Hb=hemoglobin; Ht=hematocrit; Plt=blood platelets; Sem=standard error of mean; P=probability; ML 0%=control ration (with no *M. esculenta* leaf meal); ML 4% = ration containing 4% *M. esculenta* leaf meal; ML 6% = ration containing 6% *M. esculenta* leaf meal; ML 8% = ration containing 8% *M. esculenta* leaf meal.

3.3. Effect of Cassava Leaf Meal on Biochemical Parameters of Broilers During Growth-Finishing Phase

3.3.1. Protein Content of Broilers

The protein level went down as follows: ML 4%>ML 6%>Control>ML 8% (Figure 2).

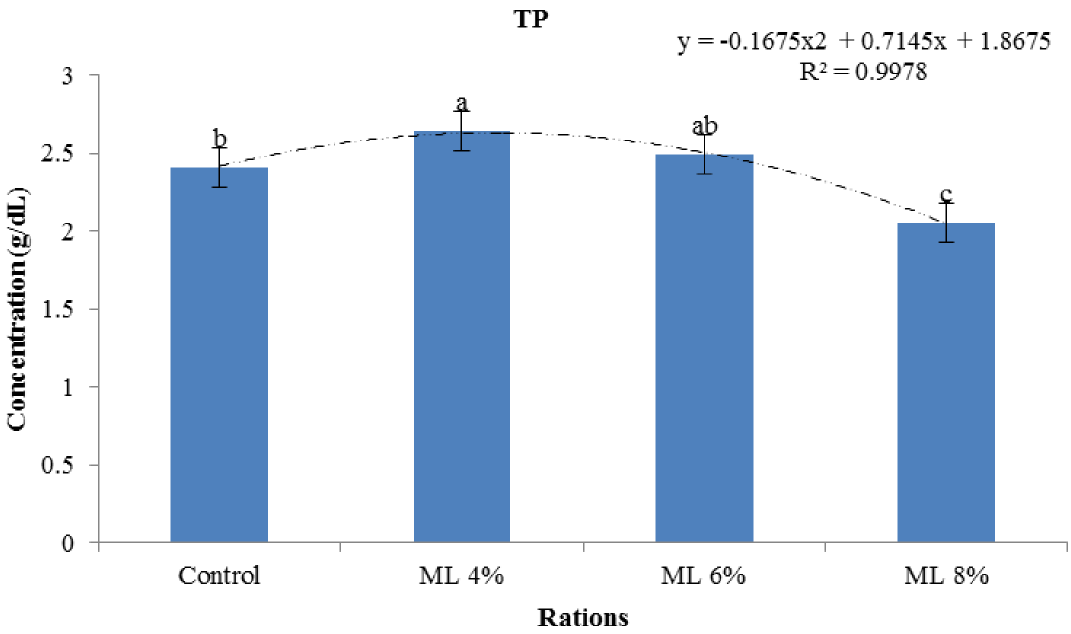

Figure 2. *Protein content of broilers during growth-finishing phase according to the experimental diets.*

ML 0% = control ration (with no *M. esculenta* leaf meal); ML 4% = ration containing 4% *M. esculenta* leaf meal; ML 6% = ration containing 6% *M. esculenta* leaf meal; ML 8% = ration containing 8% *M. esculenta* leaf meal.

3.3.2. Lipid Profile of Broilers

CLM supplementation induced higher total cholesterol level (TOT CHOL) ($P<0.05$) in birds of ML 4% batch and lower level ($P<0.05$) in birds of ML 8% batch (Figure 3). HDL level was higher ($P<0.05$) and comparable in supplemented groups and lower ($P<0.05$) in the Control batch. Animals subjected to 6% ML obtained higher ($P<0.05$) triglyceride (TRIG) content and those of the Control group lower ($P<0.05$) content.

