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Feeding Rabbits (*Oryctolagus cuniculus*) Based on *Ocimum gratissimum* and *Vernonia amygdalina* Leaves in West Africa: Biochemical and Hematological Aspects

Pascal Abiodoun Olounladé[1, *], Basile Boni Saka Konmy[1, 2], Erick Virgile Bertrand Azando[3], Lamine Baba-Moussa[2], Sanni-yo Doko Allou[1]

[1]Zootechnical Research and Livestock System Unit, Laboratory of Animal and Fisheries Science (LaSAH), Doctoral School of Agricultural and Water Sciences (EDSAE), National University of Agriculture, Porto-Novo, Benin

[2]Laboratory of Biology and Molecular Typing in Microbiology, Faculty of Science and Technology, University of Abomey-Calavi, Cotonou, Benin

[3]Laboratory of Ecology, Health and Animal Production, Department of Animal and Fisheries Production Sciences and Techniques, Faculty of Agronomy, University of Parakou, Parakou, Benin

Email address:
abiodouno@yahoo.fr (P. A. Olounladé)

[*]Corresponding author

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Abstract: Several medicinal plants are used like fodder in the animal diet. The impact on the blood parameters of these plants is not always known. The present study aims to determine the influence of *Ocimum gratissimum* and *Vernonia amygdalina* leaves meal on the biochemical and haematological parameters of growing rabbits. Ninety-six rabbits New Zealand with 35 to 40 days old were used. The animals were randomly divided into 8 groups of 12 rabbits. Groups O_0, O_5, O_{10}, O_{15}, V_0, V_5, V_{10} and V_{15} received the ration contain 0%, 5%, 10% and 15% of *Ocimum gratissimum* or *Vernonia amygdalina* leaf meals respectively. All the rabbits had a blood sample taken. For *Ocimum gratissimum*, the RBC number, haemoglobin levels, glycaemia, total protein content, and PCV of the animals were not significantly different ($p<0.05$). However, White Blood Cells number was significantly ($p <0.001$) raised in group O_{10} ($7.97\pm0.15 \times 10^3$/L) Aspartate Aminotransferase levels (48.67±1.53 UI) increased significantly ($p <0.001$). Alanine Aminotransferase, blood creatinine and total cholesterol were significantly decreased in the test groups ($p <0.001$). For *Vernonia amygdalina*, the Haemoglobin and PCV values did not change significantly ($P> 0.05$). The results showed significant ($P <0.05$) differences in the average values of AST, ALT and ALP of treatments tested compared to control. The average values of glycaemia, urea, creatinine and total cholesterol presented a significant ($P <0.05$) decrease between the control and the treatments. These results revealed that *Vernonia amygdalina* leaf meal in rabbits' diet had ameliorative and protective health benefits up to 15%. It has no hepatotoxic and nephrotoxic effects. In conclusion, *Ocimum gratissimum* and *Vernonia amygdalina* supplemented feed significantly increased the growth performance, biochemical, and haematological profiles of the animals and was shown to have no harmful effects in rabbits.

Keywords: Rabbits, Haematological, Biochemical, *Ocimum gratissimum*, *Vernonia amygdalina*, Leaf Meal

1. Introduction

One of the breeding accessible to the rural and peri-urban population is rabbit breeding. Indeed, the rabbit offers several advantages to the population and to breeders [1]. It is a rapidly expanding activity in Benin. This breeding is

subjected to food constraints which slow down its development. Feeding is one of the important factors that determine the success and profitability of breeding. Feed represents 70% of the total production costs in breeding [2]. Rabbit breeders are also encountering feeding problems because of the increase in the number of livestock industry in most African countries. Nowadays rabbit breeding has solely depended on concentrates for their animals. Incorporating unconventional food into rabbit feed remains an alternative solution to overcome this difficulty. In the world the species of flowering plants recorded are more than 300,000. So far, fewer than 1% of them have been explored for use in feeding and against diseases [3]. Plants can be used as a replacement for current antiparasitic drugs [4].

