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# Research Article
# Effect of Aqueous Extract of *Moringa oleifera* Leaves on Hematological and Biochemical Parameters in Broilers (Cobb 500)

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## Abstract

**Objective:** This study was designed to evaluate the effect of the aqueous extract of *Moringa oleifera* leaves on the hematological and biochemical parameters of broilers. **Materials and Methods:** The study was conducted at T3F Poultry Farm, Makong from February to April 2024. A total of 150 day-old chicks of Cobb 500 strain, with an average weight of 40.09±3 g, were randomly divided into 20 experimental units according to a completely randomized design with 4 replicates of 7 to 8 subjects each for a period of seven weeks. The experimental rations were formulated such that the aqueous extract of *Moringa oleifera* leaf was included in the diet at 0 g ($R_0$), 0.25 g ($R_1$), 0.50 g ($R_2$), 0.75 g ($R_3$) and 1 g ($R_4$) both at the beginning and at the end of the study. $R_0$ served as the control ration. **Results:** The main results showed that the addition of *Moringa oleifera* leaf extracts in the diet had no significant effect on mean platelet volume levels. On the other hand, mean corpuscular volume, hematocrit and hemoglobin levels increased with increasing levels of *Moringa oleifera* leaf extract in the diet, while platelet levels decreased with increasing levels of extract. The HDL cholesterol, triglycerides, ALT and AST decreased with the inclusion of aqueous extract of *Moringa oleifera* leaves in the diet. The addition of aqueous extracts of *Moringa oleifera* leaves to the diet had no significant effect on total protein, albumin, globulins, total cholesterol, glucose, creatinine and urea. **Conclusion:** Meat with fewer bacterial residues was obtained when *Moringa oleifera* leaf extracts were included in the diet, which is beneficial for health-conscious consumers.

Key words: Biochemical parameters, broiler chicken, hematological parameters, leaf meal, *Moringa oleifera*

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

## INTRODUCTION

The average animal protein consumption in Cameroon is 11.1 g/day/capita, one third of the 33 g/day/capita recommended by the FAO[1]. In addition, animal protein consumption in Cameroon is 15 kg/capita/year compared to the 32 kg/capita/year recommended by the FAO[2]. In the face of this problem, the promotion of livestock, especially species with short reproductive cycles such as poultry, remains a necessity for meat production[3]. Poultry, with its ease of rearing and high productivity, is one of the species that can effectively contribute to increasing the availability of much needed animal proteins for African populations[4,5]. Antibiotic-based additives have been used to improve growth parameters in poultry farming but their use has been officially banned in the European Union since 2006[6]. In fact, the latter have caused resistance problems in humans[7]. In fact, a wide range of alternatives to antibiotics based on live microorganisms and plant extracts have been developed in recent years to address this problem. The use of unconventional resources (*Moringa oleifera*, *Leucaena leucocephala*, etc.) as feed additives in poultry diets has become an exciting area of research, as evidenced by the large number of papers on the subject. A study by Ravva and Korn[8] showed that 5% neem leaf extract, when incorporated in feed, eliminated pathogenic *Escherichia coli* O157 in cattle in less than 10 days. Similarly, Tendonkeng *et al*.[9] and Olugbemi[10] showed that the use of *Moringa oleifera* leaf meal at low incorporation rates (6-15%) improved growth performance in broilers and egg production in laying hens. Although, this plant is widely available, there have been very few studies using its extract to examine the hematological and biochemical parameters of broilers, so this study is of particular interest.

## MATERIALS AND METHODS

**Study area:** The study was conducted at the FERAVI T3F poultry farm from February to April 2024. The farm is located in the village of Makong in the West Region of Cameroon. The village of Makong is located between latitudes 5°26'49" and 10°0'57" North and longitudes 10°0'57" East and 10°0'57" West. The region's climate is equatorial Cameroonian, modified by altitude, with a rainy season from mid-March to mid-November and a dry season from mid-November to mid-March. Rainfall varies between 1,500 and 2,000 mm per year and the average temperature is 21°C, with relative humidity varying between 40 and 97%[11].

