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Eucalyptus globulus as an Alternative to Antibiotics for *Isa brown* Laying Hens during the Starter Phase

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ABSTRACT

Identification of antibiotic residues in meat and eggs of laying hens in Togo and the ban in 2006 on using antibiotics growth promoter (AGP) in animal production by the World Health Organization induce the use of medicinal plants with antimicrobial effects, such as AGP alternatives in poultry production. For the same purpose, this study was conducted to contribute to studies using phytobiotics as alternatives to AGP in poultry production. Indeed, antibiotics have been substituted by *Eucalyptus globulus* leaf powder (ELP) during the starter phase. Polyphenolic compounds from ELP were determined, and the effects of different rates of ELP supplementation on growth performance, mortality, and hematological and biochemical parameters were evaluated. A total of 460 one-day-old laying chicks (*Isa brown*) were randomly allocated to 5 groups, each consisting of 4 replications, with 23 chicks in each replication. Treatments consisted of the basal diet (BD) without ELP and antibiotics, a negative control (group EGO-), BD with antibiotics and no ELP, a positive control (group EGO+), BD + 0.25% of ELP without antibiotic (group EG1), BD with 0.50% of ELP without antibiotic (group EG2), and BD with 1% ELP without antibiotic (group EG3). The rates of 0.25%, 0.5%, and 1% mean 0.25 kg, 0.5 kg, and 1 kg of ELP for 100 kg of BD, respectively. The study revealed that ELP contains flavonoids (4.85 µg QE/mg), tannins (30.34 µg CE/mg), and total phenols (165.2 µg AGE/mg). Supplementation did not affect feed intake (FI), body weight gain (BWG), feed conversion ratio, and mortality of *Isa brown* laying hens during the starter phase (8 weeks) in all treatment groups. However, the chicks that received ELP had the best FI and BWG, which was not significantly different from the control groups. The biochemical parameters such as total proteins, albumin, triglyceride, total cholesterol, and glycemia were not affected by ELP supplementation. Among the hematology parameters, the leukocyte decreased in the groups fed with ELP, while mortality was unaffected. The results of the present study indicated that ELP inclusion rate of 0.25% could serve as the best antibiotic replacement for *Isa brown* laying hens during the starter phase.

Keywords: *Eucalyptus globulus*, Growth parameters, *Isa brown*, Starter

INTRODUCTION

In Togo, the poultry sector mainly focuses on egg consumption production and produced 30,668,872 eggs in 2005 (FAO, 2008). This level does not meet producers' expectations and national demand due to many avian diseases, which negatively impact productivity (Atakpama et al., 2016). Generally, the use of antibiotics is frequent and leads to the formation of residues in livestock products such as eggs and meat (Niyibizi, 2012). The World Health Organization banned the use of antibiotic growth promoters (AGP**)** in livestock production in 2006, although AGP improves animal performance and reduces the spread of disease (Mashayekhi et al., 2018). In Togo, one of the reasons is that AGPs are responsible for the presence of antibiotic residues in chicken meat and eggs from laying hens, which constitutes a food security problem (Gambogou et al., 2020). Furthermore, the use of antibiotics as growth promoters induces the dissemination of antibiotic-resistance genes (Bedkelabou et al., 2020). To limit the use of antibiotics in hen production and improve public health, nutritionists and animal health actors need to find alternatives that have the potential to mitigate the negative effects related to AGP (Lillehoj and Lee, 2012). In this dynamic, studies have been conducted to supplement plant products (*Moringa oleifera*, *Carica papaya*, *Eucalyptus*

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globulus) *in poultry feed as an alternative to antibiotic* growth promoters. Supplementation of *Moringa oleifera* leaves improved broilers' zootechnical performance (Teteh et al., 2013) and increased production performance in laying hens while causing a decrease in hematological parameters (Voemesse et al., 2018).

The studies about *Eucalyptus globulus* have related to the replacement of antibiotics by *Eucalyptus* leaves powder in broiler chicken production and the laying phase of hens (Mashayekhi et al., 2018; Abd El-Hack et al., 2023). *Eucalyptus globulus* is a medicinal plant belonging to the *Myrtaceae* family. It was discovered in Australia but is found worldwide, especially in tropical and subtropical regions (Salari et al., 2006). *Eucalyptus globulus* contains compounds such as cineol (60%), flavonoids (4.4%), and tannins (19.6%), which have antioxidant and antimicrobial properties that have led to improved appetite, health, and growth performance of broiler chickens (Mashayekhi et al., 2018). The metabolic and aqueous extracts from *Eucalyptus globulus* leaves showed antimicrobial activity in *Staphylococcus*, *Pseudomonas*, *Bacillus*, and *Escherichia coli* (Boukhalfoun, 2012). *Eucalyptus globulus* is effective against microorganisms that cause food intoxication (Takahashi et al., 2004). It can potentially improve the immune response of broiler chickens (Farhadi et al., 2017). The supplementation of 0.30% *Eucalyptus globulus* leaf powder in the laying hens' diet increased egg production and shell thickness (Kaur et al., 2022). However, there is little scientific data on *Eucalyptus globulus* usage in the starter phase of *Isa brown* laying hens although its use is widespread in Africa (Gaston and Parfait, 2017). This study aims to evaluate the effects of *Eucalyptus globulus* leaves on growth performance, and hematological, and biochemical parameters of *Isa brown* laying hens during the starter phase.

