Int.J.Curr.Microbiol.App.Sci (2025) *14*(08): 1-9

International Journal of Current Microbiology and Applied Sciences
ISSN: 2319-7706 **Volume 14 Number 8 (2025)**
Journal homepage: http://www.ijcmas.com

Original Research Article https://doi.org/10.20546/ijcmas.2025.1408.001

Impact of Plant Extracts on Zootechnical, Microbiological and Biochemical Parameters of Broiler Chicken of the Cobbs 500 Breed (*Gallus gallusdomesticus*)

Bonny Aya Carole[1], Assandi Kouamé Rivière[2*], Ake Moussan Désirée Francine[3] and Koffi Kouamé Sébastien[3]

[1]Laboratory of Biotechnology, Agriculture and Valorization of Biological Resources (LBAVRB), Biosciences Training and Research Unit, Félix Houphouët-Boigny University, BP V34 Abidjan, Côte d'Ivoire
[2]Laboratory of Biochemistry, Biotechnology and Food Sciences (LaBBSA), Science and Technology Training and Research Unit, Alassane OUATTARA University, BP V 1801, Bouaké, Côte d'Ivoire
[3]Laboratory of Food Biotechnology and Microbiology (LBMA), Food Science and Technology Training and Research Unit, NanguiAbrogoua University, 02 BP 801 Abidjan 02, Côte d'Ivoire

**Corresponding author*

Keywords

Broiler chickens, plant extracts, antimicrobials, *Salmonella* sp, *Eimeria tenella*

Article Info

Received:
20 May 2025
Accepted:
25 July 2025
Available Online:
10 August 2025

A B S T R A C T

The use of antimicrobials in animal production has promoted resistance of microorganisms, transmissible to humans, thus compromising any treatment of pathologies resulting. The aims of this study is to contribute to the fight against the proliferation of avian pathogens resistant to antibiotics, by the use of plant extracts in broiler chickens. Thus, hydro-ethanolic and aqueous extracts of the plant *Mallotus oppositifolius* were administered to chicks during breeding. Zootechnical, microbiological parameters and blood constants were evaluated. The search for *Salmonella* and *Eimeria tenella* oocysts was carried out on fecals samples of the subjects at the 10th, 21st and 38th days. Weight evolution, feed consumption, average daily gain, evaluated in the subjects of the control group, are similar to those obtained in the subjects of the test groups. No contamination by *Salmonella*, and no infestation by the parasite *Eimeria tenella* in all the experiments. Also, creatinine, urea, aspartate aminotransferase, alanine aminotransferase, Blood Sugar and C-reactive protein, subjects are normal, compared to the threshold values. In summary, the extracts of the plant *Mallotus oppositifolius* have an inhibitory effect on the contamination of potential avian pathogens such as *Salmonella*sp and *Eimeria tenella*.

Introduction

Poultry farming in Ivory Coast, like in West African countries, is constantly threatened by various pathologies such as coccidiosis, colibacillosis, avian flu and salmonellosis (Filliat & Souvestre, 2016). The use of antibiotics in animal nutrition, the only beneficial option for improving both zootechnical performance parameters

and for the treatment of diseases, is the source of biosecurity threats to both human and animal health. These threats, resulting from the increase in resistant pathogens and the accumulation of antibiotic residues in animal products and in the environment, are becoming increasingly worrying.

Numerous scientific studies reveal the interest of medicinal plant derivatives for poultry production (Jyotsana *et al.,* 2019). In Ivory Coast, the work of Bonny *et al.,* (2021) have shown that aqueous and ethanolic extracts of the plant *Mallotus oppositifolius*, which have antibacterial properties, also have potential for solving infectious problems in poultry farming. This would reduce the emergence and spread of antibiotic-resistant pathogens. In addition, Assandi *et al.,* (2024) report that these extracts own phytochemicals compounds, which are beneficial for the digestive health of broiler chickens.