Africans and Asians widely use *Ocimum gratissimum* for food and health purposes. Much has been done globally on the use of supplements and feed additives in animal health and nutrition [5]. The haematological parameters are important indicators of change in the physiological condition of animals, and are equally used to monitor physiological responses to various exogenous substances [6]. In broiler chickens for instance, *Ocimum gratissimum* leaf extracts are used to reduce microorganisms loads. These extracts are also used to stabilize blood composition and improve growth performance [7].

Among those plants is *Vernonia amygdalina*. This plant, known as "African bitter leaf" is a plant which has several nutritional and pharmacological properties. It is very frequently used in West Africa as food and culinary herb in soup in many parts of Africa [8]. The ingestion effect determination of food components on an animal's blood composition is established by an analysis of the animal's blood parameters. This analysis also makes it possible to establish the state of animal health [9]. Thus, the present study aims to evaluate the effect of *Ocimum gratissimum* and *Vernonia amygdalina* leaves as a fodder plant on the blood parameters of domestic rabbits (*Oryctolagus cuniculus*).

2. Materials and Methods

2.1. Study Area

The study was conducted in the Department of Production and Animals Health, and Research Laboratory of Biotechnology and Animals Improvement of Faculty of Agronomic Sciences, University of Abomey-Calavi, Cotonou, Benin.

2.2. Collection, Identification, Preparation and Processing of the Plant's Materials

Identification of the plant material was done in the national herbarium of the University of Abomey-Calavi, Calavi Benin. The collection, preparation, processing and preservation of *Ocimum gratissimum* and *Vernonia amygdalina* leaves were done practically as reported by previous studies [10, 11].

2.3. Formulation and Preparation of Experimental Diets

Ocimum gratissimum and *Vernonia amygdalina* leaves were obtained from the Faculty of Agronomic Sciences farm of University of Abomey-Calavi, Benin. The leaves were harvested in batches and air-dried under a shed until they were crispy to touch while retaining their greenish coloration. The leaves were then milled to obtain a product herein referred to as of *Ocimum gratissimum* and *Vernonia amygdalina* leaf meal. The experimental diets were formulated using algebraic method along with least cost formulae as reported by previous studies [12]. The diets of rabbits were prepared using the various calculated dosages to produce *Ocimum gratissimum* and *Vernonia amygdalina* meal diets and coded as follows: O_5, O_{10}, O_{15}; V_5, V_{10}, V_{15} and control diets respectively as shown in Table 1. The rabbit feed was prepared following the methods as reported by previous studies [12].

Composition of diets of rabbits

Table 1. *Ingredient Composition of the experimental diet.*

Ingredients (%)	Control	V_5	V_{10}	V_{15}	O_5	O_{10}	O_{15}
Maize	14	14	14	14	14	14	14
Bran	20	20	20	20	20	20	20
cottonseed meal	7	7	7	7	7	7	7
Soya bean meal	4	4	4	4	4	4	4
Corn bran	14	14	14	14	14	14	14
Palm karnel cake	40	40	40	40	40	40	40
Lysine	0.1	0.1	0.1	0.1	0.1	0.1	0.1
Methionine	0.1	0.1	0.1	0.1	0.1	0.1	0.1
oyster shell	0.6	0.6	0.6	0.6	0.6	0.6	0.6
Phosphate	0.2	0.2	0.2	0.2	0.2	0.2	0.2
Vernonia amygdalina leaf meal	-	5	10	15	-	-	-
Ocimum gratissimum leaf meal	-	-	-	-	5	10	15
Total	100	105	110	115	105	110	115
Proximate composition of experimental diets							
Dry matter	89.10	96.4	100.9	105.4	92.88	97.38	101.88
Cendres brutes	4.40	6.6	7.09	7.59	5.5	5.99	6.49
Protéine brute	26.78	25.44	26.34	27.24	23.79	24.69	25.59
Matière grasse	2.07	1.69	1.87	2.05	2.7	2.88	3.06
Cellulose brute	8.05	10.14	11.22	12.3	11.15	12.23	13.31

2.4. Experimental Design

A total of 96 white growing rabbit New Zeland of average weight of (653±45 g) were acclimated for 14 days in holing farms of the Department of Production of Animal Health of the Faculty of Agronomic Science. After acclimatisation, the growing rabbit were randomly allocated in the experiment cages in triplicates, using completely randomised design.

