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RESEARCH ARTICLE

Lippia multiflora (Verbenacae, Mold.) Leaves Dietary Supplementation Effect on Broiler Chickens' Growth, Hematological Parameters, and Serum Biochemistry

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Abstract: The continued overuse of synthetic antibiotics has led to microbial resistance, responsible for numerous therapeutic failures in both animal and human health. This resistance has prompted the search for natural alternatives to the use of synthetic antibiotics in the poultry sector. The essay evaluated *Lippia multiflora* (Lm) leaves' effects on chickens' growth, health, and fatty acid profile. The rearing lasted 10 weeks, with 5 start-up weeks and 5 weeks of growth. At the start-up end, 6 experimental chicken groups were formed, including a control group without Lm leaves (0% Lm) and 5 additional groups, within which were 5% Lm, 7% Lm, 9% Lm, 11% Lm, and 13% Lm, receiving 5, 7, 9, 11, and 13% *Lippia multiflora* leaves, respectively. Throughout the test, the chickens and the feed leftovers were weighed for the growth performance determination. On day 70, 6 chickens were slaughtered per experimental group for the hematological and biochemical blood parameters analysis. Diet 5%Lm allowed the heaviest chicken weight of 1,824.51 g compared to the control diet for 1,773.01 g ($p = 0.134$). In addition, diets 5% Lm, 11% Lm, and 13% Lm leaves promoted the white blood cell count reduction from 121×10^3/µL with the control to 110.7, 108.48, 106.60×10^3/µL, respectively. Furthermore, diet 5% Lm led to the highest HDL cholesterol for 76.11±1.38 mg/dL, triglycerides for 132.85±6.83 mg/dL, and protein for 5.47±0.22 g/dL. Adding 5% *Lippia multiflora* dried leaves in growing and finishing broilers' diets can improve chickens' growth performance and their health status.

Keywords: Blood Biochemistry, Broiler, *Lippia multiflora,* HDL Cholesterol, Hematological Parameters

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Introduction

Traditional plants and their derivatives have long been used as sources of antibiotics in indigenous poultry production [1]. In modern poultry production, synthetic antibiotics are used as growth promoters by combating strong pathological constraints [1]. The misuse of these synthetic antibiotics has led to an increased development of resistant microorganisms. Also, synthetic antibiotics are associated with potential carcinogenicity and hepatotoxicity in both animals and humans. Moreover, the use of synthetic antibiotics has adverse effects on the chickens' health and represents a potential risk to human health [2]. These numerous disadvantages have led to increasing traditional plant use. Indeed, the immunomodulatory action of plants' photobiotic makes them a potential alternative to synthetic antibiotics [3]. Indeed, medicinal plants are used as growth promoters, antibiotics, antivirals, and antiparasitic agents [4]. For example, [5] have reported the use of many plants, such as green tea, lavender, neem, and garlic, as growth promoters and an alternative to synthetic antibiotics in poultry farming. Similarly, [6] reported a considerable positive effect of Jasmine sambac on chickens' growth, blood characteristics, and immune system. Nevertheless, depending on each medicinal plant, there is an optimal incorporation rate into broiler chicken diets. For example, when [7] introduced 0.2%, 0.5%, and 1.2% Salvia officinalis leaf powder into broiler chicken diets, they concluded that 0.2% and 0.5% levels performed better compared to 1.2%. Similarly, when [8] used 100, 150, and 200 mg/kg of a plant extract (Oleobiotec®) in broiler chicken diets, the 150 mg/kg diet provided the best results for chicken growth and health. So, depending on the medicinal plant and its concerned part, the incorporation rate in broiler diet varies. Meaningfully, overuse depresses chicken growth. Coming back to *Lippia multiflora*, very few studies have been conducted on its leaf's incorporation in broiler chicken diets. In fact, this plant has various biological properties, including anti-hypotensive, anti-analgesic, anti-microbial, anti-pyretic, and anti-malarial activities [9]. Also, [10] reported the good antioxidant activity of *Lippia multiflora* leaves. Given their numerous properties and non-toxicity reported by [10], these leaves could therefore improve broiler chickens' growth. So, this test assumed that *Lippia multiflora* dried leaves may positively impact growing-finishing broilers' growth and health status. The test objective was to evaluate *Lippia multiflora* dried leaves' effects on broilers' growth, blood cell count, and health status.

Materials and Methods

Lippia multiflora dried leaves were produced, some experimental materials were used, and broilers were reared.