With the growth of poultry production in Côte d'Ivoire, the risk of the emergence and spread of resistant pathogens is becoming increasingly high. So, it is important to promote research into alternatives to the use of antimicrobials in livestock farming. Plant extracts represent a new class of products of interest in this area.

The aims of this work is to contribute to the fight against the proliferation of avian pathogens resistant to antibiotics, by the use of plant extracts as an alternative to the antibiotics commonly used in the Ivorian poultry sector.

Materials and Methods

The plant material consists of ethanolic and aqueous extracts of the dry leaves of the plant *Mallotus oppositifolius*, with antibacterial potential on avian *Salmonella* and non-toxic to broiler chickens (Table 1). The animal material consisted of one-day-old chicks with an average weight of 50.65 ± 2 g, from the Ivorian Society of Animal Production (SIPRA).Chicken treatment products, namely Tetracolivit (10g oxytetracycline + 7MUI colistin) and Anticox (74g sulfadimidine + 8g diaveridine) were used.

This study was carried in an experimental farm of the Central Veterinary Laboratory of Bingerville, located in the west of the city of Abidjan. The peripheral location and the climate make Bingerville a suitable site for agricultural and poultry activities.

Management of breeding and administration of extracts

This work is an experimental study in which subjects, treated with plant extracts, are followed for a period of 35 days, compared to a control group not exposed to plant extracts (Merazi, 2019). Health prophylaxis methods such as hygiene and crawl space were implemented. The animals were fed with a feed marketed by the company Ivograin of Côte d'Ivoire. The different groups were monitored prospectively, carrying out associated microbiological and parasitic analyses. To do this, 99 chicks were randomly divided into 3 batches of 33 chicks, including 3 repetitions of 11 chicks per batch. The batches consisted of a control batch (batch T), a batch treated with ethanolic extract of *Mallotus oppositifolius* (batch ME) and a batch treated with aqueous extract of *Mallotus oppositifolius* (batch MA). Batch T received prophylactic treatment based on antibiotics (COVIT) and anticoccidial (Anticox), according to the prophylactic schedule in place. MA and ME batches received prophylactic treatment with aqueous and ethanolic extracts of *Mallotus oppositifolius*, respectively.

The extracts were incorporated into the drinking water at a concentration of 200 mg/L throughout the breeding period. The subjects of each group were weighed at the beginning of the experiment, and at seven-day intervals until the end of the experiment. At the end of the experiment, zootechnical parameters such as weight gain, feed consumption, and average daily gain were determined (Merazi, 2019).

Determination of zootechnical parameters

Weight gain

To monitor the growth of the chickens, weighings were carried out on D0 (day of the arrival of the chicks), then every week, using a TEFAL brand scale with a sensitivity of 1g. The weekly weight change was obtained by taking the difference between the average weekly weights of one week and those of the previous week (Merazi, 2019).

Average daily gain

The average daily gain (ADG), which represents the growth rate of a subject, was calculated weekly according to the following formula:

$$ADG = \frac{\text{weight f} - \text{weight d}}{t}$$

ADG = average daily gain; weight f = weight at the end of the period; weight d = weight at the beginning of the period; t = duration of the period in days.

Determination of the effectiveness of plant extracts on microbiological parameters

Microbiological analysis for *Salmonella* detection during the test

On the 10th, 21st, and 35th day of experimentation, fecal samples from subjects in each batch were taken for *Salmonella* testing according to the standard ISO-6579-1 (2017). Three sample per repetition group of each batch were taken, for a total of 27 samples per day of analysis. The search for *Salmonella* was carried out in 4 stages:

- ✓ Pre-enrichment: 10 g of samples were diluted in 90 mL of buffered peptone water, then incubated at 37°C for 18 h;
- ✓ Selective enrichment: 0.1 mL of the pre-enrichment was diluted in 9.9 mL of Vassiliadis Rapaport, then incubated at 40°C for 24 h;
- ✓ Selective isolation: The enrichment culture was inoculated onto Hektoen and XLD agar, then incubated at 37°C for 24h;
- ✓ Biochemical identification: Presumptive colonies were analyzed by Malditof mass spectrometry.