MATERIALS AND METHODS

Ethical approval

The animal care guidelines recommended by the Animal Ethics Committee of the University of Lome in Togo were followed (008/2021/BC-BPA/FDS-UL).

Study design

To begin, 6 kg of *Eucalyptus globulus* leaves were collected in the canton of Badja in Avé prefecture, Togo, and were dried and sheltered from light for 72 hours with natural ventilation (at a room temperature of 20-25°C and relative air humidity of 42-54%). The leaf samples were analyzed for their composition in flavonoids, tannins, and phenols. The dried leaves were ground into powder and added at different rates to the basal diet of *Isa brown* laying hens at the starter phase. The supplementation rates were 0.25%, 0.50%, and 1% per 100 kg of feed. The *Eucalyptus globulus* leaf powder was added to the feed, and the antibiotic was given through drinking water according to the described dosage (Bouassi et al., 2020). A total of 460-day-old chicks at 30.79 g average weight from CERSA-UL hatchery unity of Lomé, Togo, were used for the study. During the experiment, the chicks were raised 7 days and were randomly allocated to 5 treatments with 4 repetitions of 23 chicks, each housed in floor pens of identical size (3 × 2.5 m). Treatments consisted of a basal diet (BD) without ELP and antibiotics, a negative control (group EGO-), BD with antibiotics and no ELP, a positive control (group EGO+), BD + 0.25% of ELP, without antibiotic (group EG1), BD with 0.50% of ELP without antibiotic (group EG2) and BD with 1% ELP without antibiotic (group EG3). The rates of 0.25%, 0.5%, and 1% mean respectively 0.25 kg, 0.5 kg, and 1 kg of ELP for 100 kg of BD.

Table 1. Composition and characteristics of diets of *isa brown* chicks in the starter phase (8 weeks) per treatment

Ingredients (kg)	**EGO-**	**EGO+**	**EG1**	**EG2**	**EG3**
Maize	56	56	56	56	56
Soya roasted	12	12	12	12	12
Wheat bran	17	17	17	17	17
Beer grains	7.5	7.5	7.5	7.5	7.5
Fish meal	5	5	5	5	5
Lysine	0.3	0.3	0.3	0.3	0.3
Methionine	0.2	0.2	0.2	0.2	0.2
Oyster shell	2	2	2	2	2
ELP	0	0	0.25	0.50	1
Characteristics					
ME (Kcal/kg)	2841.04	2841.04	2841.49	2841.95	2842.87
CP (%)	17.05	17.05	17.06	17.07	17.09
Lysine (%)	0.95	0.95	0.95	0.95	0.95
Methionine	0.59	0.59	0.59	0.59	0.59
Methionine + cysteine (%)	0.81	0.81	0.81	0.81	0.81
Calcium (%)	0.97	0.97	0.97	0.97	0.97
Phosphorus (%)	0.65	0.65	0.65	0.65	0.65

ELP: *Eucalyptus globulus* leaf powder, ME: Metabolizable energy, CP: Crude proteins, EG0-: Groups fed basal diet (BD) without ELP and no antibiotics, EGO+: BD without Eucalyptus leaf powder (ELP) but with antibiotic, EG1: BD + 0.25% ELP and without antibiotic, EG2: BD+ 0.50% ELP and without antibiotic, EG3: BD+1% ELP without antibiotic.

The feed compositions and the nutritional values are presented in Table 1. The animals were raised on the floor, and feed and water were provided *ad libitum*. The lighting program consisted of 24 hours of light for the first day,

followed by 23 hours of light until day 7. The 21.30 hours of light was used in the second week and decreased by 1.30 hours weekly from the third week to 12 hours of light in week 8 (Voemesse et al., 2018). Vaccines against Marek, Newcastle, Gumboro, infectious bronchitis, and smallpox from the LAPROVET laboratory of France were administered to each group following the prophylaxis program (Table 2). The feed offered was weighed every day, and the feed not consumed was weighed at the end of the week. At 8 weeks, four animals were stunned and slaughtered per treatment for blood sampling and weighing of organs (heart, liver, gizzard, proventriculus, kidney, and pancreas). The amount of 1.5 ml blood samples was collected and analyzed to determine the hematological and biochemical parameters.