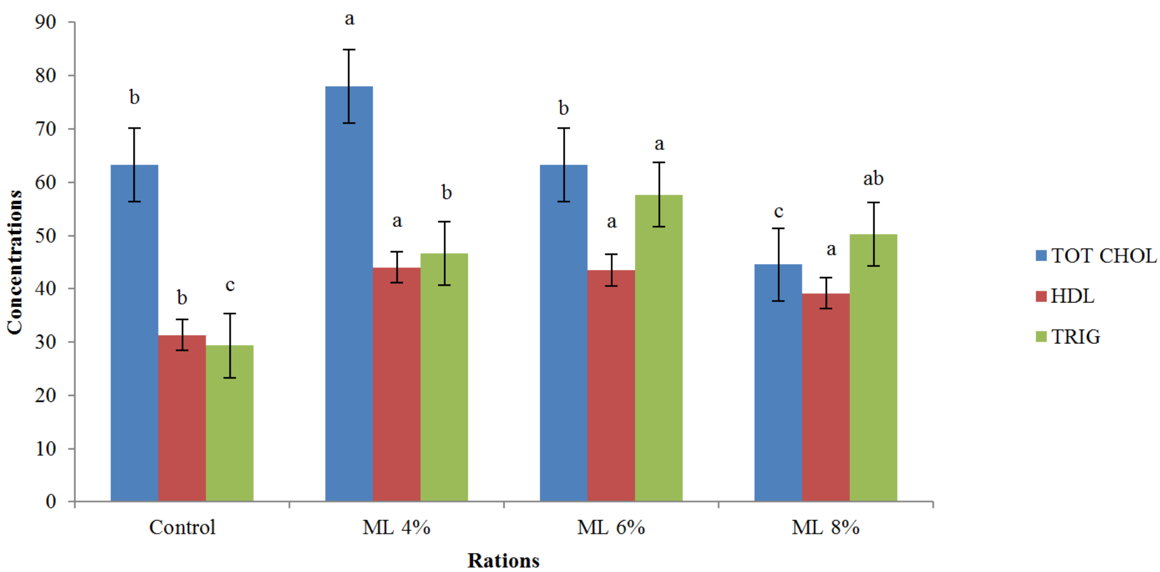

Figure 3. *Lipid profile of birds during growth-finishing phase according to experimental diets.*

ML 0% = control ration (with no *M. esculenta* leaf meal); ML 4% = ration containing 4% *M. esculenta* leaf meal; ML 6% = ration containing 6% *M. esculenta* leaf meal; ML 8% = ration containing 8% *M. esculenta* leaf meal; TOT CHOL= total cholesterol; HDL= High density Lipoproteins; TRIG= triglycerides.

3.3.3. Toxicity Contents of Broilers

The incorporation of CLM in the ration resulted in higher ($P<0.05$) AST level in animals fed ML 4% diet and lower ($P<0.05$) in animals fed ML 8% diet (Figure 4). ALAT content of birds consuming 0%, 6% and 8% CLM presented comparable values and statistically ($P<0.05$) lower than those of birds consuming 4% CLM.

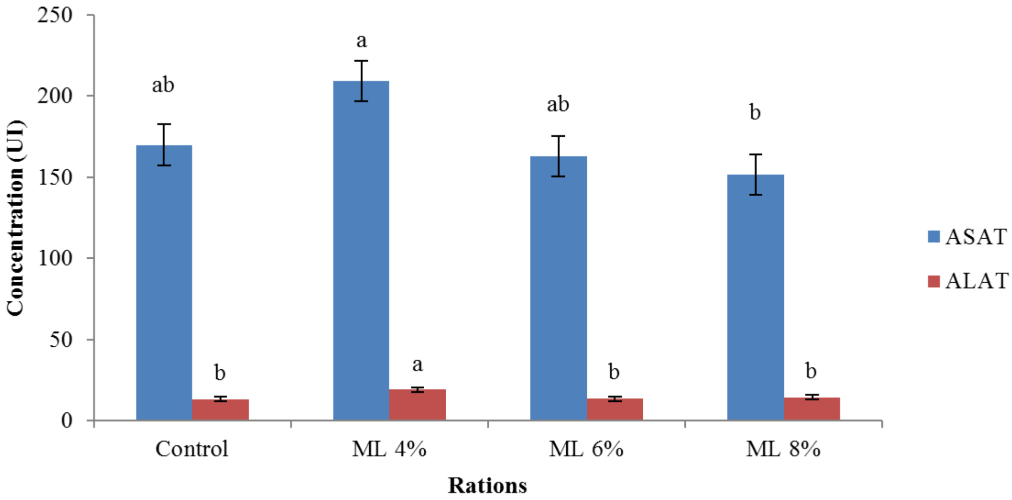

Figure 4. *ASAT and ALAT contents of birds during growth-finishing phase according to experimental diets.*

ML 0% = control ration (with no *M. esculenta* leaf meal); ML 4% = ration containing 4% *M. esculenta* leaf meal; ML 6% = ration containing 6% *M. esculenta* leaf meal; ML 8% = ration containing 8% *M. esculenta* leaf meal.

3.3.4. Effect of Cassava Leaf Meal on Mineral Contents of Broilers During Growth-Finishing Phase

Chickens from the Control batch recorded higher ($P<0.05$) P content while those receiving 6% and 4% CLM recorded higher ($P<0.05$) Ca and Mg values respectively (Table 5).

Table 5. *Mineral characteristics of broilers during growth-finishing phase according to experimental rations.*

Parameters (mEq/L)	Control 0%	Rations with Manihot leaf meal 4%	6%	8%	Sem	P
P	7.35	5.75	6.09	5.61	0.5	0.05
Ca	8.83	9.68	9.83	8.78	0.3	0.00
Mg	1.88	2.10	1.70	1.69	0.1	0.04

P=phosphorus; Ca=calcium; Mg=magnesium; Sem= standard error of mean; P=probability; ML 0% = control ration (with no *M. esculenta* leaf meal); ML 4% = ration containing 4% *M. esculenta* leaf meal; ML 6% = ration containing 6% *M. esculenta* leaf meal; ML 8% = ration containing 8% *M. esculenta* leaf meal.