The rabbits were starved for 24 hours before the commencement of the feeding trial, and not fed on the weighing day as recommended [13]. The rabbits were fed, twice daily between 7:00 am and 6 pm for 8 weeks. The rabbits were monitored for mortality daily.

2.5. Blood Parameters Evaluation of Feeding Trials of Rabbits

At the end of the culture period the rearing, venous blood samples were aseptically from all rabbits and placed in sterile tubes. An initial 2 ml blood was collected into labelled sterile vacuum tube containing Ethylene Diamine-Tetra-acetic Acid (EDTA) as an anticoagulant which was used for haematological analysis. Another 3 ml of blood was collected into labelled sterile sample bottles without anticoagulant and used for the serum biochemical analysis.

The haematological variables were assessed [14]. In the complete blood samples, evaluations of Red Blood Cells (RBCs), Packed Cell Volume (PCV), haemoglobin (Hb), Mean Corpuscular Volume (MCV), Mean Corpuscular Haemoglobin Concentration (MCHC), White Blood cells, basophils, eosinophils, lymphocytes, and monocytes were analysed using methods describe [14, 15].

The biuret method of total protein determination was employed in this assay [16]. Alkaline Phosphates (ALP), Alanine Amino Transferase (ALT), Aspartate Amino Transferase (AST), glycaemia, total cholesterol, creatinine and urea were determined using spectrophotometric methods.

2.6. Statistical Analyses

The data (biochemical and haematological parameters) obtained from experiments analysed by R software (version 3.6.3) with a general linear model procedure for ANOVA. Differences between averages were analysed with Duncan's multiple tests. The significant difference statements were based on the probability ($P<0.05$).

3. Results

3.1. Effect of Ocimum gratissimum and Vernonia amygdalina Leaves Meal on Biochemical Parameters of Rabbits

The results of the effect of *Ocimum gratissimum* leaf meal on the biochemical parameters of rabbits are presented in Table 2.

Biochemical parameters of rabbits fed with diet containing *Ocimum gratissimum* leaves

Table 2. *Biochemical parameters of rabbits fed with diet containing Ocimum gratissimum leaves meal.*

Parameters	Diets			
	O_0	O_5	O_{10}	O_{15}
Glucose (mg/dl)	1.06±0.04[a]	1.12±0.02[a]	1.03±0.06[a]	1.03±0.08[a]
Urea (g/L)	0.45±0.03[b]	0.34±0.02[a]	0.53±0.04[c]	0.42±0.03[b]
Creatinine (g/L)	14.60±0.87[d]	9.31±0.97[b]	6.33±0.12[a]	11.70±0.58[c]
Total Cholesterol (g/L)	1.23±0.21[b]	0.82±0.04[a]	0.92±0.03[a]	0.94±0.04[a]
Total Protein (g/L)	57.33±5.13[a]	58.67±4.04[a]	60.00±2.00[a]	57.33±2.52[a]
AST (UI)	26.33±6.03[a]	27.00±2.65[a]	48.67±1.53[c]	39.67±2.52[b]
ALT (UI)	91.06±9.10[d]	42.00±2.00[a]	71.67±3.06[c]	60.67±5.13[b]
ALP (UI)	251.33±7.09[d]	219.33±2.31[c]	137.00±1.73[a]	180.00±2.65[b]