### Materials

**Plant materials and extract preparation:** Fresh young *Moringa oleifera* leaves were harvested from the Bafia campus of Dschang University. The leaves were shade-dried at room temperature to a constant weight and ground to a fine powder using a grinder. The powder thus obtained was sieved to obtain a finer powder, which was then used to prepare the aqueous extract. For this purpose, 100 g of the powder was placed in a container containing 1 liter of distilled water and the contents were left to stand for 48 hrs. The maceration was then filtered through muslin for the first filtrate and cotton for the second. The filtrate obtained was dried in an oven at 45°C until a solid extract was obtained, which was stored in a sealed container away from light and moisture until use. Figure 1 shows the different forms of *Moringa oleifera*.

**Phytochemical analysis of the aqueous extract of *Moringa oleifera*:** The phytochemical composition of this extract is shown in Table 1.

**Animal material:** In this study, 150 day-old chicks of the Cobb 500 strain with an average weight of 40.09 g were used. They were randomly divided into 20 experimental units in a completely randomized design with 4 replicates of 7 to 8 chicks each, reared on litter at a density of 20 chicks/$m^2$ at the beginning and 10 chicks/$m^2$ at the end.

**Housing and equipment:** The start-up and completion phases were conducted in a building (divided into four rooms) at FERAVI T3F in Dschang. The building consists of a 1 m high half wall covered with wire mesh and a corrugated iron roof. One room is 18.20 m long and 10 m wide, with a surface area of 182 $m^2$. Two weeks before the arrival of the chicks, the rearing building and the various equipment were cleaned and disinfected with bleach, Cresyl (20 mL per litter of water) and TH4 solution (20 mL per litter of water), which were spread throughout the room and on all the cages. The room was divided into 1 $m^2$ cubicles, each equipped with a 3l plastic siphon drinker, a linear feeder for young birds and a 100 W incandescent light bulb as a heat source. After disinfection, a sanitary vacuum was maintained for two weeks before birds were introduced. The rooms and equipment used were cleaned daily.

**Feed ration and experimental design:** The birds were fed with locally available feed and plenty of fresh water. The feed, composed of ingredients such as maize, remolding, bone


Fig. 1(a-c): Different forms of *Moringa oleifera*, (a) Dried moringa leaves, (b) Moringa leaf powder and (c) Aqueous extract of Moringa leaves

Table 1: Phytochemical composition of *Moringa oleifera* aqueous extract

| Phenolic compounds | *Moringa oleifera* |
|---|---|
| Alkaloids | - |
| Phenols | + |
| Flavonoids | + |
| Sterols | + |
| Triterpenoids | + |
| Tannin | + |
| Saponins | + |
| Anthraquinone | + |
| Anthocyanin | + |

+: Present and -: Absent

meal, cottonseed cake, soybean cake, fish meal, shell powder and 5% broiler concentrate, was purchased from the Dschang market.

A control ration ($R_0$) was prepared at both the beginning and end of the diet (Table 2) and the other four rations were obtained by incorporating different levels of *Moringa oleifera* leaf extract, corresponding to 0.25 g, 0.50 g, 0.75 g and 1 g aqueous extract per kg of diet, respectively. Each of the five rations was randomly assigned to 20 experimental units in a completely randomized design. Only the level of extract was considered in the formulation of the rations, which were composed as follows:

Table 2: Composition of experimental rations in the start and finish periods

| Feed ingredients (%) | Starter | Finisher |
|---|---|---|
| Corn | 59 | 65 |
| Remolding | 5 | 5 |
| Cotton cake 50 | 6 | 4 |
| Soybean meal 49 | 20 | 15 |
| Fish meal 60 | 4 | 5 |
| Shell | 0.75 | 1 |
| Bone meal | 0.25 | 0 |
| CMAV 5%* | 5 | 5 |
| **Chemical composition (analyzed)** | | |
| Metabolizable energy (kcal/kg) | 2951.91 | 3006.85 |
| Crude protein (%) | 22.53 | 20.38 |
| **Chemical composition (calculated)** | | |
| Energy/protein | 130.99 | 147.54 |
| Lysine (%) | 1.32 | 1.19 |
| Methionine (%) | 0.46 | 0.45 |
| Calcium (%) | 1.07 | 1.15 |
| Available phosphorus (%) | 0.5 | 0.48 |
| Calcium/phosphorus | 2.16 | 2.41 |
| Crude fiber (%) | 4.96 | 4.94 |

*CMAV: Mineral-Nitrogen Vitamin Complex, PB: 40%, Calcium: 8%, Phosphorus: 2,05%, Lysine: 3,3%, Methionine: 2,40%, EM: 2078 Kcal/kg, Vitamin A: 3000000 UI, Vitamin $D_3$: 600000 UI, Vitamin E: 4000 mg, Vitamin K: 500 mg, Vitamin $B_1$: 200 mg, Vitamin $B_2$: 1000 mg, Vitamin $B_6$: 400 mg, Vitamin $B_{12}$:4 mg, Iron: 8000 mg, Cu: 2000 mg, Zn: 10000 mg, Se: 20 mg and Mn: 14000 mg

$R_0$ = Negative control diet (no additives)
$R_1$ = $R_0$+0.25 g moringa leaf extract/kg feed
$R_2$ = $R_0$+0.50 g moringa leaf extract/kg feed
$R_3$ = $R_0$+0.75 g moringa leaf extract/kg feed
$R_4$ = $R_0$+1 g moringa leaf extract/kg feed

### Parameters and data collection

**Hematological parameters:** Hematological parameters are indicators of the physiological state of the animal. They make it possible to assess the state of the red blood cells and the proper functioning of the hematoprotective organs. For this purpose, 2 to 3 mL of blood was collected from the 10 animals sacrificed per treatment. This blood was collected in test tubes containing anticoagulant and then transported to "Clab labo" in Bafoussam, where these parameters were assessed using a Genius auto hematology analyzer, model K-T 6180. These parameters were red and white blood cells, hemoglobin, hematocrit and platelets.

**Biochemical parameters:** Biochemical parameters characterize the different substances (blood, urine, effusions, secretions, etc.) in the organism, whose concentration is relatively constant and whose degree of variation makes it possible to assess the functional state of the organism. For these analyses, 2 to 3 mL of blood were taken from the 10 subjects sacrificed per treatment during the carcass evaluation. This blood was collected in test tubes without anticoagulant and then centrifuged at 3,000 rpm for 15 min. The resulting serum was stored in a freezer at -20°C until the biochemical parameters were analyzed. Assays were performed using a spectrophotometer according to the protocol described in the commercial CHRONOLAB® kits.

Creatinine, urea, aspartate aminotransferase (AST) and alanine aminotransferase (ALT) levels were analyzed by the colorimetric kinetic method, while total cholesterol levels were determined by the enzymatic method as described by Pesce and Bodourian[12]. Serum triglyceride levels were determined by the colorimetric method as described by Fossati and Prencipe[13] and total protein and albumin levels were determined by the biuret and bromocresol green colorimetric methods as described by Gornall *et al.*[14]. The globulin level, which is an indicator of the animal's immune system, was obtained by subtracting the total protein level from the serum albumin level using the method recommended by Abdel-Fattah *et al.*[15]. The LDL cholesterol level was obtained by subtracting the total cholesterol level from the HDL cholesterol level and then multiplying the result by 5.