Lippia multiflora Dried Leaves Production

In October and November 2024, *Lippia multiflora* leaves were harvested at the flowering stage in the bush, near the Graduate School of Agriculture at National Polytechnic Institute Felix Houphouet Boigny, at Yamoussoukro, Côte d'Ivoire. Then, the leaves were dried in a room in the laboratory of Animal Science for 14 days and ground to get some powder. During this period, the average room temperature was 25.5oC, and the relative humidity was 77%. So, the weather was quite dry.

Experimental Diets

During the grower-finisher phase, 6 diets including 0% Lm control diet without Lm, 5% Lm, 7% Lm, 9% Lm, 11% Lm, and 13%Lm, were formulated by incorporating *L. multiflora* leaves at the corresponding rates in the diets. Thereafter, each diet was analyzed to determine the bromatology composition. So, the diet composition and their chemical compositions are presented in Table 1.

Experiment Conduct

The test took place from October to December 2024 at the Graduate School of Agronomy experimental farm, at National Polytechnic Institute Felix Houphouet Boigny (INP-HB), in Yamoussoukro, Côte d'Ivoire. The start-up phase began with 250-day-old local breed chicks. The start-up lasted five weeks, and five chicks died. At the start-up end, the chickens were weighed and the mean was (μ) computed, by considering the gender (Eq. 1). Also, the weight Variance (V) and standard error (δ) were calculated (Eqs. 2-3). Looking at [9] approach, the birds for the experiment were selected in an interval (Eq. 4):

$$mean(\mu) = \frac{1}{n} * \sum_{i=1}^{n} x_i \quad (1)$$

$$Varianceof\ the\ population = V = \frac{1}{n} * \sum_{i=1}^{n} (x_i - \mu)^2 \quad (2)$$

$$Standard\ error\ of\ the\ population = \delta = \sqrt{V} \quad (3)$$

$$Selection - Interval: \mu - \delta \leq \mu \leq \mu + \delta \quad (4)$$

The selected chickens were divided into 6 groups of 24 chickens each, consisting of 12 males and 12 females. Each group was repeated 3 times with 4 chickens per repetition. The chickens were fed on 6 diets, namely 0% Lm, 5% Lm, 7% Lm, 9% Lm, 11% Lm, and 13% Lm. During the test phase, i.e., the growth-finishing phase, the chickens were fed daily with

120 g per chicken, once a day, at midday (12 O'clock). Furthermore, from the beginning to the end, the water was distributed ad libitum.

Table 1: Diets and chemical composition

Ingredients	Diets					
	0% Lm	**5% Lm**	**7% Lm**	**9% Lm**	**11% Lm**	**13% Lm**
Yellow corn	52	52	52	52	52	52
Fish meal	17	17	17	17	17	17
Soybean meal	12	12	12	12	12	11
Wheat bran	8	3	1	0	0	0
Premix	4	4	4	4	4	4
Eggshell	3	3	3	3	3	3
Parkia biglobosa grains' pulp	4	4	4	3	1	0
Lippia multiflora leaf's powder	0	5	7	9	11	13
Total	100	100	100	100	100	100
			Analyses and computations			
Dry matter (%)	90.63	90.66	90.42	90.35	90.36	90.71
Ash content (%DM)	16.31	16.60	16.43	15.96	16.29	18.079
Fat (%DM)	4.20	3.60	2.30	3.75	3.20	3.20
Tot_Carb (%DM)	60.79	61.10	61.47	61.39	61.51	59.92
Crude protein (%)	18.70	18.70	18.80	18.90	19.00	18.8
M.E (kcal/kg DM)	3,579.86	3,542.17	3,536.17	3,574.53	3,537.35	3,464.15

Tot_Carb (% DM) = Total carbohydrate=100% - (CP % +Fat % +Ash %); ME: Metabolizable Energy, ME (kcal /Kg DM) = [3.87× CP (% DM) +8.37× Fat (% DM) +4.12×Tot_Carb (% DM)] ×10 [9] (Koné et al., 2025)

Chickens' Growth, Hematological, and Biochemical Parameters

During the growth-finishing phase, the birds were weighed weekly. Alongside this weighing, the feed daily intakes were assessed. At the end of the experiment, in order to analyze the blood, 6 chickens, including 3 males and 3 females, were slaughtered per diet. So, 6 animals having the smallest absolute interval were chosen (Eq. 5):

$$Broilers\_Absolute\_interval = |\ \mu_j - x_j| \quad (5)$$

Where μ_j was the group ''j'' mean, and x_j was each individual weight in the group ''j''.