4. Discussion

Proteins are one of the main components of cells, they have a building role and are involved in the growth of organisms [27]. The comparable weight increase observed in birds fed Control, 4% and 6% ML rations during the 5th week of the trial suggests that high inclusion level of CLM (8%) may not support growth performance of broilers at growth-finishing phase due to the presence of cyanogenetic glycosides and tannins which are predominant anti-nutrients in cassava leaves [3, 28, 29] responsible of retarding growth [10, 30] and lowering the digestibility and absorption of dietary nutrients [3, 10, 12, 13].

The observed increase in FI in animals fed up to 8% ML could be the result of the treatment applied to *M. esculenta* leaves because sun drying have been reported to improve palatability, nutrient and vitamin contents [9, 10]. Our results agree with those of Melesse et al [31] who reported that Rhode Island Red chicks fed on 2%, 4% and 6% *Moringa Stenopetala* leaf meal showed a significantly higher FI than control ones. This result is in contrast with the findings of Iheukwumere et al. [3] who supported the fact that above 5% inclusion in broilers diet, CLM depressed FI. Several works supported observations of these authors [32-35]. However, Tesfaye et al. [36] reported comparable FI in Ethiopia in broiler finishing ration (*Hubbard Classic*) fed increasing levels of *Moringa oleifera le*af meal (0%, 5%, 10%; 15% and 20%); Similarly, Oloruntola et al. [37] made the same observations in rabbits in Nigeria fed increasing levels of *Mucuna pruriens* leaf meal (0%, 4%; 8% and 12%); Findings of Eichie et al. [30] in Nigeria observed identical FI in all chickens fed graded levels (25%, 50%; 75% and 100%) of *Leucaena leucocephala* in finishing broilers compare to the control group. FI values of this study (147.88 g: ML 6% and 146.77 g: ML 8%) are all lower than the reference value of the Cobb$_{500}$ strain during the same period: 203 g reported by Cobb-Vantress [38] and higher than that reported by Zanu et al. [39]: 116.5 g; by Aka-Tanimo et al. [33]: 53.19 g. This difference can be explained by the low palatability of the other types of leaves used in these different studies. Indeed, the low consumption of the leaf-based ration would be due to the anti-nutritional factors present in the leaves such as terpenoids, lactones, pyrans and tannins (at the origin of the bitterness in a plant) which reduce the appetite of animals thus lowering their food consumption [40]. Our results confirm the high palatability of rations containing *M. esculenta* leaves [10, 12, 13, 41]. The drying time, the nature the composition of the different leaves, the strain and the age of animals used in these different works may also explain the differences.

The decrease in ADG of birds fed *M. esculenta* leaf meal could be attributed to anti-nutritional factors present in the feed that reduce the bioavailability of nutrients during digestion. These substances either act by complexing nutrients and preventing their absorption along the gastrointestinal tract, inhibiting the activity of enzymes responsible for their hydrolysis, or inducing toxicity at high doses [42]. The ADG obtained at the end of the trial (45 days) in birds receiving ML 4% (67.93 g) and ML 6% (68.09 g) treatments are comparable and significantly higher (P<0.05) than that reported by Aka-Tanimo et al. [33] in broilers fed 2.5% *M. indica* leaf meal as protein source (53.19g); by Tesfaye et al. [36] in broilers fed 5% *M. oleifera* leaf meal (24.7 g) and by Zanu et al. [39] in broilers fed 5% *L. leucocephala* leaf meal (34.2g).

The feed conversion ratio (FCR) is an important index of performance which is a direct indication of how best feed offered to birds was utilized for meat production. The lower the FCR value, the better the feed utilization [35]. Replacing groundnut cake with CLM at higher proportions in the diet was accompanied with low feed utilization compare to the Control. This result corroborates with earlier findings of several authors [30, 33, 35, 43]. The FCR (2.37: CL 8%) of this study is closed to values reported by Ansari et al. [43] (2.10), Eichie et al. [30] (2.66), Adedeji et al. [35] (2.56), Aka-Tanimo et al. [33] (2.09) and Sese et al. [42] (2.43) but lower than the value 3.4 reported by Zanu et al. [39]. But Beg et al. [32] reported no significant (P>0.05) difference in FCR among supplemented neem leaf meal groups of broiler chicken than antibiotic and control groups. Similarly, Alam et al. [44] found non-significant FCR in all Neem treated groups compared to that of control group of broiler. However, FCR alone is not sufficient to predict the efficiency of a protein as the amount of feed ingested does not always reflect the amount of protein ingested or assimilated. For this reason, other growth parameters that take into account the amount of protein ingested, such as the PEC, have been determined.