Search for parasites

Fecal samples from the different subjects (test and control) were collected on the 17th and 21st day and then analyzed. Three batches of samples per replication group were created, for a total of 27 samples per day of analysis. The analysis was carried out according to the Mac MASTER coccidia counting method, in order to determine the intensity of infestation of these parasites (Thienpont *et al.,* 1986). Thus, 3 grams of fecal samples were homogenized in a mortar with 42 mL of sterile distilled water. The resulting mixture was poured through a tea strainer. Then the filtrate was centrifuged at 3000 rpm for 5 minutes. Each collected base was suspended in 20 mL of 40% (w/v) sodium chloride (NaCl) solution. Using a pipette, 0.20 mL of this suspension was taken and then introduced into a Mac MASTER cell. Then, observation under an optical microscope with an X10 objective revealed the presence or absence of parasite eggs in the cells of Mac MASTER.

Biochemical analyses

At the end of the experiment, blood samples were taken to check the blood biochemical constants of the chickens treated with the plant extracts. The blood samples were taken directly via the wing vein as described by the technique of Merazi (2019), in 3 chickens per repetition group of each batchon day 35. Blood collected in an EDTA tube was centrifuged at 3,500 rpm using an EAB-20 centrifuge (Hettichzentrifugen, Germany). The plasma obtained was used for the determination, by the Cobbas® C111 analyzer (Roche, France), of alanine aminotransferases (ALAT), aspartate aminotransferases (ASAT), C-reactive protein (CRP), urea, creatinine and blood glucose.

Statistical analyses

Excel 2016 software was used for data recording and coding. Analysis of variance of hematological and biochemical parameters was performed with XLSTAT version 2022 software.

Results and Discussion

Effects of extracts on zootechnical parameters of broiler chickens

Zootechnical parameters concern feed consumption, weight gain and average daily gain.

Food consumption

On day 7, food consumption was between 18.06 g and 18.27 g in the groups. On day 14, food consumption increased to 27.39 g for batch T; to 26.92 g for batchMA; to 26.96 g for batchME. On day 21, food consumption in batchT and batchME was 55.51 g and 55.41 g. That of batchMA was 57.13 g. On day 28, subjects consumed 68.15 g, 69.81 g and 69.47 g respectively in batches ME, MA and T. At the 5th week of the experiment, the observed consumption was 127.71 g for batchT, 122.54 g for batchMA and 120.11 g for batchME. (Figure1). The average daily feed consumption per chicken was 59.67 ± 43.29g in batchT, 58.90 ± 41.40g in batchMA, and 57.73 ± 40.39g in batchME. However, statistical analysis of feed consumption values shows that there is no

significant difference (p=0.9982 > 0.05). In view of these results, the extracts administered in drinking water have no significant influence on the feed consumption of broilers.

Weight gain

Figure 2 shows the weekly weight evolution of the different batches. At the end of the breeding, in the different batches, the average weight of the subjects of batch T was 1315.83 ± 358.16g, that of batch MA was 1235.67 ± 312.42g. Batch ME recorded an average weight of 1200.35 ± 314.44g.

Average Daily Gain (ADG)

During the five weeks of experimentation, the results relating to the cumulative daily average gains, recorded throughout the conduct, reveal that the subjects of batch T expressed a ADG of35.6±19.4g. Those in the MA batch expressed a ADG of 33.4±19.4g. A ADG of32.4±19.6g for the ME batch. Statistical analysis showed that these differences are not significant (p>0.05). (Table 2). In summary, the administration of plant extracts in drinking water has no influence on the GMQ of chickens.