[a, b, c] averages in the same row with different superscript as significantly different ($P<0.05$). ALT=Alanine Aminotransferase; AST=Aspartate Aminotransferase; ALP=Alkaline phosphatase. O_0=0% *Ocimum gratissimum* leaf meal; O_5=5% *Ocimum gratissimum* leaf meal; O_{10}=10% *Ocimum gratissimum* leaf meal; O_{15}=15% *Ocimum gratissimum* leaf meal

There were no significant differences in blood sugar and total protein levels across the groups. Creatinine, urea and cholesterol levels were significantly decreased ($p<0.05$) in *Ocimum gratissimum* treatment groups *as* compared to the control. Aspartate transferase (AST) levels were significantly increased in tested subjects compared to the control. The highest values of AST were recorded in subjects O_{10} (48.67±1.53 IU) and O_{15} (39.67±2.52) while subjects O_0 showed the lowest values (26. 33±6.03); unlike ASAT, ALT and ALP which experienced a significant decrease in the test subjects. The highest concentration of ALT and ALP was noted in the control subjects. The lowest ALT value was recorded in O_5 subjects (42.00±2 UI) and that of ALP in O_{10} subjects (137.00±1.73 UI).

The biochemical parameters of the experimental animals are presented in Table 3.

Biochemical parameters of growing rabbits fed diets containing *Vernonia amygdalina* leaves meal.

Table 3. *Biochemical parameters of rabbits fed with diet containing Vernonia amygdalina leaves meal.*

Parameters	Diets			
	V_O	V_5	V_{10}	V_{15}
Glucose (mg/dl)	1.07±0.01[a]	1.06±0.01[a]	1.06±0.02[a]	0.83±0.03[b]
Urea (g/L)	0.44±0.02[c]	0.31±0.02[b]	0.33±0.01[b]	0.26±0.01[a]
Creatinine (g/L)	20.06±0.03[d]	11.58±0.04[b]	10.96±0.03[a]	13.92±0.09[c]
Total Cholesterol (g/L)	1.42±0.02[a]	1.32±0.06[b]	0.90±0.02[c]	0.62±0.05[d]
Total Protein (g/L)	53.33±0.58[a]	54.33±2.08[a]	52.67±30.60[a]	54.67±2.52[a]
AST (UI)	92.35±0.70[a]	159.35±1.17[d]	143.66±1.04[c]	169.67±0.81[b]
ALT (UI)	100.20±0.70[d]	80.31±1.47[b]	67.67±0.71[a]	89.37±1.15[c]
ALP (UI)	258.00±5.57[c]	125.33±4.04[b]	113.67±3.51[a]	125.67±2.52[b]

(AST=Aspartate amino transaminase, ALT=Alanine amino transaminase, ALP=Alkaline phosphate). V_0=0% *Vernonia amygdalina* leaf meal; V_5=5% *Vernonia amygdalina* leaf meal; V_{10}=10% *Vernonia amygdalina* leaf meal; V_{15}=15% *Vernonia amygdalina* leaf meal

a, b, c: averages in the same row with different superscript as significantly on the same row bearing different superscripts are significantly different (P <0.05).

The glucose (V_{15}=0.83±0.03 mg/dl) was significantly (P <0.01) lower than (V_0=1.07±0.01 mg/dl; V_5=1.06±0.01 mg/dl and V_{10}=1.06±0.02 mg/dl) which were similar. The urea (V_0=0.44±0.02 g/l 0.31±0.02 g/l; V_{10}=0.33±0.01 g/l; V_{15}=0.26±0.01 g/l), the creatinine (V_0=20.06±0.03 g/l; V_5=11.58±0.04 g/l; V_{10}=10.96±0.03 g/l; V_{15}=13.92±0.09 g/l) and the Total Cholesterol (V_0=1.42±0.02 g/l; V_5=1.32±0.06 g/l; V_{10}=0.90±0.02 g/l; V_{15}=0.62±0.05 g/l) were significantly (P <0.05) decrease, and lower value were recorded respectively on V_{15} (0.26±0.01), V_{10} (10.96±0.03 g/l) and V_{15} (0.62±0.05 g/l). The total protein was not significantly different (P> 0.05) among treatment groups. Furthermore, the Aspartate Amino Transferase of V_5 (159.35±1.17 UI), V_{10} (143.66±1.04 UI), V_{15} (169.67±0.81 UI) were significantly (P <0.05) higher than V_0 (92.35±0.70 UI). On the other hand, Alanine aminotransferase of V_0 (100.20±0.70 UI) and Alkaline phosphatase of V_0 (258.00±5.57 UI) were significantly (P <0.05) higher than V_5 (80.31±1.47 UI), V_{10} (67.67±0.71 UI), V_{15} (89.37±1.15 UI) and V_5 (125.33±4.04 UI), V_{10} (113.67±3.51 UI) and V_{15} (125.67±2.52 UI) respectively.