Table 2. Prophylaxis protocol of *Isa brown* laying hens during the starter phase

Treatments	Period
Marek, gumboro, Newcastle, and infectious bronchitis vaccines	Day 1
Newcastle, infectious bronchitis, and gumboro	Day 7
Newcastle, infectious bronchitis, and gumboro	Day 21
Newcastle, infectious bronchitis, and gumboro	Day 35
Smallpox+Newcastle	Week 7
Vitamins (Aminogrow ws, introchick oral, and Amin'total)	Weeks 1, 2, 4, 5, and 8
Anticoccidial (Amprolium 20 %)	Weeks 4 and 8
Deworming (Levalap)	Week 6
Antibiotics (Aliseryl ws) only for the positive control group (EGO+)	Weeks 3 and 8

Vitamin, anticoccidial, and deworming are produced by the LAPROVET laboratory of France, and aliseryl ws, aminogrow ws and introchick oral produced by Interchemie werken 'De Adelaar' BV Holland. The products were given according to the manufacturer's dosage.

Determination of total flavonoids

The flavonoid was evaluated with the colorimetric method based on the aluminum trichloride (AlCl3), using the protocol described by Mahmoudi et al. (2013). A volume of 200 µl of each extract of concentration 500 µg/mL, was added to 1,600 µL of distilled water, 120 µL of sodium nitrite (NaNO2 = 5%), then 80 µL of aluminum trichloride hexahydrated (AlCl3; 6H2O = 10%). After vortex stirring with each addition, the mixture was incubated at laboratory temperature in the dark for 5 min. The absorbance of the final solution obtained was read with the UV-5200PC spectrophotometer (METASH, China) against a blank at the wavelength of 510 nm. The tests were carried out in triplicate for each sample. A calibration range from 0 to 700 µg/mL was performed under the same conditions using quercetin as the reference standard. The total flavonoid contents of the samples were in mgEqQt/gES.

Determination of total condensed tannins

The rate of total tannins condensed in dry extracts was measured using vanillin and concentrated hydrochloric acid (HCl) by adopting the experimental protocol of Bahorun et al. (1996). A mixture was made using 400 µL of dry extract, 2000 µL of vanillin solution, 4% of methanol, and 750 µL of hydrochloric acid (37%). The mixture was thoroughly stirred and incubated at 18°C for 30 minutes. The absorbance of the final solution obtained was measured at the wavelength of 500 nm. The tests were performed in triplicate for each sample. A standard catechin solution was used for the calibration curve to measure tannin total condensed values, expressed in milligrams (mg) equivalent (Eq) of catechin (CA) per gram (g) of dry extract (ES), either mgEqCA/gES.

Determination of total polyphenols

The total polyphenols were determined through the method described by Suman et al. (2017). According to the experimental approach, a solution of an initial concentration equal to 1 mg/mL must be prepared for each sample to be determined, followed by a freshly prepared sodium carbonate solution (6%: w/v). On 500 µL of each previously prepared solution, a volume of 500 µL of Folin's reagent Ciocalteu (10%: v / v) was added. The mixture was stirred by a vortex and then stood for 5 minutes. A volume of 500 µL of the sodium carbonate solution (6%: w / v) was added to the mixture. The final mixture was left to rest for 30 minutes at room temperature and protected from light. The reading was performed at a wavelength of 725 nm against a blank, using the UV-5200 PC spectrophotometer (METASH, China). A calibration curve was plotted under the same operating conditions using gallic acid as standard in a concentration range from 0 to 300 µg/mL. The polyphenol total contents of the samples analyzed were expressed in mg EqAG/gES.

Physicochemical analysis

The moisture content of the leaves was determined by the NF EN ISO 665 (2000) method (Aziato et al., 2021). This determination is based on drying leaves at 103°C ± 2°C for 4 hours in the dry oven until practically constant mass was obtained. For this, 5 g of the *Eucalyptus globulus* (*EG*) leaves were weighed in a beaker and placed in the dry oven at 103°C ± 2°C for 4 hours.

The moisture content was determined by following Formula 1.

H = (m1 – m2)/ (m1 – m0) x 100 (Formula 1)

Where, m0 means mass of the empty beaker, m1 signifies the mass of the beaker with the test portion before drying, m2 denotes mass of the beaker with the test portion after drying.

The ash content was determined according to AOAC 923.03 (AOAC, 1999). It consisted of the incineration of *EG* leaves in the muffle oven at the temperature of 550°C until whitish ash was obtained. For this, 5 g of EG was placed in a dry porcelain crucible previously weighed. The whole (crucible and test portion) was then subjected to incineration in the furnace at 550°C for 4 hours. At the end of the incineration, the crucible containing the ash was cooled with a moisture analyzer for 30 minutes and then weighed. The ash content was determined according to Formula 2.

Ash content (%) = m1/m0 x 100, (Formula 2)

Where, m0 is the mass of the test portion, and m1 determines mass of the incineration residue.