Effect of extracts on Salmonella contamination

The effectiveness of plant extracts in preventing *Salmonella* contamination was assessed by microbiological analyses. The results show that on the 10th, 21st and 35thday of the experiment, the search for *Salmonella* in fecals samples of batch T was positive, while that of batches MA and ME was negative. Table 3 shows the results of the microbiological analysis carried out in chicken fecals samples for the search for *Salmonella*. These results show that plant extracts have an effective preventive action against *Salmonella* contamination.

Effect of extracts on parasite Eimeria tenella proliferation

The effectiveness of the extracts in the treatment of coccidiosis was compared with that of a reference product, Anticox (Sulfadimidine and Diaveridine).

Parasite analysis of fecals samples showed that subjects in batch T were positive with very high infestation levels. A total of 70,000 oocysts per gram of fecals samples (OPG) were counted. Subjects in batches MA and ME showed no infestation (0 OPG).

Effect of extracts on biochemical parameters

The urea concentration in all subjects is 0.01g/L. The normal concentration in chickens is 0.01g/L. The normal concentration of creatinine in chicken blood is less than 10 mg/L. It is noted that the creatinine concentration at the end of the test is less than 10 mg/L in all experimental groups.

The normal concentration of aspartate aminotransferase (ASAT) in chickens is less than 270 IU/L and that of alanine aminotransferase (ALAT) is less than 10 IU/L. The AST concentration of batches MA, ME and T have their concentrations below the standard values. The ASAT concentration of batch T was 201.2± 8.34 IU/L. The ME batch recorded a concentration of 257.2± 8.76 IU/L and the MA batch, 215.5± 8.06 IU/L. Alanine aminotransferase (ALAT) concentrations in blood samples from all subjects in the different batches were less than 10 IU/L. The ALAT concentration recorded in batch T was 7.60 IU/L. These concentrations were 7.85 IU/L for batch MA and9.3 IU/L for the ME batch.

The normal blood glucose concentration of chickens is between 2 and 5 g/L. The values obtained with all experimental batches were within the normal range.

The C-reactive protein (CRP) concentration values obtained with all experimental batches were less than 6 mg/L (normal value). The lowest concentration was recorded with batch ME (0.155 mg/L) followed by batch MA (0.21 mg/L). The control batch recorded an estimated CRP value of 0.24 mg/L. (Table 4).

This study was conducted with the aim of finding a palliative to the use of antibiotics in chicken farming.

The study of the growth performance character of the extracts was carried out by adding the extracts to the drinking water of the subjects. At the end of the practice, the average daily gains of the different experimental groups varied between 31.76±18.33 g and 35.64±19.42 g. These results would mean that the plant extracts had no impact on the performance parameters of the broiler chickens. The work of Dosso (2015) which focused on SELKO-pH, which is a combination of organic acids (formic acid, ascorbic acid, acetic acid, propionic acid,

citric acid) supposed to improve the quality of drinking water by preventing the development of germs, reported a ADG of the group exposed to the products higher than that of the control (35.64 ± 19.42g). Larbi & Lakhdari (2016) in their work carried out on the study of the effect of origanum extract (product obtained by distillation of the plant and recognized as a growth promoter) on the zootechnical performance of broiler chickens, reported identical ADG between the group exposed to the extract and the non-exposed group.

On the other hand, Achour & Saifi (2017) obtained a higher ADG of the group exposed to a plant extract (VOLARUM, extract obtained by distillation of 5 plants) than that of the unexposed group. During this breeding, no antimicrobial products other than plant extracts were administered to the experimental groups. In addition to bacterial infections, parasitic infections are a problem in poultry farming. Coccidiosis, the world's leading scourge in poultry farming, is a parasitic infection caused by internal parasites called coccidia. There are several types depending on their location in the digestive system (Saarinen *et al.,* 2001).