3.2. Effect of Ocimum gratissimum and Vernonia amygdalina Leaves Meal on Haematological Parameters of Rabbits

The results of the effect of *Ocimum gratissimum* leaf meal on the haematological parameters are presented in Table 4.

Table 4. *The haematological parameters of rabbits fed with diet containing Ocimum gratissimum leaves.*

Parameters	Diets			
	O_0	O_5	O_{10}	O_{15}
RBC ($x10^6/mm^3$)	4.43±0.40[a]	4.83±0.09[a]	4.95±0.05[a]	4.84±0.15[a]
Haemoglobin (g/dl)	10.62±0.58[a]	9.83±0.15[a]	10.57±0.40[a]	10.73±0.64[a]
PCV (%)	32.60±2.42[a]	29.87±1.50[a]	31.67±1.22[a]	32.47±1.47[a]
MCV (fl)	61.87±1.67[a]	66.80±0.44[a]	62.53±1.36[a]	63.83±0.85[a]
MCH (pg)	20.07±0.60[a]	20.80±0.17[a]	20.17±0.96[a]	20.53±0.50[a]
MCHC (g/dl)	30.77±0.68[a]	30.40±0.79[a]	31.87±1.33a[b]	33.23±0.97[b]
Blood Platelets ($x10^3/mm^3$)	127.67±7.09[c]	99.33±5.13[a]	175.33±4.51[d]	114.67±5.51[b]
WBC ($x10^9/L$)	5.63±0.60[b]	4.07±0.11[a]	7.97±0.15[c]	5.80±0.20[b]
Neutrophils (%)	42.67±2.52[b]	31.33±1.53[a]	35.67±1.53[a]	34.67±2.52[a]
Eosinophils (%)	1.67±0.58[a]	1.67±0.58[a]	2.00±1.00[a]	2.33±0.58[a]
Basophils (%)	0.00±0.00[a]	0.00±0.00[a]	0.00±0.00[a]	0.00±0.00[a]
Lymphocytes (%)	51.00±3.61[a]	62.00±1.73[b]	58.00±1.73[b]	59.67±3.51[b]
Monocytes (%)	4.67±0.58[a]	4.33±0.58[a]	4.00±1.00[a]	3.67±1.53[a]

a, b, c averages on the same row bearing different superscripts is significantly different (P <0.05). O_0=0% *Ocimum gratissimum* leaf meal; O_5=5% *Ocimum gratissimum* leaf meal; O_{10}=10% *Ocimum gratissimum* leaf meal; O_{15}=15% *Ocimum gratissimum* leaf meal. RBC=Red Blood Cells, PCV=Packed Cell Volume, MCV=Mean Corpuscular Volume, MCH=Mean Corpuscular Haemoglobin, MCHC=Mean Corpuscular Haemoglobin Concentration, WBC=White Blood Cell.

There were no significant differences in Red Blood Cells, haemoglobin, Packed Cell Volume, and Red Blue Cell indices between the groups. However, the number of White Blood Cells revealed a significant variation with its greatest value recorded in subjects fed with O_{10}. This variation was recorded as the percentage of lymphocytes. This study noticed a total absence of basophils. The Red blue cell indices (MCV, MCH) did not demonstrate any significant difference, although the MCHC increased significantly in subjects fed with O_{10} and O_{15}.