The blood was collected in a dry tube and an EDTA tube containing an anticoagulant. First, the hematological analysis was carried out with the blood from the tube containing EDTA, with the Mindray Robot, BC 30 (China). Thus, the White Blood Cells (WBC), the Red Blood Cells (RBC), hemoglobin, hematocrit, Mean Corpuscular Volume (MCV), platelets (PLT), and procalcitonin (PCT) contents were assessed. Thereafter, the blood from dried tubes was centrifuged at 3700 rpm for 1 min to obtain blood serum. Then, this blood serum was used to determine the total and HDL cholesterol, as well as total protein and triglycerides with a kit (Linear Chemicals, Barcelona). Optical densities were read on a UVIS spectrophotometer (Jenway 7410, UK).

Statistical Analyses

Each datum was generated 3 times by using 3 chickens per gender and per diet. So, the calculation of parameters such as Feed Conversion Ratio (FCR), feather yield, total cholesterol, triglycerides, and HDL cholesterol was performed using Excel software. All data were analyzed using XLSTAT software version 2014.5.03. The impact of the different diets was assessed by an analysis of variance (ANOVA). Then, the means were classified into homogeneous groups using the Newman- Keuls test within a 95% confidence interval.

Results and Discussion

The section concerns the broilers' growth; their blood cells' count and blood serum constituents.

Broilers' Growth

Statistical analyses revealed a difference in the weights following the diets (Table 2). Referring to the weights, no difference was observed between diets 5% Lm, 9% Lm, 7% Lm, 0% Lm (Table 2), and the least squares means were 1,824.51; 1,797.24; 1785.94 and 1773.01 g, respectively ($0.134 \leq p \leq 0.427$). It should be noted that, from 0% Lm to 5% Lm, the mean weight increased by 51.50 g, which represented a 2.9% gain. So, the incorporation of *Lippia multiflora* (Lm) leaves up to 9% had no depressive effects on the chickens' weight. But, over 9%, *L. multiflora* incorporation rates had an impairing effect on chickens' growth. For example, at 11% and 13%, the derived weights were 1,710.39 and 1,756.48 g, respectively. Moreover, these weights from 11%Lm and 13%Lm diets were highly different from that of 5%Lm ($p<0.0001$).

Table 2: Broilers' weights according to the gender, weeks, and diets

1. Diets	**LSM (g) µ±δ**	**3. Weeks*Gender**	**LSM (g) µ±δ**	**4. Week-10*Diets*Gender**	**LSM (g) µ±δ**	**5. Diets*Gender**	**LSM (g) µ±δ**
5% Lm	1,824.51±16.78^{a}	10*M	2,332.86±22.02^{a}	0% Lm*M	2,426.54±53.25^{a}	5% Lm*M	1,858.97±23.63^{a}
9% Lm	1,797.25±17.17^{ab}	10*F	2,207.96±22.17^{b}	5% Lm*M	2,347.25±55.43^{ab}	11% Lm*M	1,847.57± 27.43^{a}
7% Lm	1,785.95±16.91^{ab}	9*M	2,087.93±22.17^{c}	11% Lm*M	2,342.67±64.00^{ab}	0% Lm*M	1,840.75±23.28^{a}
0% Lm	1,773.01±16.91^{ab}	9*F	1,988.54±21.74^{d}	7% Lm*M	2,336.29±51.32^{ab}	7% Lm*M	1,827.86±22.95^{ab}
13% Lm	1,756.48±16.59^{bc}	8*M	1,852.41±21.74^{e}	0% Lm*F	2,305.50±55.43^{abc}	9% Lm*M	1,827.30±24.19^{ab}
11% Lm	1,710.89±16.65^{c}	8*F	1,764.38±21.74^{f}	13% Lm*M	2,284.23±53.25^{abc}	13% Lm*M	1,800.22±23.11^{abc}
		7*M	1,576.65±21.74^{g}	9% Lm*M	2,273.20±49.58^{abc}	5% Lm*F	1,789.52±23.82^{abcd}
		7*F	1,503.75±21.08^{h}	9% Lm*F	2,257.18±57.89^{abcd}	9% Lm*F	1,766.71±24.38^{abcd}
2. Gender	LSM (g) µ±δ	6*M	1,337.44±21.74^{i}	5% Lm*F	2,253.15±53.25^{abcd}	7% Lm*F	1,736.22±25.00^{bcd}
M	1,832.93±9.79^{a}	6*F	1,181.38±21.20^{j}	7% Lm*F	2,206.78±64.00^{abcde}	13% Lm*F	1,710.05±23.82^{cd}
F	1,717.19±9.65^{b}			13% Lm*F	2,160.08±53.25^{bcdef}	0% Lm*F	1,697.49±24.58^{d}
				11% Lm*F	2,109.94±46.57^{fg}	11% Lm*F	1,631.15±20.95^{e}