Proteins are firstly consumed to support body growth, and in this respect the ratio of growth weight to the ingested protein, known as PEC, above 2 are of good nutritional quality and allow good growth. In this study, groundnut cake meal proteins with a PEC of 2.51 are of good nutritional quality and *M. esculenta* leaf meal proteins with PEC of 2.30 (ML 4% and ML 6% respectively) and 2.10 (ML 8%) are also of good nutritional quality and hence can allow for good growth. Fasuyi et al. [45] observed a reduced PEC in broiler chicks fed varying levels of *A. cruentus* leaf meal which is concomitant to our results. However, Nworgu and Fasogbon [46] observed increased PER in growing pullets fed 2, 4 and 6% *Centrosema pubescens* leaf meal which is not in accordance with the current study.

CLM substitution to groundnut cake in broilers ration up to 6% increased yield of most carcass characteristics (LW, CW and CY) in the current study which appeared to be a consequence of increased FI and growth rate of birds suggesting a high protein uptake responsible for the weight increase [44]. On the other hand, the rise in weight gain is due to the presence of macro and micro minerals in *M. esculenta* leaf meal [7, 28]. The higher body weight gain in broilers consuming leaf meal could also be due to its appetite and digestion stimulating, anti-bacterial properties, which aid to lessenthe microbial load of birds and enhanced the feed consumption and feed efficiency of birds [47]. Indeed, as reported by Granum et al. [48], the beneficial effect of cassava leaves is thought to be due to their richness in condensed tannins, which give them anthelmintic properties. It seems that tannins act on the metabolism of larvae by preventing their migration in the organism, which would inhibit their development process.

Our result agrees with findings of Iheukwumere et al. [3] who reported improved carcass traits at 5% CLM in the diet of broilers in Nigeria contrary to Tesfaye et al. [36] who reported that *Moringa olifera* leaf meal substitutions in broilers ration reduced yield of most carcass characteristics. On the other hand, Ansari et al. [43] and Gayathri and Panda [47] reported improved carcass characteristics with graded levels of *Azadirachta indica* leaf meal fed to broilers. The values of ML 6% subjects in CY (86.04%) are higher than the values reported in other studies: 78.89% [35]; 72.41% [32], 72.60% [33]; 77.85% [34] and 81.3% [39] but our values are lower than the 91.03% value [36].

A proportionate increase in the size of heart and gizzard with increase in the level of CLM in the ration has been observed. Indeed birds fed with 6 and 8% CLM recorded the highest weights in these organs. This observation was also made by Gayathri and Panda [47] who reported higher relative weights of the heart and gizzard in broilers. Similarly, Onibi et al. [11] reported higher relative weight of the gizzard in broilers chicken finishers fed partial equi-protein replacement of soyabean meal with cassava and Leucaena leaf meals; Okpanachi et al. [49] also noted a proportionate increase in the size of gizzard with increase in the level of urea-treated and untreated rice milling waste in broilers. This increase could be as a result of increasing dietary fiber as CLM is gradually incorporated into the diet, leading to increased peristaltic movement and muscle tone as mentioned by Onibi et al. [11]. High fiber content of feeds has been reported to stimulate activity of gizzard resulting in increased musculature [11, 49, 50]. Similarly, the marked increase in heart weight of 6 and 8% CLM birds with increase in the level of CLM in the diets could be associated with higher fibre content which might have led to intense work on this organ who has to pump a lot of oxygenated blood to help detoxify toxins in any material [49].

Hematological parameters are good indicators of physiological, pathological and nutritional status of an animal. Changes in haematological parameters have the potential of being used to elucidate the impact of nutritional factors in diet on animals. With the exception of Plt content, all other parameters (WBC, RBC, Hb and Ht) were elevated in subjects fed with 4% CLM. This implies that cassava leaves improve blood parameters in chickens. Platelets are membrane bound cell fragments which accumulate at site of broken blood vessels to form clots. They have a big role in hemostasis, by preventing blood loss. Their level in the blood also followed the quality of ingested protein, lower for 4% CLM than for other level of CLM inclusion. The values of this study are within the reference values reported in broiler chicken [51] and are close to the values reported in other studies [30, 32, 33, 34, 39].

Total serum protein has been reported as an indication of the protein retained in the animal body [2]. The increasing total serum protein content of broilers receiving dietary CLM up to 6% inclusion suggests good protein retention in these birds as observed by Tesfaye et al. [36]. The protein values of this study are within the reference values reported in broiler chicken [52].

Variations recorded in lipid profile of birds supplemented to CLM as observed in this study could be due to the fact that protein, amino acid composition, lipid fractions, fiber and phytosterols legumes may have significant impact on the metabolic mechanisms that affect the intestinal microflora, cholesterol and triglyceride synthesis and excretion of bile acids [53]. The increased HDL (good cholesterol) in birds fed CLM as determined in the result is of health benefits to the consumers, especially those predisposed to heart diseases [54]. The Cholesterol and Triglycerides contents of this study are within the reference values reported in broiler chicken [52].