Table 5 showed the haematological parameters of rabbit fed diets containing *Vernonia amygdalina* leaves meal.

Table 5. *Haematological parameters of rabbits fed with diet containing Vernonia amygdalina leaves.*

Parameters	Diets			
	V_O	V_5	V_{10}	V_{15}
RBC ($x10^6/mm^3$)	5.54±0.04[c]	4.93±0.03[b]	4.33±0.02[a]	4.32±0.01[a]
Haemoglobin (g/dl)	10.83±0.21	10.70±0.35	10.13±1.33	10.23±1.15
PCV (%)	34.60±0.53	33.57±0.21	27.53±0.32	30.77±5.43
MCV (fl)	62.43±0.45	68.10±0.36	64.30±0.40	65.17±0.57
MCH (pg)	19.53±0.15	20.57±0.25	19.50±0.40	20.67±0.21
MCHC (g/dl)	31.33±0.15	30.30±0.53	30.57±0.25	31.50±0.30
Blood Platelets ($x10^3/mm^3$)	122.67±2.52[a]	181.67±3.79[c]	163.33±1.53[b]	178.67±4.04[c]
WBC ($x10^9/L$)	5.62±0.07[b]	5.56±0.05[b]	5.52±0.05[a]	9.05±0.05[c]
Neutrophils (%)	46.00±1.00[d]	38.33±0.58[b]	40.67±0.58[c]	31.33±0.58[a]
Eosinophils (%)	2.33±0.58	3.00±0.00	2.33±0.58	2.33±0.58
Basophils (%)	0.00	0.00	0.00	0.00
Lymphocytes (%)	48.33±1.53[a]	54.33±0.58[b]	53.67±0.58[b]	62.33±0.58[c]
Monocytes (%)	3.33±1.53[a]	4.33±1.15[a]	3.33±0.58[a]	4.00±0.00[a]

a, b, c averages on the same row bearing different superscripts is significantly different (P <0.05). V_0=0% Vernonia amygdalina leaf meal; V_5=5% Vernonia amygdalina leaf meal; V_{10}=10% Vernonia amygdalina leaf meal; V_{15}=15% Vernonia amygdalina leaf meal RBC=Red Blood Cells, PCV=Packed Cell Volume, MCV=Mean Corpuscular Volume, MCH=Mean Corpuscular Haemoglobin, MCHC=Mean Corpuscular Haemoglobin Concentration, WBC=White Blood Cell

The haematological parameters of the experimental animals are presented in table 5. No significant difference (p>0. 05) was recorded in haemoglobin concentration (V_0=10.83±0.21 g/dl; V_5=10.70±0.35 g/dl; V_{10}=10.13±1.33 g/dl and V_{15}=10.23±1.15 g/dl), Packed Cell Volume (V_0=34.60±0.53%; V_5=33.57±0.21%; V_{10}=27.53±0.32% and V_{15}=30.77±5.43%), Mean Corpuscular Volume (fl), Mean Corpuscular Haemoglobin (pg), Mean Corpuscular Haemoglobin Concentration (g/dl), Eosinophils (V_0=2.33±0.58%; V_5=3.00±0.00%; V_{10}=2.33±0.58% and V_{15}=2.33±0.58%) and Monocytes (V_0=3.33±1.53%; V_5=4.33±1.15%; V_{10}=3.33±0.58% and V_{15}=4.00±0.00%). The Red Blood Cells number were negatively, significantly (p <0.05) influenced by dietary treatment. The RBC (4.32±0.01 $x10^6/mm^3$) recorded in V_{15} treatment were significantly (P <0.05) lower compared to the RBC of V_0 (5.54±0.04 $x10^6/mm^3$). However, the animals tested with the V_{15} treatment recorded significantly (P <0.05) higher in White Blood Cell (9.05±0.05 $x10^9/L$) and lymphocytes number (62.33±0.58%) than animals' treatment V_0 (5.62±0.07 $x10^9/L$ and 48.33±1.53%) and V_5 (5.56±0.05 $x10^9/L$ and 54.33±0.58). It also noted the absence of basophils in the various treatments.