**Data analysis:** Data on hematological and biochemical parameters were subjected to One-way analysis of variance (ANOVA) using the general linear model (GLM). When there were significant differences between treatments, the means were separated by the least significant difference (LSD) test at the 5% significance level.

## RESULTS

**Effect of aqueous leaf extract of *Moringa oleifera* on hematological parameters in broiler chickens:** The effect of incorporating aqueous extracts of *Moringa oleifera* leaves in the diet on a number of hematological parameters is shown in Table 3. Analysis of variance showed a significant effect ($p<0.05$) between treatments. Mean corpuscular volume, hematocrit and hemoglobin increased with increasing levels

Table 3: Effect of aqueous leaf extract of *Moringa oleifera* on hematological parameters in broiler chickens

| Hematological parameters | Treatments | | | | | p-value |
|---|---|---|---|---|---|---|
| | $R_0$ | $R_1$ | $R_2$ | $R_3$ | $R_4$ | |
| WBC ($10^9$/L) | 282.80±5.77$^a$ | 206.58±1.37$^c$ | 144.06±1.39$^e$ | 226.22±0.72$^b$ | 195.42±1.11$^d$ | 0.000 |
| RBC ($10^{12}$/L) | 1.26±0.01$^d$ | 1.67±0.07$^c$ | 2.15±0.01$^a$ | 1.36±0.04$^d$ | 1.88±0.03$^b$ | 0.000 |
| HGB (g/dL) | 13.36±0.20$^b$ | 12.88±0.22$^b$ | 12.74±0.28$^b$ | 12.84±0,18$^b$ | 15.20±0.07$^a$ | 0.000 |
| HCT (%) | 22.42±0.52$^c$ | 27.26±0.56$^b$ | 31.88±1.77$^a$ | 32.70±0.46$^a$ | 32.82±0.39$^a$ | 0.000 |
| MCV (fL) | 142.34±0.39$^d$ | 171.22±0.43$^c$ | 175.24±0.59$^b$ | 178.18±0.48$^a$ | 178.84±0.29$^a$ | 0.000 |
| MCHC (g/dL) | 105.38±0.81$^a$ | 78.12±0.80$^d$ | 89.32±0.70$^b$ | 104.32±0.61$^a$ | 80.90±0.59$^c$ | 0.000 |
| PLAT ($10^3$/µL) | 165.00±0.55$^a$ | 94.40±0.87$^b$ | 60.80±0.37$^c$ | 450.60±0.40$^d$ | 45.00±0.32$^d$ | 0.000 |
| MPV (fL) | 5.64±0.28$^a$ | 5.50±0.22$^a$ | 5.52±0.13$^a$ | 5.30±0.13$^a$ | 5.62±0.14$^a$ | 0.542 |
| Lym (%) | 78.14±0.54$^b$ | 66.92±1.66$^d$ | 82.40±0.50$^a$ | 71.04±0.34$^c$ | 76.24±1.16$^b$ | 0.000 |
| Mono (%) | 23.26±0.71$^b$ | 24.56±0.33$^a$ | 14.64±007$^d$ | 22.66±0.20$^b$ | 18.86±0.51$^c$ | 0.000 |
| Gran (%) | 2.60±0.13$^c$ | 7.12±0.27$^a$ | 4.96±0.15$^b$ | 5.32±0.18$^b$ | 2.84±0.26$^c$ | 0.000 |

$^{a-e}$Means with the same letter on the same line are not significantly different ($p>0.05$); $R_0$: control diet without extract; $R_1$: $R_0$+0.25 g aqueous extracts of *Moringa oleifera* leaves; $R_2$: $R_0$+0.5 g aqueous extracts of *Moringa oleifera* leaves; $R_3$: $R_0$+0.75 g aqueous extracts of *Moringa oleifera* leaves; $R_4$: $R_0$+1 g aqueous extracts of *Moringa oleifera* leaves; p: probability; WBC: White Blood Cells; RBC: Red Blood Cells; HGB: Haemoglobin; HCT: Haematocrit; Lym: Lymphocytes; Mono: Monocytes, Gran: Granulocytes; MCV: Mean corpuscular volume; PLAT: Platelets; MPV: Mean platelet volume and MCHC: Mean corpuscular hemoglobin concentration