µ = LSM: Least Square Mean; n = 3; δ: standard error; Lm: *Lippia multiflora*; F: female (hens); M: males (roosters); mean; ^{abc}: Values in a column with different superscript letters are significantly different at α = 5% level

Similarly, when [9] used *Lippia multiflora* essential oil in broilers' diets, they reported that after an optimum rate of incorporation, the oil became highly toxic for chickens. Accordingly, [4] concluded that dosage levels are important factors in assessing the impact of phytobiotics on chicken growth. The moderate phytobiotic levels can improve chicken growth performance by stimulating nutrient utilization and health benefits, while high levels may have adverse effects including reduced feed intake and potential toxicity [4]. Incorporation of *Lippia multiflora* leaves above 9% resulted in decreased chicken weights. These ongoing results corroborate [11] observations. By all means, [11] observed reduced growth performance in chickens when Moringa oleifera leaves were mixed at 10 and 15% in the diet. Likewise, when [1] used Acacia auriculiformis dried leaves in broilers' diets, they said that over a 1.5% incorporation rate, the leaves had adverse effects on broilers' growth. Again, 1% Dendrobium officinale leaf addition led to an improvement in the chickens' growth, while 5% had no effect, and 10% led to a reduction in growth [12].

For these authors, the decrease in growth observed at 10% would be due to an overload of certain bioactive compounds, which can have adverse effects on metabolic processes and nutrient absorption [12]. Additionally, [13] reported that despite the beneficial effects of polyphenols, they can inhibit pancreatic enzymes and reduce body weight at certain doses. *Lippia multiflora* dried leaves addition at levels lower than 5% could therefore have more beneficial effects on the broilers' growth.

Indeed, [3] reported an improvement in mean body weight gain by progressive supplementation of 0.5 and 1% Ocimum tenuiflorum and Ocimum sanctum leaves in 2, 4 and 6-week-old broiler chickens. Similarly, improved body weight gains were reported following Olea europaea leaves incorporation at 0.25, 0.50, 0.75 and 1% [14].

Because of the cooking habits in Côte d'Ivoire, the broilers were reared for 10 weeks with the aim of producing some hard meat. This long-term run revealed the sexual dimorphism between roosters and hens. At day 70, the males and females weighed 1,832.93 and 1,717.19 g, respectively ($p<0.0001$; Table 2). Looking at the week effect on the broilers' weights (Table 2), it appears that, at each week, the roosters were heavier than the hens. To clarify, on week 10, the males and females weighed 2,332.86 and 2,207.96 g, respectively ($p<0.0001$). This observation was also established on weeks 6, 7, 8 and 9. So, through the weeks, the growing gap between roosters and hens was more and more important (Table 2). Similarly, though the birds were reared in different boxes based on gender, within any interaction, the roosters performed better than the hens (Table 2). Regardless of *Lippia multiflora* dried leaves incorporation rates, roosters performed better than hens. Indeed, with diet 5%Lm, roosters and hens weighed 1,858.97 and 1,789.52 g, respectively ($p = 0.117$). Though these weights were similar, the males weighed 69.45 g more than the hens. This tendency was already reported by [9], because with 1.5 mL of *L. multiflora* essential oil per kilogram of feed, the roosters and hens weighed 1,678.4 g and 1,462 g at week 8, respectively ($p<0.0001$).

Broiler Chickens' Hematological Parameters

Hematology and serum biochemistry are essential factors in determining the animals' sanitary state. So, at week 10, the chickens' hematological parameters were analyzed and recorded (Table 3). Thus, the hematological analysis checked the white and red blood cell counts. Also, hematocrit (HCT), hemoglobin (HGB), and the mean corpuscular volume (MCV) were determined. The mean corpuscular volumes were stable (Table 3) according to the diets ($0.23 \le p \le 0.96$), the gender ($p=0.64$), and the interaction of both factors ($0.14 \le p \le 1.00$). So, with MCVs ranging between 113.17 and 121.37 fL, these results were similar to those of [1] and [15]. When they put some Acacia auriculiformis leaves [1], or some Zingiber officinale powder [15] in broilers' diets, MCVs ranged between 121.63 and 128.43 with A. auriculiformis, and 118.19 and 122.30 with Z. officinale. In fact, when the phytochemicals don't have a depressive effect on the chickens, age remains the main effect on MCVs [16]. For example, [16] discovered that hens' MCVs moved from 96.50 fL at 3 months of age to 99.20 fL at 6 months of age.