ALT and AST are important in the diagnosis of heart liver diseases and also the transamination in the metabolism of specific amino acids [55]. The elevated level of serum ALT in birds fed 4% CLM is indicative of liver damage. This result is not in agreement with that of Ansari et al. [43] who observed that decreased in the activities of ASAT and ALAT in serum evidenced the positive effect of leaf meal on liver parenchyma of broiler chicks fed *Azadirachta indica* dried leaf meal as phytogenic feed additive. The abnormally rising AST concentration indicates liver and biliary system disease, skeletal muscle disease, myocardial injury/diseases, haemolytic disorder and haemolysis [37]. ALT and AST contents of this study are within the reference values reported in broiler chicken [52].

Minerals are essential for broiler growth, and are involved in many physiological and biosynthetic processes in the body [56]. They function primarily as catalysts in enzyme systems, and are constituents of hundreds of proteins involved in intermediary metabolism, hormone secretion pathways, and immune defense system [57, 58]. Calcium is required for bone ossification, regulation of skeletal and cardiac muscle activity, activation of several enzymes, transmission of nerve impulses, hormone mediation, membrane permeability, blood coagulation and maintenance of osmotic pressure [59]. Phosphorus is also an important constituent of bones, but also of nucleic acids and phospholipids [60]. Magnesium is important, like calcium, in the transmission of nerve impulses and muscle contraction. Without it, phosphorus could not be well used by the body when it is involved in reactions that produce energy, because it is an activator of many ATPases. It is also active at the cellular level and has a role to play in protein synthesis. Generally, there was a pronounced improve of the serum mineral content of broilers that received dietary CLM up to 6% inclusion. This presumably may be due to the fact that leaf meals do not only provide protein, but also some essential vitamins such as vitamins A and C, minerals, etc. [7]. The mineral contents of this study are within the reference values reported in broiler chicken [52].

5. Conclusion

M. esculenta leaf meal can be included in the diet of broilers up to 6% without any deleterious effects on their performances, haematological indices, serum biochemical constituents and mineral content. The use of CLM should be encouraged and may lead to broilers low-cost production.

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References

[1] O. J. Alabi, A. D. Malik, J. W. Ng'Ambi, P. Obaje, and B. K. Ojo, "Effect of aqueous Moringa oleifera (Lam) leaf extracts on growth performance and carcass characteristics of hubbard broiler chicken," *Rev. Bras. Cienc. Avic.*, vol. 19, no. 2, pp. 273–280, 2017.

[2] B. O. Esonu, O. O. Emenalom, A. B. I. Udedibie, U. Herbert, C. F. Ekpor, I. C. Okoli, F. C. Iheukwuumere,, "Performance and blood chemistry of weaner pigs fed raw Mucuna (velvet ban) meal.," *Trop. Anim. Prod. Invest.*, vol. 4, pp. 49–54, 2001.

[3] F. Iheukwumere, E. Ndubuissi, E. Mazi, and M. Onyekwere, "Perfomance, nutrient utilization and organ characteristics of broilers fed cassava leaf meal (*Manihot escuenta* Crantz)," *Pakistan J. Nutr.*, vol. 7, no. 1, pp. 13–16, 2008.

[4] R. O. Eggum, "The protein quality of cassava leaves.," *Br. J. Nutr.*, vol. 24, pp. 761–768, 1970.

[5] S. R. A. Adewusi and J. H. Bradbury, "Carotenoid in cassava: comparison of open column and HPLC methods of analysis.," *J. Sci. Food. Agri.*, vol. 62, pp. 375–383, 1993.

[6] B. H. O. Huy, U. I. Nguyen, T. R. Preston, B. Ogele, and J. E. Lindberg, "Replacing of soya bean meal with cassava root diet for growing pigs.," *Res. Rural Dev.*, vol. November-D, 1995.

[7] A. O. Fasuyi, "Nutrient Composition and Processing Effect on Cassava Leaf Antinutrients," *Pakistan Journal of Nutrition*, vol. 4. pp. 37–42, 2005.

[8] W. R. Reeds, S. K. Sathe, and D. K. Salunkhe, "Phytater in legumes and cereals.," *Adv. Food Res.*, vol. 28, pp. 1–9, 1982.

[9] M. Dahouda, S. S. Toleba, A. K. I. Youssao, A. A. M. Ali, S. Ahounou, and J. L. Hornick, "Utilisation des cossettes et des feuilles de manioc en finition des pintades (Numida meleagris, L): Performances zootechniques, coûts de production, caractéristiques de la carcasse et qualité de la viande," *Ann. Med. Vet.*, vol. 153, no. 2, pp. 82–87, 2009.