Attacking the intestinal mucosa, they cause bloody diarrhea, malabsorption, loss of feed efficiency, a drop in egg production and defects in the quality of meat and eggs. It can also cause the death of poultry. So one part of this work consisted of evaluating the effect of plant extracts on parasitic infections due to *Eimeria tenella*, a parasite responsible for avian coccidiosis. The results obtained show that daily consumption of 200 mg/L of water of aqueous and ethanolic extract of *Mallotus oppositifolius* prevents infestation by *Eimeria tenella*.

These results are in agreement with those of Kouakou *et al.,* (2010) which showed that the aqueous extract of *Thonningiasanguinea* (THOS) reduces the rate of infestation of chickens by *Eimeria tenella*. Studies conducted by Saarinen *et al.,* (2001) showed that the use of plant extracts could reduce oxidative stress in chickens, thus reducing lesions and delaying the development of the parasite. In addition to the effects of the secondary metabolites contained in these extracts, the cytotoxic effects of these substances can also be mentioned to explain the positive effects of plant extracts. Makkar (2003) and Sahraoui *et al.,* (2016) working on the effect of *Yucca Schidigera* plant extract on oockystal excretion in broiler chicken droppings, concluded that supplementing this extract in the diet significantly reduces coccidia carriage. Deffas & Achelache (2021), in their work mention medicinal plant extracts with anticoccidial activity and reveal that their bioactive substances reduce the excretion of *Eimeria* spoocysts, reduce the severity of lesions, and prevent mortality.

According to the same authors, this anticoccidial effect is attributed to their richness in active ingredients, including phenolic compounds, essential oils, and alkaloids. It is important to note that such a reduction could contribute to improving the health of broiler chickens and solve the mortality problem in the poultry sector.

Transaminases are enzymes present in many tissues, located in the cytoplasm (ASAT and ALAT) and mitochondria (ASAT), whose increase in the blood indicates the release of cellular contents following cytolysis. Their determination in serum allows the detection of pathologies characteristic of liver damage (hepatitis, cirrhosis, drug toxicity, etc.), muscle and heart disease. Non-significant increases in the activities of the two transaminases have been demonstrated in poultry during bacterial infection (Koynarski *et al.,* 2010). The observed ALAT and ASAT levels mean that the extracts used do not present any toxicity to the liver and muscle of the subjects. Birds have significant metabolic activity (Koochaksaraie *et al.,* 2010). Glucose is used by birds as a source of energy, for glycogen synthesis, for fatty acid synthesis, for non-essential amino acid synthesis, and for vitamin C synthesis (Braun & Lefebvre, 2008).

The blood glucose results showed statistically identical values between the different study batches. The observed results mean that the extracts used have no effect on the rate of glucose absorption in broiler chickens. Both creatinine and urea are important indicators of protein metabolism and result from a breakdown of muscle creatine (Wyss & Kaddurah-Daouk, 2000). It is eliminated in the urine by filtration at the kidney level. When the kidneys' ability to eliminate waste decreases, the amount of creatinine and urea in the blood increases. Low levels of creatinine and urea in the blood are therefore a reflection of kidney function, and in certain conditions, of liver function. A high level of urea and creatinine can also be attributed to a condition that reduces blood flow to the kidneys (heart failure, shock, severe stress, recent heart attack, obstruction of the urinary flow, dehydration). Some medications can also cause an increase in urea level. In summary, the extracts used in our study have no effect on the biochemical parameters of broiler chickens.