It must be remembered that White Blood Cells (WBC) are part of the immune system and are involved in fighting infections in the body [17]. Looking at the white blood cells' count (Table 3), there was no difference between 0% Lm and 7% Lm diets. In these diets, WBC counts were 126.38 and 121x103/µL, respectively, for 7% Lm and 0% Lm diets ($p = 0.28$). Because the chickens did not receive any synthetic antibiotic during the growing-finishing phase, a high WBC content could indicate the presence of an infection. So, diets 0% Lm and 7% Lm were not appropriate for broilers. In contrast, 5% Lm, 9% Lm, 11% Lm, and 13% Lm formed a homogeneous bloc, and WBCs' contents fluctuated between 105.22x103/µL for 9% Lm and 110.70 x103/µL with 5% Lm diet ($0.652 \le p \le 0.768$). Particularly, by increasing *Lippia multiflora* leaves incorporation to 5%, 11%, and 13%, WBCs' cell counts gradually decreased and were 110.70, 108.48, and 106.60x103/µL, respectively. These WBC count reductions could show that no infection occurred. So, there was no need to synthesize more WBC cells for a higher immune response.

These results were consistent with [1] conclusions. They observed a decrease in the broilers' WBC counts following dietary supplementation with Acacia auriculiformis leaves at rates of 0.75, 1.5, and 3% [1]. Similarly, it could be deduced that *L. multiflora* dried leaves had an antibiotic effect on the broilers. This decrease in WBC count could be due to its antibiotic action. Indeed, [18] reported that plants produce saponins to protect themselves from parasite attacks. Likely, [18] announced that saponins are involved in immune system protection once the plants are ingested. In addition, certain secondary compounds present in plants, including tannins, alkaloids, and saponins, have antioxidant activities.

Lippia multiflora leaves phytochemical screening revealed the presence of tannins, alkaloids, and saponins. Moreover, [19] discovered important total flavonoid and total polyphenol contents in *Lippia multiflora* leaves' extracts, at 26.2 mg QE/g and 462.2 mg GAE/g, respectively. These phytochemicals could justify the important inhibition diameters they observed [19]. According to [20], Morinda lucida leaves protective effect against diseases is related to the antioxidant activity of their tannins, alkaloids, and saponins.

Red Blood Cells (RBC) transport oxygen from the lungs to the respiratory tissues and carbon dioxide in the opposite direction. This transport is possible via RBCs' hemoglobin. Some differences were observed in RBC counts (Table 3). Like WBC count, the 7% Lm diet performed better on RBC than the 5% Lm diet. Again, RBC counts were 3.62 and 2.56×10^6/µL, respectively, for 7% Lm and 5%Lm diets ($p<0.0001$). Thus, the 7% Lm diet was the best compared to the 5%Lm diet. Looking at the interaction between the diets and the gender, unlike 7% Lm*F, which scored 4.74×10^6/μL, other diets delivered similar RBC contents. When observing WBC and RBC contents, it may be concluded that diets 5%Lm and 7%Lm were both suitable for the chickens.