[10] N. N. Mweugang, F. Tendonkeng, N. E. F. Matuimini, E. Miégoué, B. Boukila, and T. E. Pamo, "Influence of the Inclusion of Graded Levels of Cassava Leaf Meal in the Diet on Post Partum Weight and Pre- Weaning Growth of Guinea Pigs (*Cavia Porcellus* L.)," *Int. J. Agric. Innov. Res.*, vol. 2, no. 6, pp. 939–945, 2014.

[11] G. E. Onibi, O. R. Folorunso, and C. Elumelu, "Assessment of partial equi-protein replacement of soyabean meal with cassava and Leucaena leaf meals in the diets of broilers chicken finishers," *Int. J. Poult. Sci.*, pp. 408–413, 2008.

[12] N. N. Mweugang, F. Tendonkeng, E. Miégoué, F. E. N. Matuimini, G. T. Zougou, A. F. Fonteh, B. Boukila, T. E. Pamo, "Effects of inclusion of cassava leaf meal (*Manihot esculenta* Crantz) in the diet on reproductive performances of Cameroonian local guinea pig (*Cavia porcellus* L.).," *Int. J. Biol. Chem. Sci.*, vol. 10, no. 1, pp. 269–280, 2016.

[13] N. N. Mweugang, J. Lemoufouet, K. G. Nkana, E. Miegoue, A. F. Fonteh, and T. E. Pamo, "Intake and in vivo digestibility of Pennisetum purpureum K. Schum supplemented with graded level of cassava (*Manihot esculenta* Crantz) leaf meal in guinea pigs (*Cavia porcellus* L.)," *J. Exp. Appl. Trop. Biol.*, vol. 1, no. 1, pp. 35–45, 2021.

[14] National Research Council (NRC), *Nutrient Requirements of Poultry*, vol. 9th Editio, no. 1. 1994.

[15] AOAC, Association of Official Analytical Chemists. Official methods of analysis, 15th edition. Washington, DC. 1990.

[16] J. Del Carmen, A. G. Gernat, R. Myhrman, and L. B. Carew, "Evaluation of raw and heated velvet beans (Mucuna pruriens) as feed ingredients for broilers," *Poult. Sci.*, vol. 78, no. 6, pp. 866–872, 1999.

[17] I. A. Iyayi and V. O. Taiwo, "The effect of diets incorporating mucuna (*Mucuna pruriens*) seed meal on the performance of laying hens and broilers," *Trop. Subtrop. Agroecosystems*, vol. 1, pp. 239–246, 2003.

[18] N. N. Mweugang E. Miegoue, K. F. Djitie, M. Yacouba, M. Pelyang, T. Youssoufa, S. Madjou, Y. N. Njintang, T. E. Pamo, "Growth and Hematological Performances of Broilers Fed on Meal and Protein Isolate of *Mucuna pruriens* Seeds," *Anim. Vet. Sci.*, vol. 8, no. 6, pp. 117–123, 2020.

[19] V. P. Röschlau, E. Bernt, and W. Gruber, "Die Enzymatische Bestimmung Des Cholesterins Im Serum," *J. Clin. Chem. Clin. Biochem.*, vol. 12, no. 226, pp. 403–407, 1974.

[20] P. Fossati and L. Prencipe, "Serum Triglycerides Determined Colorimetrically with an Enzyme ThatProduces Hydrogen Peroxide," *Clin. Chem.*, vol. 28, no. 10, pp. 2077–2080, 1982.

[21] C. A. Burtis and E. R. Ashwood, Tietz textbook of clinical chemistry (3rd ed.). Philadelphia: W. B. Saunders Company, 1999.

[22] O. H. Lowry, N. J. Rosebrough, L. A. Farr, and R. J. Randall, "Protein measurement with the folin phenol reagent," 1951.

[23] S. M. D. Reitman and S. Frankel, "A colorimetric method for the determination of serum glutamic oxalacetic and glutamic pyruvic transaminases," *Planta Med.*, vol. 28, pp. 56–63, 1957.

[24] I. Hochleithner, M Schwendenwein, "Evaluation of two dry chemistry systems in pet bird medicine. Assoc Avian Vet Today," *Assoc Avian Vet Today*, vol. 2 (1), pp. 18-23, 1988.

[25] R. G. D. Steel and J. H. Torrie, "Principles and Procedures of Statistics. A Biometric Approach.(2nd ed.). McGraw-Hill Publishers, New York.," pp. 1–633, 1980.

[26] D. B. Duncan, "New Multiple Range Test. Biometrics," vol. 11, pp. 1–42, 1955.

[27] Y. Henry, B. Sève, A. Mounier, and P. Ganier, "Growth Performance and Brain Neurotransmitters in Pigs as Affected by Tryptophan, Protein, and Sex," *J. Anim. Sci.*, vol. 74, no. 11, pp. 2700–2710, 1996.