The protein total content was determined by the Kjeldahl method according to NF V18-100 (1977) (Ayssiwede et al., 2011). The principle consists of mineralization of the material by concentrated sulfuric acid in the presence of a protein catalyst followed by alkalization of the reaction products. The ammonia released is distilled and collected in a boric acid solution and titrated with a solution of normal sulfuric acid (1 N). One gram of crushed EG leaves was weighed in a mineralization flask to which 25 ml of concentrated 96% sulfuric acid and 5 g of protein catalyst (1000 g of K2SO4 + 30 g of TiO2 + 30 g of Cu2SO4) were added. The final mixture was placed on a mineralization ramp and mineralized until the complete discoloration of the solution, which was initially black. After mineralization, the solution was cooled and then introduced into the nitrogen distiller (Lab Logistics Group of Germany). Then, it underwent neutralization with 40% soda. The released nitrogen was collected in an Erlenmeyer containing a boric acid solution at saturation, methylene blue, and methyl red. The distillate obtained was defined with 1 N sulphuric acid. The total protein content was determined by the Formula 3.

Tp = (V-V0) /P x T x ß x 0.014 x 100 (Formula 3)

Where, Tp is total protein content (%), V means the volume of sulphuric acid used for the test portion (ml), V0 signifies the volume of sulfuric acid used for the white (ml), T denotes the concentration of the sulphuric acid solution used for titration (1 N), ß refers to the nitrogen-to-total protein conversion factor (6.25), P is test portion (g), 0.014 determines conversion coefficient of the titer of the sulphuric acid solution used (normality) into mass titer.

Lipids were extracted from leaves using the Soxhlet method (gravimetric method) according to AOAC 960.39 (AOAC, 1999). A test portion of 3 g of *EG* sheets was fed into an extraction cartridge. The cartridge was corked with cotton wool and then placed in an extraction cup containing 100 ml of n-Hexane or light petroleum at 40-60°C. The entire extraction system was placed in the Soxhlet, and extraction was performed for 1 hour and 30 minutes. After extraction, the flask containing the lipids was placed in the oven for 1 H at 105°C and then in a moisture analyzer for cooling. A series of weighing, steaming, and drying of the flask was carried out alternately until a constant mass was obtained. The mass of lipids was obtained by the difference between the mass of the balloon containing the fat and the empty mass of the balloon. The lipid content (Tl) was determined by Formula 4.

Tl = (MF – M0) / ME x 100. (Formula 4)

Where, MF represents mass of fat matter + flask, M0 corresponds to mass of the vacuum balloon, ME indicates mass of the test portion.

The carbohydrate content of EG leaf was determined by differential calculation according to the method of Al-Hooti et al. (1998). It was obtained according to the following Formula 5.

Carbohydrates (%) = 100 -% (water + protein + fat + ash) (Formula 5)

The energy value was determined by the following Formula 6.

E = (protein %) × 4 + (carbohydrates %) × 4 + (Fat %) × 9 (Formula 6)

Growth performance

Feed intake (FI), body weight gain (BWG), and feed conversion ratio (FCR) were calculated per treatment. The offered and residual feed was weighed weekly. Each chick was weighed per treatment at the beginning and end of the week.

Data for FI and BWG were used to calculate the FCR. These parameters were determined by formulas 7, 8, and 9 (Guembo et al., 2021).

FI = (quantity of food distributed - quantity refused) / number of chicks (Formula 7)

BWG = (final weight - initial weight) / duration (Formula 8)

FCR = amount of feed consumed by the animal / (final weight - initial weight) (Formula 9)

Determination of hematological and biochemical parameters

At 8 weeks of age, 20 chicks were randomly selected at the rate of 4 chicks per treatment. Blood samples were taken between 09:00' and 10:00' in the Ethylenediaminetetraacetic acid (EDTA) tubes to determine hematological parameters and in dry tubes to determine biochemical parameters.

An average of 1.5 ml of blood was taken from each chicken and used within 3 hours of collection to determine hematological parameters using ABX Micros 60, a fully automated hematology analyzer from Sysmex Corporation Corporation Company (Japan). Biochemical parameters were determined using an enzymatic colorimetric method on 200 and 10 µl of serum according to the protocols provided by the reagent (Voemesse et al., 2018).

Statistical analysis

The collected data was saved in Excel software version 2019 and was processed in Software R version 3.6.0 (2019-04-26). The SHAPIRO TEST was used to check whether the data followed a normal distribution. The differences between mean values were evaluated using the ANOVA test followed by the Tukey test, and the significant level was set at $p < 0.05$. The results were presented as means ± standard error mean.

RESULTS

Phytochemicals compounds

The analysis showed the presence of flavonoids, tannins, and total phenols. The composition varied from one compound to another according to the reference standard or standard solution. Total flavonoids (4.85 µg/mg) were less represented than tannins (30.34 µg/mg) and total phenols (165.2 µg/mg, Table 3).

Table 3. Composition of *Eucalyptus globulus* leaves in polyphenols

Parameters	Concentration
Total flavonoids	4.85 µg QE/mg
Tannins condensed Totals	30.34 µg CE/mg
Total phenols	165.2 µg GAE/mg

QE: Quercetin equivalent, CE: Catechin equivalent, GAE: Gallic acid equivalent, µg: Microgram, mg: Milligram

Physicochemical compounds

The physicochemical composition of *EG* leaves is diverse. Table 4 shows water, total nitrogen, protein, fat, carbohydrate, and ash *EG* leaves content. The *Eucalyptus globulus* leaf contained carbohydrates (38.85%), lipids (1.12%), proteins (4.37%), nitrogen (0.70%), and ash (3.04%).