Table 3: Broilers' hematological parameters

	2.RBC (10^6/μL)	3.HCT (%)	4.HGB (g/dL)		5.MCV (fL)
Diets					
7% Lm	126.38±3.59^{a}	3.62±0.22^{a}	42.04±2.64^{a}	18.72±1.12^{a}	115.88±1.76
0% Lm	121.00±3.27^{a}	3.13±0.20^{ab}	37.17±2.41^{a}	16.75±1.02^{a}	118.40±1.6
5% Lm	110.70±3.59^{b}	2.56±0.22^{bc}	28.98±2.64^{b}	13.24±1.12^{b}	113.80±1.76
11% Lm	108.48±3.27^{b}	2.35±0.20^{c}	26.78±2.41^{b}	12.37±1.02^{b}	113.93±1.60
13% Lm	106.60±3.27^{b}	2.28±0.20^{c}	25.80±2.41^{b}	12.18±1.02^{b}	113.17±1.60
9% Lm	105.22±3.27^{b}	2.19±0.20^{c}	25.40±2.41^{b}	11.88±1.02^{b}	116.10±1.60
p-value	<0.0001	<0.0001	<0.0001	<0.0001	0.23≤p≤0.96
μ±δ					115.21±1.65
Gender					
Male	115.11±2.00	2.62±0.12	30.36±1.48	13.99±0.63	115.58±0.98
Female	110.63±1.89	2.70±0.12	31.13±1.39	14.17±0.59	114.93±0.93
p-value	0.12	0.64	0.71	0.83	0.64
μ±δ	112.87±1.95	2.66±0.12	30.74±1.43	14.08±0.61	115.25±0.95
Diets*gender					
7% Lm*F	143.77±4.63^{a}	4.74±0.28^{a}	55.07±3.41^{a}	24.13±1.45^{a}	116.37±2.27
5% Lm*M	132.25±5.67^{a}	3.44±0.35^{b}	38.15±4.17^{bc}	17.50±1.77^{bc}	111.35±2.78
0% Lm*M	129.43±4.63^{a}	3.35±0.28^{b}	40.80±3.41^{b}	18.03±1.45^{b}	121.37±2.27
0% Lm*F	112.57±4.63^{b}	2.90±0.28^{b}	33.53±3.41^{bc}	15.47±1.45^{bc}	115.43±2.27
11% Lm*M	111.23±4.63^{b}	2.41±0.28^{b}	27.70±3.41^{bc}	12.80±1.45^{bc}	114.93±2.27
13% Lm*M	110.67±4.63^{b}	2.48±0.28^{b}	27.63±3.41^{bc}	13.23±1.45^{bc}	111.53±2.27
9% Lm*M	107.57±4.63^{b}	2.15±0.28^{b}	25.33±3.41^{bc}	11.80±1.45^{bc}	117.57±2.27
11% Lm*F	105.73±4.63^{b}	2.29±0.28^{b}	25.87±3.41^{bc}	11.93±1.45^{bc}	112.93±2.27
9% Lm*F	102.87±4.63^{b}	2.22±0.28^{b}	25.47±3.41^{bc}	11.97±1.45^{bc}	114.63±2.27
13% Lm*F	102.53±4.63^{b}	2.09±0.28^{b}	23.97±3.41^{c}	11.13±1.45^{bc}	114.80±2.27
7% Lm*M	100.30±5.67^{b}	1.96±0.35^{b}	22.50±4.17^{c}	10.60±1.77^{bc}	115.15±2.78
5% Lm*F	096.33±4.63^{b}	1.97±0.28^{b}	22.87±3.41^{c}	10.40±1.45^{c}	115.43±2.27
p-value					0.14≤p≤1.00
μ±δ					115.13±2.35

μ = LSM: Least square mean; n = 3; δ: standard error; WBC: White Blood Cells; RBC: Red Blood Cell; HCT (%): Hematocrit; HGB: Hemoglobin; MCV: Mean corpuscular volume; Lm: *Lippia multiflora*; F: female (hens); M: males (roosters); mean; ^{abc}: Values in a column, and the same section (Diets, Gender, Diets*gender), with different superscript letters are significantly different at α = 5% level

Furthermore, variations were observed in the hematocrit (HCT) and hemoglobin (HGB) levels between the diets (Table 3). Because the 7% Lm diet had higher scores on WBC and RBC counts, its HCT and HGB were higher than the other diets' results. Thus, 7% Lm could have improved the hematopoiesis system. While 7% Lm allowed 42.04% and 18.72 g/dL for HCT and HGB, respectively, 5% Lm diet results were 28.98% and 13.24 g/dL ($p<0.0001$). Following this, there was no difference between the hemoglobin contents from 5% Lm, 9% Lm, 11% Lm, and 13% Lm diets, which were 13.24, 11.88, 12.37, and 12.18 g/dL, respectively. These levels were consistent with the reference range for broiler chickens, which ranged from 11.60-13.68 g/dL [21]. Supplementation with 5, 9, 11, and 13%Lm leaves maintained the hemoglobin levels of the chickens at normal levels. The hemoglobin and hematocrit levels of 5% Lm, 9% Lm, 11% Lm, and 13% Lm groups were in accordance with the reference standard, which fluctuates between 35.9-41% [21]. The supplementation with *Lippia multiflora* leaves has led to an

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improvement in hematological parameters. It therefore allows the chickens' health improvement. For example, [6] reported an improvement in the immune system by the addition of Jasmine sambac leaves at 3, 6, and 9% in the chickens' diet.