[28] O. A. Dada and O. O. Oworu, "Mineral and Nutrient Leaf Composition of Two Cassava (Manihot esculenta Crantz) Cultivars Defoliated at Varying Phenological Phases," *Not. Sci. Biol.*, vol. 2, no. 4, pp. 44–48, 2010.

[29] I. U. Udo and J. F. John, "Effect of processing methods on the utilization of cassava (Manihot esculenta Crantz) leaf meal (CLM) by african catfish (*Clarias gariepinus*)," *Livest. Res. Rural Dev.*, vol. 27, no. 8, pp. 1–8, 2015.

[30] F. Eichie, D. Akinyemi, and M. Adegbenro, "Effect of Replacement of Soybean Meal with Leucaena leucocephala Leaf Meal on Performance, Haematology, Carcass Measures and Organ Weight in Broiler Chickens," *Am. J. Exp. Agric.*, vol. 8, no. 5, pp. 327–334, 2015.

[31] A. Melesse, W. Tiruneh, and T. Negesse, "Effects of feeding Moringa stenopetala leaf meal on nutrient intake and growth performance of Rhode Island Red chicks under tropical climate," *Trop. Subtrop. Agroecosystems*, vol. 14, no. 2, pp. 485–492, 2011.

[32] A. H. R. M. Beg, Z. M. Uddin, M. Aftabuzzaman, T. A. M. Nahid, and M. Begum, "Efficacy of Neem Leaf (*Azadirachta indica)* Meal as an Alternative to Antibiotic in Broiler Ration," *Asian J. Res. Anim. Vet. Sci.*, vol. 4, no. 2, pp. 1–10, 2018.

[33] H. A. Aka-Tanimo, D. O. Oshibanjo, V. O. Adelowo, M. A. Akwashiki, I. W. Azi, C. J. Oguche, S. Haruna, Y. S. Afiniki, "Growth Performance, Carcass Characteristics and Heamatology Indices of Broiler Chicken Fed Graded Levels of (*Mangifera indica)* Mango Leaf Meal," *Asian J. Res. Anim. Vet. Sci.*, vol. 6, no. 2, pp. 20–27, 2020.

[34] S. G. A. Ramadan, "Impact of Supplementation of Moringa Oleifera in Diet of Broiler Chicks on Their Behavior, Welfare, Performance and Immune Responses," *Alexandria J. Vet. Sci.*, vol. 55, no. 2, pp. 50–59, 2017.

[35] O. S. Adedeji, S. Amao, and K. S. Olugbemiga, "Effect of *Sesamum indicum* leaves on growth performance and carcass characteristics of broiler chicken," *Niger. J. Anim. Sci. 2*, vol. 21, no. 1, pp. 222–232, 2019.

[36] E. Tesfaye, G. Animut, M. Urge, and T. Dessie, "Moringa olifera Leaf Meal as an Alternative Protein Feed Ingredient in Broiler Ration," *Int. J. Poult. Sci. 1*, vol. 12, no. 5, pp. 289–297, 2013.

[37] O. D. Oloruntola, S. O. Ayodele, S. A. Adeyeye, and J. O. Agbede, "Performance, haemato-biochemical indices and antioxidant status of growing rabbits fed on diets supplemented with Mucuna pruriens leaf meal," *World Rabbit Sci.*, vol. 26, no. 4, pp. 277–285, 2018.

[38] Cobb-Vantress, "Performances et recommandations nutritionnelles: poulet de chair," *cobb-vantres.com*, 2008.

[39] H. K. Zanu, M. Mustapha, and M. A. Nartey, "Response of broiler chickens to diets containing varying levels of leucaena (*Leucaena leucocephala*) leaf meal," *Online J. Anim. Feed Res.*, vol. 2, no. 2, pp. 108–112, 2012.

[40] N. Chauynarong, A. Elangovan, and P. Iji, "The potential of cassava products in diets for poultry," *Worlds. Poult. Sci. J.*, vol. 65, no. 1, pp. 23–35, 2009.

[41] T. R. Preston and L. Rodríguez, "Production and utilization of cassava foliage for livestock in integrated farming systems," *Livest. Res. Rural Dev.*, vol. 16, no. 5, pp. 1–9, 2004.

[42] B. T. Sese, M. Okpeku, and I. Patani, "Tropical Velvet Bean (Mucuna Utilis) Leaf Meal as Unconventional Protein Supplement in the Diet of Broiler," *J. Anim. Sci. Adv.*, vol. 3, no. 11, pp. 575–583, 2013.

[43] J. Ansari, S. H. Khan, A. Ul Haq, and M. Yousafb, "Effect of the levels of *Azadirachta indica* dried leaf meal as phytogenic feed additive on the growth performance and haemato-biochemical parameters in broiler chicks," *Journal of Applied Animal Research*, vol. 40, no. 4. pp. 336–345, 2012.