Table 4. Physicochemical composition of *Eucalyptus globulus* leaves

Physico-chemical characteristics	Result
Moisture content (% m/m)	52.60
Total Nitrogen content (% m/m)	0.70
Protein content (% m/m)	4.37
Fat content (% m/m)	1.12
Ash content (% m/m)	3.04
Carbohydrate content (% m/m)	38.85
Energy value (kcal)	183.04

Kcal: Kilocalorie

Growth performance

Feed intake

The chick's feed intake by treatment is presented in Table 5. The average consumption in grams (g) of chicks during the starter phase was 35.24 ± 3.16, 35.47 ± 3.22, 35.36 ± 3.14, 34.28 ± 2.88, and 35.65 ± 3.11 for *EG*O-, *EG*O+, *EG*1, *EG*2, and *EG*3 groups, respectively. Overall, the chick's consumption increased weekly during the starter phase. The weekly average feed intake of the chicks showed no significant difference across groups ($p > 0.05$). The group that received 1% of *Eucalyptus globulus* leaf powder had the highest level of feed consumption (35.65 g).

During the first 2 weeks, all chicks had a similar feed intake. The chicks of the EGO- and EG3 groups had the same feed intake in the last weeks. This similarity was related to the increase in feed intake from one week to another during the starter phase, but for groups that took the highest level of ELP (1%), the average individual feed intake decreased from 53.56 g to 49.14 g in the weeks 7 and 8, respectively. The feed intake evolution of *EG*O+, *EG*1, and *EG*2 groups was similar at the starter phase.

Weekly evaluation of chick's weight

The chick's weight at the starter phase is shown in Graph 1. The average weight per chick at the starter phase was 177.56 ± 24.21 g, 176.96 ± 23.46 g, 182.68 ± 24.09 g, 178.26 ± 23.87 g, and 167.88 ± 22.21 g for groups *EG*O-, *EG*O+, *EG*1, *EG*2 and *EG*3, respectively. The chicks that received 0.25% ELP had the average highest weight in all groups, followed by groups that received 0.50% ELP.

Chicks fed with 0.25% ELP had a higher weight (388.45 g), and the groups that received 1% of ELP had the lowest weight (337.15 g) at 8 weeks of age, but the difference between weights was not significant ($p > 0.05$).

Body weight gain

The BWG of the chicks during the starter phase is shown in Graph 2. The control groups *EG*0- and *EG*0+ indicated the BWG of 7.51 ± 1.21 and 7.34 ± 0.96 g/day/chick. For chicks who received *Eucalyptus globulus* leaf powder treatment *EG*1, *EG*2, and *EG*3, the BWG values were 8.21 ± 1.65, 7.80 ± 1.25, and 7.73 ± 1.57 g/day/chick, respectively. The differences between the values for the different groups were not significant at the starter phase ($p > 0.05$).

Feed conversion ratio and mortality rate

The FCR and mortality rate for each treatment is presented in Table 6. No significant differences were found between the FCR of the different groups ($p > 0.05$). The *EG*1 group had the lowest FCR (4.31 ± 0.35), followed by *EG*2 (4.40 ± 0.49) and *EG*3 (4.61 ± 0.56). The Control groups, EGO- and EGO+, had the highest FCR (4.69 ± 0.52 for *EG*0- and 4.83 ± 1.03 for *EG*0+). The Mortality rate values varied between the groups. The negative control group had the same mortality as the group that received 1% of ELP (3% of chick mortality). The chicks that received antibiotics and the groups that received 0.25% and 0.5% of ELP showed 2% mortality. The values were not significantly different among the groups ($p > 0.05$).

Table 5. Effect of *Eucalyptus globulus* leaves on feed intake of *Isa brown* laying hens during the starter phase

Age \ Treatments	EGO-		EGO+		EG1		EG2		EG3	
	$\bar{X}$	SEM	$\bar{X}$	SEM	$\bar{X}$	SEM	$\bar{X}$	SEM	$\bar{X}$	SEM
W1	9.91	0.04	9.93	0.04	9.93	0.04	9.93	0.04	9.93	0.04
W2	20.17	0.07	20.15	0.02	20.15	0.02	20.16	0.02	20.17	0.03
W3	27.68	1.73	31.62	1.95	31.76	1.49	33.58	1.39	31.30	4.55
W4	33.09	1.07	27.40	1.87	29.36	1.44	29.93	0.81	36.91	4.87
W5	44.52	2.29	50.85	1.72	43.28	1.67	39.11	1.23	37.05	5.42
W6	44.54	1.87	49.82	4.35	50.98	1.80	45.26	3.08	47.15	9.82
W7	48.31	5.50	46.95	3.29	48.50	1.66	48.01	1.82	53.56	6.28
W8	53.73	8.83	47.01	6.00	48.92	4.14	48.22	1.63	49.14	4.26
$\bar{X}$ (g)	35.24	3.16	35.47	3.22	35.36	3.14	34.28	2.88	35.65	3.11