Table.1 Properties of ethanolic and aqueous extracts of *Mallotus oppositifolius* from the study

Excerpts	Minimum Inhibitory/Bactericidal Concentration on *Salmonella* (mg/mL)		Phytochemicals compounds	Toxicity of extracts
	(mg/mL)	CMB (mg/mL)		
MA	12.5≤CMI≤100	50≤CMB≤100	Polyphenols, catechin tannins	None
ME	CMI=6.25	50≤CMB≤100	Anthraquinones, saponins, polyphenols, sterols, catechin tannins	None
References	(Assandi *et al.*, 2021)		(Assandi *et al.*, 2024)	(Assandi *et al.*, 2024)

MA: Aqueous extracts of *Mallotus oppositifolius*; **ME**: Ethanolic extracts of *Mallotus oppositifolius*

Table.2 Weekly evolution of the Average Daily Gain of chickens during the experiment

Lots	Week 1	Week 2	Week 3	Week 4	Week 5	Average QMC
Witness	6.1 ± 0.2a	31.4 ± 0.7 a	35.09 ± 0.9 b	49.3 ± 1.1 a	56.4 ± 2.9 a	35.6±19.4a
MY	5.1 ± 0.2 a	27.9 ± 0.6 ab	35.7 ± 0.9 b	40 ± 0.9b	58.6 ± 3.5 a	33.4±19.4 a
ME	4.8 ± 0.1 a	24.3 ± 0.6 b	40 ± 1 a	35 ±0.7c	57.9 ± 5.5 a	32.4±19.6 a

T: batch T; **MA**: MAbatch; **ME**: MEbatch**.** Values followed by the same letters in the same column indicate that there is no significant difference between these values.

Table.3 Microbiological analysis of fecals samples for the search for Salmonella

Batches of chickens	10th day	21st day	35th day
T	Presence	Presence	Presence
MY	Absence	Absence	Absence
ME	Absence	Absence	Absence

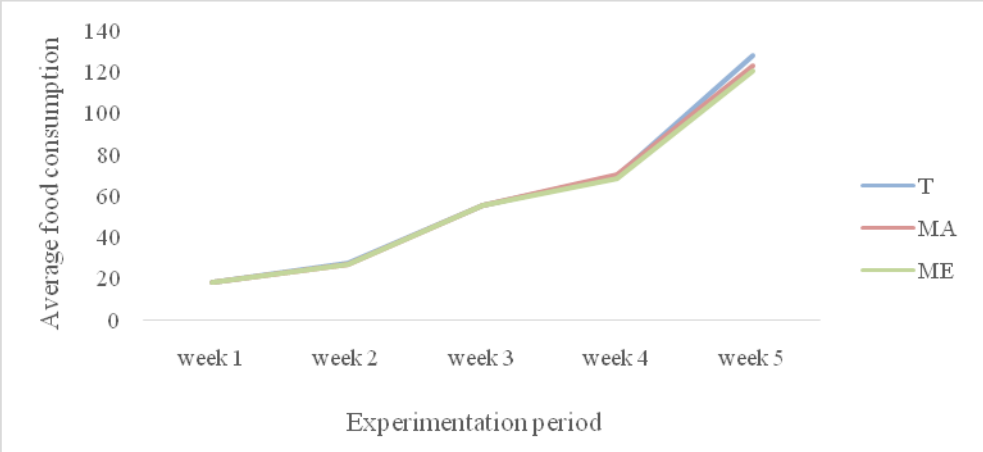
T: batch T; MA: MA batch; ME: MEbatch

Table.4 Effects of plant extracts on biochemical parameters of chickens in different experimental groups

	Creatinine (mg/L)	ASAT (UI/L)	ALAT (IU/L)	Urea (g/L)	CRP (mg/L)	Blood sugar (g/L)
T	0.035± 0.03	201.2± 8.34	7.6± 7.7	0.01± 0	0.24± 0.02	2.25± 0.04
MA	0.045± 0.02	215.5± 8.06	7.85± 0.7	0.01± 0	0.215±0.14	2.01± 0.08
ME	0.08± 0.02	257.2± 8.76	9.3± 2.82	0.01± 0	0.155± 0.14	2.15± 0
Reference values	≤ 10	≤ 275	≤ 50	0.01	≤ 6	2-5