[44] N. Md. Alam, J. Uddin, D. T. Most. Akter, Z. I. Mst. Moni, F. Alom, A. Rahman, A. Md. Al Noman, "Broiler Production By Using Polyherbal Medication (Neem, Nishyinda, Tulsi and Turmeric Extract)," *Int. J. Innov. Appl. stdies*, vol. 9, no. 3, pp. 1161–1175, 2014.

[45] A. O. Fasuyi, F. A. S. Dairo, and A. O. Adeniji, "Tropical vegetable (Amaranthus cruentus) leaf meal as alternative protein supplement in broiler starter diets: Bionutritional evaluation," *J. Cent. Eur. Agric.*, vol. 9, no. 1, pp. 23–33, 2008.

[46] F. Nworgu and F. Fasogbon, "Centrosema (Centrosema pubenscens) leaf meal as protein supplement for pullets chicks and growing pullets," *Int. J. Poult. Sci.*, vol. 6, no. 4, pp. 255–260, 2007.

[47] S. L. Gayathri and K. A. Panda, "Utilization of Neem Leaf Meal in Poultry," *Poult. Line*, pp. 57–59, 2019.

[48] G. Granum, M. Wanapat, P. Pakdee, C. Wachirapakorn, and W. Toburan, "A comparative study on the effect of cassava hay supplementation in swamp buffaloes (*Bubalus bubalis*) and cattle (*Bos indicus*)," *Asian-Australasian J. Anim. Sci.*, vol. 20, no. 9, pp. 1389–1396, 2007.

[49] U. Okpanachi, P. U. Boyi, C. F. Egbu, and A. Oyibo, "Performance and Internal Organ Characteristics of Broiler Chickens Fed Urea-Treated and Untreated Rice Milling Waste," *Am. Inst. Sci.*, vol. 1, no. 4, pp. 130–135, 2015.

[50] J. C. Acton, L. Breyer, and L. D. Satterlee, "Effect of Dietary Fiber Constituents on the In Vitro Digestibility of Casein," *J. Food Sci.*, vol. 47, no. 2, pp. 556–560, 1982.

[51] A. Chettouh and S. Riabi, " Etude de quelques paramètres hématologiques et morphométriques chez le poulet de chair (Gallus gallus domesticus) face à une perturbation du régime alimentaire en région d'Ain Zaatout –Biskra-," Mémoire de Master, Université Mohamed Khider de Biskra, 59 p, 2019.

[52] N. Lakehal, "Normes et interpretations des dosages des paramètres biochimiques sanguins chez le poulet de chair," 2013.

[53] J. M. Martins, M. Riottot, M. C. De Abreu, M. J. Lança, A. M. Viegas-Crespo, J. A. Almeida, J. B. Freire, O. P. Bento, "Dietary raw peas (*Pisum sativum* L.) reduce plasma total and LDL cholesterol and hepatic esterified cholesterol in intact and ileorectal anastomosed pigs fed cholesterol-rich diets," *J. Nutr.*, vol. 134, no. 12, pp. 3305–3312, 2004.

[54] K. Mulatu, N. Ameha, and M. Girma, "Effects of feeding different levels of baker's yeast on performance and hematological parameters in broiler chickens," *J. World's Poult. Res.*, vol. 9, no. 2, pp. 38–49, 2019.

[55] O. M. Akanbi, "Performance and health implication of feeding Fungi treated cocoa pod husk meal on broiler," *Bull. Natl. Res. Cent.*, vol. 7, pp. 43–55, 2019.

[56] A. Piotrowska, K. Burlikowska, and R. Szymeczko, "Changes in blood chemistry in broiler chickens during the fattening period," *Folia Biol. (Praha).*, vol. 59, no. 3–4, pp. 183–187, 2011.

[57] A. G. Abdallah, O. M. El-Husseiny, and K. O. Abdel-Latif, "Influence of some dietary organic mineral supplements on broiler performance," *Int. J. Poult. Sci.*, vol. 8 (3): 291, no. 1682–8356, pp. 291–298, 2009.

[58] X. J. Yang, X. X. Sun, C. Y. Li, X. H. Wu, and J. H. Yao, "Effects of copper, iron, zinc, and manganese supplementation in a corn and soybean meal diet on the growth performance, meat quality, and immune responses of broiler chickens," *J. Appl. Poult. Res.*, vol. 20, no. 3, pp. 263–271, 2011.

[59] R. De Matos, "Calcium Metabolism in Birds," *Vet. Clin. North Am. - Exot. Anim. Pract.*, vol. 11, no. 1, pp. 59–82, 2008.

[60] M. Ansar, S. A. Khan, Z. I. Chaudhary, N. A. Mian, M. Y. Tipu, and M. F. Rai, "Effects of High Dietary Calcium and Low Phosphorus on Urinary System of Broiler Chicks," *Pak. Vet. J.*, vol. 24, no. 3, pp. 24–27, 2004.