Blood Plasma Biochemical Parameters

The serum biochemical indices are effective indicators for determining the metabolic, nutrient deposition, and livestock health status. Then, the biochemical parameters such as total cholesterol, HDL cholesterol, triglycerides, and total protein were determined at week 10. On the whole, *Lippia multiflora* leaves influenced these parameters (Table 4). The total and HDL cholesterol, as well as triglycerides and protein, results are in Table 4. First of all, following 5% Lm, 7% Lm, and 9% Lm diets, the total cholesterol contents decreased gradually and were 208.30, 206.95, and 205.13 mg/dL ($2.064 \le p \le 2.498$). So, these incorporation rates decreased the total cholesterol contents. This tendency was reported with leek leaves when they were incorporated at 0, 0.05, 0.10, 0.15, and 0.20%; the total cholesterol contents were 130.37, 102.90, 102.27, 94.93, and 94.17 mg/dL, respectively [22]. [22] concluded that the hypocholesterolemic effect of these leek leaves was through a progressive inhibition of hepatic cholesterol biosynthesis and an increase in fecal bile secretion.

The total cholesterol contents did not totally follow [22] result trends. In fact, blood plasma total cholesterol contents decreased alongside an increase in leek leaves incorporation rates in the diets [22]. As an illustration, with over 9% *Lippia multiflora* leaves incorporation, total cholesterol contents increased. For instance, with 9% Lm, 11% Lm and 13% Lm diets, total cholesterol contents were 205.13, 208.92 and 210.85 mg/dL, respectively. On a broad view, the highest total cholesterol levels were recorded in the 13% Lm diet for 210.85, followed by the 11% Lm diet for 208.92, and the 5% Lm diet for 208.3 mg/dL ($0.140 \le p \le 0.298$). Finally, the lowest total cholesterol level was recorded in the 0% LM diet for 188.33 mg/dL ($p<0.0001$). It could therefore be assumed that some bioactive compounds contained in the leaves stimulated the cholesterol synthesis metabolism. These results are different from those of [11]. In fact, they reported a decrease in chickens' serum cholesterol levels following the addition of Moringa oleifera leaves in the diet. [17] attributed the decrease in cholesterol to the hypocholesterolemic properties of Carica papaya leaves. So, above a 9% incorporation rate, *Lippia multiflora* dried leaves induce a hypercholesterolemic effect.

According to [23], High-Density Lipoprotein Cholesterol (HDL-Chol) and triglycerides play an important role in the progression of fat disorders in the body. So, looking at HDL-cholesterol contents, an increase in HDL cholesterol was observed after *L. multiflora* leaves addition (Table 4). The highest HDL cholesterol level was recorded in diet 5%Lm for 76.11±1.38 mg/dL. The lowest levels were recorded in the control diet (0% Lm) and the 7%Lm diet, and the results were 54.258 mg/dL and 57,832 mg/dL, respectively ($p = 0.08$). Because the main ingredients in various diets were the same, it may be concluded that the optimum incorporation rate of *L. multiflora* leaves was 5%. In this case, *L. multiflora* dried leaves induced the formation of more HDL-Cholesterol from chylomicron remnant via the ApoC-II pathway [23]. It should be reminded that HDL-cholesterol's first job is to collect and transport back the fat to the liver [23]. Indeed, because HDL-cholesterol structure contains triglyceride molecules, the highest HDL-cholesterol content of 76.11±1.38 mg/dL was associated with the highest triglyceride content of 132.85±6.83 mg/dL with diet 5% Lm. This good health status may explain the heaviest weight recorded with diet 5% Lm for 1,824.51±16.78 g (Table 2). [24] also observed an increase in HDL cholesterol after Moringa oleifera leaves addition in the chicken diets. Nevertheless, the 5%Lm diet did not have the same effect regarding the gender. Because while the hens had 84.62±1.96 mg/dL of HDL-cholesterol, the roosters had 67.60±1.96 mg/dL ($p<0.0001$). Regarding protein levels, again, the 5% Lm diet had the best content for 5.47±0.22 g/dL. Interestingly, all diets' chickens' serum protein levels were in accordance with [1] findings when they used Acacia auriculiformis as a natural antibiotic source.

Table 4: Broiler blood's biochemical parameters

	Total cholesterol (mg/dL)	HDL-cholesterol (mg/dL)	Triglycerides (mg/dL)	Total protein (g/dL)
1.Diets				
13% Lm	210.85±0.91^{a}	64.19±1.38^{c}	84.64±6.83^{c}	4.70±0.22^{ab}
11% Lm	208.92±0.91^{ab}	70.89±1.38^{b}	53.95±6.83^{d}	5.27±0.22^{a}
5% Lm	208.30±0.91^{abc}	76.11±1.38^{a}	132.85±6.83^{a}	5.47±0.22^{a}
7% Lm	206.95±0.91^{bc}	57.83±1.38^{d}	113.00±6.83^{ab}	4.61±0.22^{ab}
9% Lm	205.13±0.91^{c}	63.06±1.38^{c}	96.30±6.83^{bc}	3.95±0.22^{b}
0% Lm	188.33±0.91^{d}	54.26±1.38^{d}	107.32±6.83^{bc}	4.3±0.22^{b}
2.Gender				