EG0-: Groups received basal diet (BD) without ELP and no antibiotics, EGO+: BD without Eucalyptus leaf powder (ELP) but with antibiotic, EG1: BD + 0.25% ELP and without antibiotic, EG2: BD + 0.50% ELP and without antibiotic, EG3: BD + 1% ELP without antibiotic, W: Week, SEM: Standard error of Means, $\bar{X}$: Average in g

Table 6. Effect of *Eucalyptus globulus* leaves on feed conversion ratio and mortality of *Isa brown* laying hens during the starter phase

Parameters	EGO-	EGO+	EG1	EG2	EG3
FI(g)	35.24 ± 3.16	35.47 ± 3.22	35.36 ± 3.14	34.28 ± 2.88	35.65 ± 3.11
BWG (g)	7.5 1 ± 1.21	7.34 ± 0.96	8.21 ± 1.65	7.80 ± 1.25	7.73 ± 1.57
FCR	4.69 ± 0.52	4.83 ± 1.03	4.31 ± 0.35	4.40 ± 0.49	4.61 ± 0.56
Mortality (%)	3	2	2	2	3

FI: Feed intake, BWG: Body weight gain, FCR: Feed conversion ratio, g: Gram, EG0-: Groups were received basal diet (BD) without ELP and no antibiotics, EGO+: BD without Eucalyptus leaf powder (ELP) but with antibiotic, EG1: BD+0.25% ELP and without antibiotic, EG2: BD+ 0.50% ELP and without antibiotic, EG3: BD+1% ELP without antibiotic.

Weights vital organs

The weights of the organs, including the pancreas, kidney, and proventriculus, were identical ($p > 0.05$) among all treatment and control groups (Table 7).

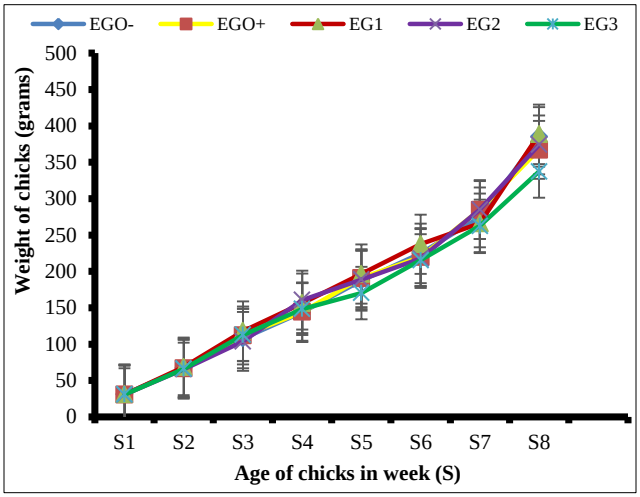
Chicks that received 0.50% ELP had the lowest liver weight, and chicks that received 1% ELP had the highest gizzard weight among the investigated groups ($p < 0.05$).

Hematological parameters

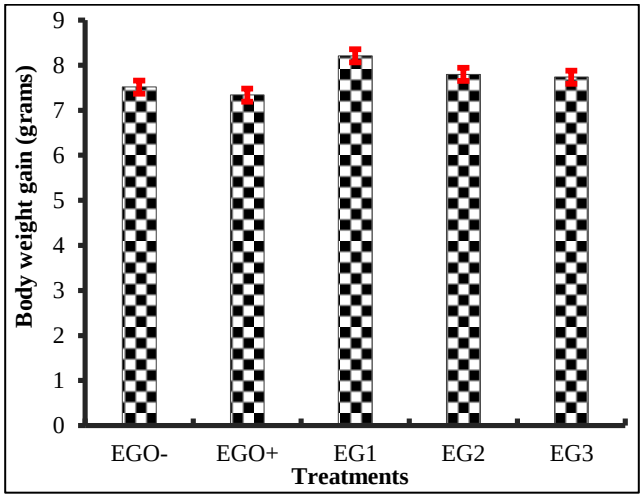
The hematological parameter is presented in Table 8. The study showed that the groups treated with *Eucalyptus globulus* leaf powder had the significantly lowest white blood cell count, compared to the control groups ($p < 0.05$). The value of red blood cell, hematocrit, mean corpuscular volume, mean corpuscular hemoglobin content, mean corpuscular hemoglobin concentration, and platelet was similar for the groups treated, compared to the control ($p > 0.05$).

Effect of *Eucalyptus globulus* leaves powder on biochemical parameters

The biochemical parameters (total protein, albumin, triglycerides, total cholesterol, glycemia, alanine aminotransferase, aspartate aminotransferase) are presented in Table 9. The findings indicated that there was no significant difference in the biochemical parameters between the control and treatment groups ($p > 0.05$).