4. Discussion

Glucose and total protein showed no significant variation amongst the groups. The renal parameters (urea, creatinine) and the total cholesterol level experienced significant variation (p <0.05) between groups. The decrease in creatinine and urea in the blood would be due to the nephroprotective property of *Ocimum gratissimum* [17]. The decrease in cholesterol levels in different rations compared to the control is similar to the results as reported by previous studies [18]. Liver enzymes such as AST, ALT and ALP had significant variations in the different test groups compared to the control. However, the values of these parameters were within the range of normal values according to [19]. The AST level increased significantly in the *Ocimum gratissimum* tested groups compared to the controls and the effect was dose dependent. ALT and AST were liberated into the blood whenever liver cells were damaged and enzyme activity in the plasma is increased [20]. In addition, Çam et al. [21] found that serum levels ALT and AST were dependent on hepatocellular damage and cholestasis. The results of this study were similar to the work as reported by the previous studies [22]. The use of *Ocimum gratissimum* is beneficial for biochemical parameters in rabbits in the present study as well as in Carnivorous fish as reported in previous studies [40] The fact that the enzyme activity was reduced indicates that the extract did not have any necrotic effect on the liver of the rabbits fed *Ocimum gratissimum* leaf meal.

Biochemical results were revealed no significant increase in the total protein concentration. The total cholesterol was showing a significant decrease (P <0.05) according to the treatments. This result is similar to these [23]. In addition, the Liver function tests revealed that the activities of ALT and APL decreased significantly (P <0.05) in the groups treated compared to the control in which there is a high concentration of these parameters. On the other hand, the activity of AST revealed a significant increase (P <0.05) in the treated groups. These parameters were showing that *Vernonia amygdalina* leaf meal has a non-hepatotoxic effect according to [24]. The ALT is a more reliable marker of liver integrity than AST. The significant increase observed in the activity of AST alone may therefore be of extra hepatic origin according to the result as reported by previous studies [24] in rats. The result showing the decrease of ALT level was contradicted with results of previous studies [25] which express the increase of ALT levels in rabbits which receive aqueous extract of *Vernonia amygdalina* leaves; translating so the hepatotoxic proprieties at *Vernonia amygdalina* leaves [25]. Glucose levels, total cholesterol levels, urea and creatinine levels were presented a significant dose-dependent decrease (p <0.05) in the treated groups compared to the control. The decrease in glucose and cholesterol levels could mark the hypoglycaemic and cholesterol-lowering properties of the plant according to the results of previous studies [26,

27]. The decrease of the creatinine and urea in the groups treated could relate that this plant is not nephrotoxic. Those results were similar to [28] who found that *Vernonia amygdalina* have a hypoglycaemia action and could protect against kidney impairments in the diabetic rats. The same result has obtained with [29] in diabetic rats also. [29] conferred the biological activities of this plant in polyphenols presence in the leaves.

With regard to blood index, changes in haematological and biochemical parameters in animals indicate their physiological status and allow the evaluation of the physiological impact of ingested feed [30, 31]. The haematological components were therefore useful in monitoring food toxicity by food constituents, which could affect the constitution of the blood [30]. The PCV and haemoglobin levels obtained in the present study were not significantly different ($p > 0.05$) and were located within the reference margins [32, 33]. There was no significant difference in the number of Red Blood Cells between the different groups. However, the number of Red Blood Cells recorded in this study in the different groups was below the normal value according to [31].