Table 4: Effects of *Moringa oleifera* leaf extracts in the ration on biochemical parameters

| Biochemical parameters | Treatments | | | | | |
|---|---|---|---|---|---|---|
| | $R_0$ | $R_1$ | $R_2$ | $R_3$ | $R_4$ | p-value |
| Total protein (g/dL) | 2.49±0.14[a] | 2.31±0.03[a] | 2.53±0.15[a] | 2.58±0.15[a] | 2.57±0.13[a] | 0.632 |
| Albumin (g/dL) | 1.43±0.14[a] | 1.35±0.14[a] | 1.31±0.11[a] | 1.39±0.17[a] | 1.21±0.13[a] | 0.829 |
| Globulin (g/dL) | 1.45±0.05[a] | 1.46±0.07[a] | 1.44±0.09[a] | 1.49±0.12[a] | 1.56±0.11[a] | 0.447 |
| Total cholest (mg/dL) | 89.42±1.35[a] | 88.43±2.01[a] | 92.07±0.39[a] | 88.04±2.06[a] | 92.02±1.00[a] | 0.258 |
| HDL cholest (mg/dL) | 37.22±1.28[a] | 29.28±0.53[b] | 27.81±0.57[b] | 27.32±0.47[b] | 27.81±0.69[b] | 0.000 |
| Triglycerides (g/L) | 33.23±0.24[a] | 21.44±0.66[b] | 22.24±0.60[b] | 23.86±0.71[b] | 23.64±0.48[b] | 0.000 |
| LDL cholest (mg/dL) | 45.99±1.00[b] | 26.05±0.61[d] | 52.10±0.79[a] | 45.76±0.84[b] | 42.53±0.95[c] | 0.000 |
| Glucose (mg/dL) | 83.96±0.74[c] | 84.22±0.80[a] | 82.97±1.13[a] | 83.57±1.50[a] | 84.08±2.72[a] | 0.132 |
| ALT (IU/L) | 85.41±0.85[a] | 25.63±1.14[b] | 25.78±0.28[b] | 22.47±0.47[c] | 22.04±0.77[c] | 0.000 |
| AST (IU/L) | 128.45±0.59[a] | 82.84±1.03[b] | 80.61±1.00[b] | 60.76±0.53[c] | 57.01±1.67[c] | 0.000 |
| Creatinine (mg/dL) | 0.08±0.00[a] | 0.08±0.01[a] | 0.08±0.01[a] | 0.07±0.00[a] | 0.08±0.01[a] | 0.210 |
| Urea (mg/dL) | 4.78±0.27[a] | 4.43±0.02[a] | 4.52±0.23[a] | 4.77±0.38[a] | 4.31±0.22[a] | 0.723 |

[a,b,c,d]Means with the same letter on the same line are not significantly different ($p>0.05$), $R_0$: Control diet without extract; $R_1$: $R_0$+0.25 g aqueous extracts of *Moringa oleifera* leaves, $R_2$: $R_0$+0.5 g aqueous extracts of *Moringa oleifera* leaves, $R_3$: $R_0$+0.75 g aqueous extracts of *Moringa oleifera* leaves, $R_4$: $R_0$+1 g aqueous extracts of *Moringa oleifera* leaves and p: Probability