Graph 1. Effect of *Eucalyptus globulus* on the growth of *Isa brown* laying hens during the starter phase.
EG0-: Groups received basal diet (BD) without *Eucalyptus globulus* leaf powder (ELP) and no antibiotics, EGO+: BD without ELP but with antibiotic, EG1: BD + 0.25% ELP and without antibiotic, EG2: BD + 0.50% ELP and without antibiotic, EG3: BD + 1% ELP without antibiotic.



Graph 2. Effect of *Eucalyptus globulus* leaves on BWG of *Isa brown* laying hens during the starter phase.
EG0-: Groups received basal diet (BD) without *Eucalyptus globulus* leaf powder (ELP) and no antibiotics, EGO+: BD without ELP but with antibiotic, EG1: BD + 0.25% ELP and without antibiotic, EG2: BD + 0.50% ELP and without antibiotic, EG3: BD + 1% ELP without antibiotic.

Table 7. Effect of *Eucalyptus globulus* leaves on the vital organs of *Isa brown* leaves laying hens during the starter phase

Vital organs / Variable	Liver	Pancreas	Heart	Kidney	Proventriculus	Gizzard
EGO-	11 ± 0.11^{a}	1 ± 0.06	2 ± 0.18	1 ± 0.05	2 ± 0.12	21.75 ± 0.95^{b}
EGO+	10.25 ± 0.63^{a}	1.5 ± 0.29	2.25 ± 0.25	1.5 ± 0.29	2.75 ± 0.48	23.75 ± 1.55^{b}
EG1	10.25 ± 0.48^{a}	1.25 ± 0.25	1.75 ± 0.25	1.5 ± 0.29	2 ± 0.41	23 ± 2.04^{b}
EG2	8.75 ± 0.63^{b}	1.5 ± 0.29	2.25 ± 0.25	1.25 ± 0.25	2.75 ± 0.48	23.25 ± 1.65^{b}
EG3	10 ± 1.29^{a}	1.5 ± 0.28	1.95 ± 0.28	1.5 ± 0.28	2.75 ± 0.47	26.15 ± 1.52^{a}

EG0-: Groups received basal diet (BD) without ELP and no antibiotics; EGO+: BD without Eucalyptus leaf powder (ELP) but with antibiotic, EG1: BD+0.25% ELP and without antibiotic, EG2: BD+ 0.50% ELP and without antibiotic, EG3: BD+1% ELP without antibiotic. $^{a, b}$On the same column, the values assigned different letters are significantly different ($p < 0.05$).

Table 8. Effect of *Eucalyptus globulus* leaves powder on hematological parameters of *Isa brown* laying hens during the starter phase

Parameters / Variable	WBC (10^3/µL)	RBC (10^6/µL)	HB. (g/dl)	HCT (%)	CVD (FL.)	MCH (pg)	MCHC (g/dl)	PLT (10^3/µl)
EGO-	16.44[a]	2.53	6.20	29.10	117.55	26.53	21.33	2.00
EGO+	15.76[a]	3.09	7.30	29.73	118.90	25.73	20.00	3.33
EG1	7.76[b]	2.43	6.40	28.67	118.13	26.37	22.33	2.33
EG2	6.18[b]	2.38	6.30	28.47	118.93	26.63	22.23	2.33
EG3	5.82[b]	2.14	6.83	27.97	118.00	27.30	22.73	2.00

WBC: White blood cell, RBC: Red blood cell, HB: Hemoglobin, HCT: Hematocrit, CVD: Mean corpuscular volume, MCH: Mean corpuscular hemoglobin content, MCHC: Mean corpuscular hemoglobin concentration, PLT: Platelet, EG0-: Groups received basal diet (BD) without *Eucalyptus globulus* leaf powder (ELP) and no antibiotics, EGO+: BD without ELP but with antibiotic, EG1: BD + 0.25% ELP and without antibiotic, EG2: BD + 0.50% ELP and without antibiotic, EG3: BD + 1% ELP without antibiotic. [a,b]: On the same column, the values assigned different letters are significantly different ($p < 0.05$). µL: Microliter, dl: Deciliter, FL: Femtoliter

Table 9. Effect of *Eucalyptus globulus* leaves on the biochemical parameters *of Isa brown* laying hens during the starter phase

Parameters / Variable	Total protein (g/l)	Albumin (g/l)	Triglycerides (g/l)	Total cholesterol (g/l)	Glycemia (g/l)	ALT (IU/L)	AST (IU/L)
EGO-	27.40	10.93	0.28	0.83	2.52	9.89	190.33
EGO+	29.77	11.47	1.00	0.91	2.79	9.17	189.70
EG1	28.67	10.50	0.30	0.69	3.03	8.86	191.00
EG2	29.13	10.43	0.44	0.77	2.60	9.08	190.40
EG3	28.50	11.37	0.77	0.85	2.94	9.60	191.01

EG0-: Groups received basal diet (BD) without *Eucalyptus globulus* leaf powder (ELP) and no antibiotics, EGO+: BD without ELP but with antibiotic, EG1: BD + 0.25% ELP and without antibiotic, EG2: BD + 0.50% ELP and without antibiotic, EG3: BD + 1% ELP without antibiotic, ALAT: Alanine aminotransferase, ASAT: Aspartate aminotransferase, g: Gram, l: Liter.