The values of Mean Corpuscular Volume (MCV) and Mean Corpuscular Haemoglobin Concentration (MCHC) in this study were not affected by the various food treatments. The values of MCH obtained were within the normal values of healthy rabbits. These results were similar to the results of the work carried out by previous studies [34] on chickens with *Ocimum gratissimum* leaves. According to [34], *Ocimum gratissimum* leaves could have a haematopoietic effect, which would maintain normal erythrocyte numbers and haemoglobin concentrations during coccidiosis. Thus, *Ocimum gratissimum* leaves would have a beneficial effect on the haematopoiesis of rabbits. This result contrasts with these of other studies in which a reduction in haematological parameters was observed in rabbits when aqueous extracts of *Ocimum gratissimum* leaves were administered [22]. We suggest that the differences between these studies may be dependent on the type of *Ocimum gratissimum* supplement used: Crushed leaves versus aqueous extracts, but this requires further evaluation in future studies.

The levels of white blood cells did significantly increase in the tested groups, the highest value was seen in subjects fed with the O_{10} ratio and suggests that subjects in this group could be prone to inflammation. Dissimilarly results have been reported by previous studies [22] by administration of *Ocimum gratissimum* leaves aqueous extract of in rabbits, who reported that WBCs decreased in numbers in correlation with the increase of the exact dose. The leukopenia (4.07×10^3/L) noted in subjects fed with the O_5 ration could be due to an allergy, anaphylactic shock or to certain parasitism and virus according to [35] who revealed a severe leukopenia in rabbits after calicivirus infection. However, neutrophils, eosinophils and monocytes showed no difference between the groups and there was a total absence of basophils, suggesting that allergy was unlikely. Others studies indicates that the oral OG administration reversed spleen, thymus, and blood toxicity which induced by lead acetate in adult Wistar rats [41].

Vernonia amygdalina leaf meal on rabbits has shown a decrease in the number of Red Blood Cells. The reduction in the number of Red Blood Cells can be the result of a reduction in the formation of red blood cells or their destruction [36]. During the experiment, the number of white blood cells significantly increased ($P < 0.001$) and the largest number was recorded in the V_{15} treatment. An increase in the number of White Blood Cells in the treated groups may induce the stimulation of the immune response. Thus, the significant increase in White Blood Cells as a function of the gradual rate of incorporation indicated the stimulation of the immune system. The Packed Cell Volume of rabbits has remained relatively constant [36]. Indeed, the Packed Cell Volume translates the absorption of oxygen and its transfer in the tissues of the rabbit. It thus makes it possible to judge the anaemic state of the subjects. Haemoglobin and Packed Cell Volume which was not significantly different. Those results were in contrast with these [37], who had reported a reduction in Packed Cell Volume due to induced haemolysis in rats normal treated with aqueous extracts of leaves of *Vernonia amygdalina*. The difference could be explained by the animal species used and the leaves ingestion form. The results of the present study were similar to [38] which stated that *Vernonia amygdalina* was capable of normalising haematological parameters of rats. According to [39], this study could conclude that *Vernonia amygdalina* is not hematotoxic and it possesses haematopoietic activities.

These results showed that *Ocimum gratissimum* and *Vernonia amygdalina* leaves can be incorporated into the granulated feed up of rabbits at 15% without any deleterious effect on the health of the latter if good hygiene practices were maintained.

5. Conclusion

Ocimum gratissimum and *Vernonia amygdalina* can increase the White blood cell specially the lymphocytes and it does not have any hepatotoxic and nephrotoxic effects. The parameters have not shown any degeneration of liver and kidney of animals. The rabbit breeders could include those plant in the rabbit's diet. However, dietary inclusion should be limited to 15% of incorporation. Histologic studies will be considered in order to evaluate the histopathology of this incorporation in the liver and kidneys of animals.

Ethical Approval

The Research Ethics Committee of the National University of Agriculture of Benin evaluated the research protocol. The decision of this Ethics Committee is favourable and registered under N° 143-2018/President -CER/SA of 08/11/2018.

Conflict of Interests

All the authors do not have any possible conflicts of interest.

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