of *Moringa oleifera* leaf extract in the diet, while platelet count decreased with increasing levels of extract. Animals fed diet without *Moringa oleifera* leaf extract and those fed diet supplemented with 0.5 g extract had the highest levels of white blood cells and lymphocytes, respectively ($p<0.05$), while those fed diet supplemented with 0.5 g and 0.25 g *Moringa oleifera* extract had low levels of both parameters. Monocyte and granulocyte levels were significantly higher ($p<0.05$) in animals fed 0.25 g *Moringa oleifera* extract in the diet, while the lowest levels were obtained in animals fed 1 g extract in the ration. Red blood cell levels were significantly higher ($p<0.05$) in animals fed 0.5 g *Moringa oleifera* leaf extract in the diet, while the lowest levels were observed in animals fed no extract and 0.75 g extract in the diet, which were otherwise comparable. Addition of *Moringa oleifera* aqueous extracts to the diet had no significant effect ($p>0.05$) on mean platelet volume.

**Effects of *Moringa oleifera* leaf extract on biochemical parameters of broiler chickens:** The results of the inclusion of aqueous extracts of *Moringa oleifera* leaves in the diet are presented in Table 4. Analysis of variance revealed a significant effect ($p<0.05$) on HDL cholesterol, triglycerides, LDL cholesterol, ALT and AST. HDL cholesterol, triglycerides, ALT and AST decreased as the amount of extract in the diet increased. LDL cholesterol levels were highest ($p<0.05$) in animals fed 0.5 g *Moringa oleifera* leaf extract and lowest in animals fed 1 g extract in the diet. The addition of aqueous extract of *Moringa oleifera* leaf to the diet had no significant effect ($p>0.05$) on total protein, albumin, globulins, total cholesterol, glucose, creatinine and urea.

## DISCUSSION

Blood parameters are considered to be the most important pathophysiological[16] and nutritional[17] indices for assessing the state of an organism. Any change in blood constituents compared to normal values is an important index not only for interpreting the physiological or metabolic state of the animal but also the quality of the diet[18]. In the present study, mean corpuscular volume, hematocrit and hemoglobin count increased with increasing levels of aqueous extract of *Moringa oleifera* leaf in the diet, while platelet count decreased with the addition of extract. These results are consistent with those of Bleyere *et al.*[19], where phytobiotics (aqueous extract of *Bidens pilosa L.*) affected blood cells *in vitro*. The addition of *Moringa oleifera* aqueous extract to the diet had no significant effect on mean platelet volume. The results are consistent with those of Alam *et al.*[20], who observed no significant differences between the treatments for hematological parameters with the incorporation of neem leaf powder at levels of 1, 2 and 3 g/kg in broiler diets.

Biochemical parameters characterize the various substances (blood, urine, effusions, secretions, etc.) of the organism whose concentration is relatively constant and whose degree of variation allows to assess the functional state of the organism. In this study, the addition of aqueous leaf extract of *Moringa oleifera* to the ration had no significant effect on total protein, albumin, globulins, total cholesterol, glucose, creatinine and urea. Creatinine and urea are waste products of protein metabolism that are excreted by the kidneys. Kidney function is commonly determined by creatinine and urea levels[21]. Messaadia *et al.*[21] reported that creatinine and urea levels increase in renal failure. Our results show that increasing the intake of *Moringa oleifera* leaf

extract in the diet has no nephrotoxic effect and kidneys of broiler function normally. There were no significant differences in serum total cholesterol levels. However, the highest levels were obtained in animals fed a diet supplemented with 1 g of aqueous leaf extract of *Moringa oleifera*. This could be explained by the presence of antioxidants in the aqueous extracts of *Moringa oleifera* leaves, which not only contribute to the scavenging of free radicals but also to the good metabolic health of the animal[22]. Serum AST and ALT levels were significantly lower than that of the control batch. Our results show that the addition of aqueous extract *Moringa oleifera* leaf to the diet has no destructive effect on broiler hepatocytes.

## CONCLUSION

The addition of aqueous extract of *Moringa oleifera* leaves to the ration of broilers (Cobb 500) significantly affected hematological and biochemical parameters. Although, these parameters were affected, the indicators showed no adverse effect on renal function or hepatocytes in broilers fed this extract.

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