M	212.08±0.53^{a}	65.84±0.80^{a}	88.75±3.94^{b}	4.53±0.13
F	197.41±0.53^{b}	62.94±0.80^{b}	107.27±3.94^{a}	4.90±0.13
p-value	<0.0001	<0.0001	<0.0001	0.052
μ±δ				4.72±0.13^{a}
3.Diets*gender				
13% Lm*M	217.60±1.29^{a}	64.39±1.96^{cde}	52.07±9.65^{de}	5.34±0.31^{ab}
11% Lm*M	215.37±1.29^{ab}	77.26±1.96^{b}	67.03±9.65^{cde}	5.28±0.31^{ab}
0% Lm*M	214.15±1.29^{ab}	57.82±1.96^{ef}	72.68±9.65^{cde}	3.84±0.31^{bc}
5% Lm*M	212.03±1.29^{b}	67.60±1.96^{cd}	119.40±9.65^{ab}	5.37±0.31^{ab}
7% Lm*M	208.20±1.29^{c}	59.66±1.96^{de}	134.80±9.65^{a}	3.95±0.31^{bc}
7% Lm*F	205.70±1.29^{c}	56.00±1.96^{ef}	91.20±9.65^{bcd}	5.27±0.31^{ab}
9% Lm*F	205.15±1.29^{c}	57.82±1.96^{ef}	106.11±9.65^{abc}	4.5±0.31^{abc}
9% Lm*M	205.10±1.29^{c}	68.30±1.96^{c}	86.49±9.65^{bcd}	3.41±0.31^{c}
5% Lm*F	204.57±1.29^{c}	84.62±1.96^{a}	146.30±9.65^{a}	5.56±0.31^{a}
13% Lm*F	204.10±1.29^{c}	63.98±1.96^{cde}	117.20±9.65^{ab}	4.06±0.31^{bc}
11% Lm*F	202.47±1.29^{c}	64.52±1.96^{cde}	40.87±9.65^{e}	5.26±0.31^{ab}
0% Lm*F	162.50±1.29^{d}	50.70±1.96^{f}	141.95±9.65^{a}	4.77±0.31^{abc}

μ = LSM: Least square mean; n = 3; δ: standard error; Lm: *Lippia multiflora*; F: female (hens); M: males (roosters); mean; ^{abc}: Values in a column, and the same section, with different superscript letters are significantly different at α = 5% level

Conclusion

In Côte d'Ivoire, researchers are looking for more and more natural solutions for poultry sanitary aspects. So, with the aim of reducing the use of synthetic antibiotics, the experiment assessed *Lippia multiflora* dried leaves' antibiotic potential. So, *L. multiflora* leaves powder was added at 5, 7, 9, 11, and 13% in broilers' diets. Then, the broilers' growth, their blood cells' count, and their blood plasma cholesterol were assessed. On a broad view, the experiment revealed that 5% *Lippia multiflora* leaves addition in broilers' diet was the best incorporation rate. Under this circumstance, *Lippia multiflora* leaves allowed the heaviest live weight of 1,824.51 g versus 1,773.01 g for the control diet without *L. multiflora* leaf powder (p = 0.134), at 10 weeks of age. In addition, this diet led to the highest HDL-cholesterol contents for 76.11±1.38 mg/dL, while the control diet exhibited 54.26±1.38 mg/dL (p<0.0001). Because the 5% incorporation rate, which was the lowest, gave the best results, another experiment must be conducted with lower incorporation rates like 2, 2.5, 3, 3.5, 4, 4.5, 5, and 5.5%.

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Author's Contributions

Tagouèlbè Tiho: Is the poultry section supervisor at the Graduate School of Agriculture, National Polytechnic Institute Felix Houphouet Boigny. Wrote the research proposal, and we, 3 Associate Professors, corrected it. She ran the experiment and collected the data under TT's observations and guidelines. Thereafter, she wrote the first draft corrected it. We all read the final text and agreed to send it for publication.

Kelemin Awa Koné: Advisers for her Doctorate Degree studies achievement. Wrote the research proposal, and we, 3 Associate Professors, corrected it. She ran the experiment and collected the data under TT's observations and guidelines. Thereafter, she wrote the first draft.

Yapo Magloire Yapi and Vama Etienne Tia: Advisers for her Doctorate Degree studies achievement corrected it. We all read the final text and agreed to send it for publication.

Conflict of Interest

No conflict of interest to be disclosed.

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