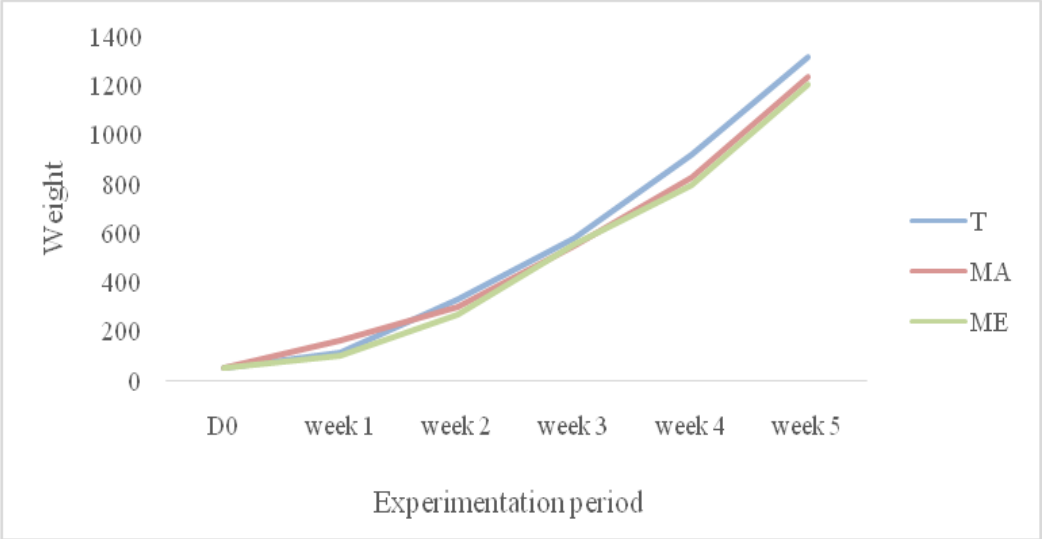
ASAT: Aspartate Aminotransferase; ALAT: Alanine Aminotransferase; CRP: C-reactive protein
T: batch T; MA: MA batch; ME: MEbatch

Figure.1 Weekly evolution of average food consumption per batch



T: batch T; **MA**: batch MA; **ME**: batch ME

Figure.2 Weekly weight evolution of chickens (in grams)



T: batch T; **MA**: batch MA; **ME**: batch ME

The results of this study show that aqueous and ethanolic extracts of *Mallotus oppositifolius* added in small quantities (200mg/L) to drinking water prevent *Salmonella* contamination and *Eimeria tenella* infestation without having an influence on the zootechnical and biochemical parameters of broiler chickens.

Ultimately, in the face of the spread of multi-resistant microorganisms of avian origin, the use of aqueous and ethanolic extracts of *Mallotus oppositifolius* a as a substitute for synthetic antimicrobials, represents an effective alternative for the treatment of pathologies due to *Salmonella* and parasites including *Eimeria tenella* in broiler chicken farming.

Author Contributions

Bonny Aya Carole: Investigation, formal analysis, writing—original draft. Assandi Kouamé Rivière: Validation, methodology, writing—reviewing. Ake Moussan Désirée Francine:—Formal analysis, writing—review and editing. Koffi Kouamé Sébastien: Investigation, writing—reviewing.

Data Availability

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethical Approval Not applicable.

Consent to Participate Not applicable.

Consent to Publish Not applicable.

Conflict of Interest The authors declare no competing interests.

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How to cite this article:

Bonny Aya Carole, Assandi Kouamé Rivière, Ake Moussan Désirée Francine and Koffi Kouamé Sébastien. 2025. Impact of Plant Extracts on Zootechnical, Microbiological and Biochemical Parameters of Broiler Chicken of the Cobbs 500 Breed (*Gallus gallusdomesticus*). *Int.J.Curr.Microbiol.App.Sci.* 14(08): 1-9.
doi: https://doi.org/10.20546/ijcmas.2025.1408.001