DISCUSSION

Eucalyptus globulus leaves contain flavonoids, tannins, and polyphenols (Amira and Amira, 2022; Drouiche and Haj Moussa, 2022). The present study confirmed the presence of flavonoids (4.85 µg QE/mg), tannins (30.34 µg CE/mg), and total phenols (165.2 µg GAE/mg) in *Eucalyptus globulus* leaves. The flavonoids were lower than the 7.57 mg QE/g flavonoids found by Amira and Amira (2022) and 20.28 mg QE/g flavonoids found by Drouiche and Haj Moussa (2022). The count of total phenols is higher than 71.98 mg GAE/g of phenols, as reported by Drouiche and Haj Moussa (2022). These differences would be due to the vegetative cycle of the plant, climate, season, and treatment methods (Dei et al., 2007; Boukhalfoun, 2012).

The feed intake of the layers chick in the starter phase was between 34 and 35 g per chick in the present study. Tayo et al. (2022) showed a value of 40.67 g/chick/day. This value is higher than the result in this study for the negative control, chicks that received only antibiotics, and chicks treated with ELP.

The BWG of chicks who received different rates of ELP is higher than those of the control groups. A similar study by Mohebodini et al. (2021) indicated a linear increase in BWG of broiler chickens that received 0.1 % of *Eucalyptus* essential oil. This finding confirms the results of Arise et al. (2009) that *Eucalyptus* leaves have multiple biochemical and physiological functions in the chick's body due to their antioxidant phytochemical active components. The highest BWG and lowest FCR of chicks that received ELP indicate that *Isa brown* laying hens in the starter phase have an enhanced performance with ELP. This corroborates with the findings of Mashayekhi et al. (2018), who indicated an improvement in the FCR of broilers supplemented with ELP by 0.50%. The improvement in growth performance in healthy chicks can be explained by the fact that the *Eucalyptus globulus* has a high ability to secrete digestive and pancreatic enzymes (Hashemipour et al., 2013). In addition, flavonoids have antimicrobial activity (Boukhalfoun, 2012) due to their

detrimental effect on the growth of harmful bacteria in the digestive tract (Gabriel et al., 2013), antioxidant activity, and the ability to scavenge free radicals (Ghedira, 2005). The role of appetite stimulators and the antimicrobial effect of ELP also explain the improvement of growth performance in the chicks treated during the study because bacteria compete with the chickens on the utilization of the feed (Windisch et al., 2008). The mean BWG obtained (7.72 g/day/chick) in this study is lower than the value of 9.34 g/day/chick found by Tossou et al. (2019). Indeed, the differences can be explained by some factors, including the age of the animals and the ingredients of diet (Halbouche et al., 2018; Khaber and Guermah, 2018). The average FCR of the different groups (4.31 to 4.83) at 8 weeks is higher than the result found by Ayodele et al. (2021); for which the FCR of *Isa brown* laying hens during the starter phase was 3.21 to 56 days of age.

The improvement in growth performance of the chick that received the *EG* leaves was not significantly different from the control groups. In the same line, Sedaghat and Karimi Torshizi (2017) found that supplementation of *EG* leaves in the quail diet did not significantly affect FI, BWG, and FCR. The studies of Kaur et al. (2022) revealed no significant differences between FI and FCR of laying hens who received *Eucalyptus* powder at different rates of 0.30%, 0.45%, and 0.60%. The same study showed that the lowest FCR was obtained in chicks that received 0.30% *Eucalyptus globulus* powder. Contrary to the result of these reports, the lowest FCR in chicks that received 0.50% ELP was obtained. This difference could be related to the fact that this study was done for the starter phase.

The mortality rate between the different treatments varied from 2 to 3%, with no significant difference between treatments over the starter phase of laying hens ($p > 0.05$). This value is considered acceptable in comparison with the rate of 5-8% indicated in hot countries (Ouedraogo et al., 2015).

The internal organs have the same weights, unlike the liver, which had a low weight for the treatment of 0.50% ELP. *Eucalyptus globulus* leaves would have improved macromolecule metabolism, which is reflected in the weight of organs that are similar to positive control treatments (Picard et al., 1999). According to Klasing (1998), the weight of the gizzard is more developed in poultry with more effort in grinding feed. In fact, during the experiment, chicks that received a high rate of ELP would have additional grinding efforts. The result is in agreement with the report of Kouadio et al. (2020) whose increase in the rate of supplementation of cassava peeling meal in broiler rations increased the gizzard muscle mass.

For hematological parameters, only the white blood cell concentrations of chicks who received the ELP decreased significantly, unlike the other parameters, which did not vary according to the rate of supplementation of *Eucalyptus globulus* leaf powder. The present findings can reflect the absence of bacterial infection in chicks during the study. The decrease in leukocytes during the experimentation would be due to the improvement of the effect of *Lactobacillus* on the infection by inhibiting the growth of harmful bacteria. *Lactobacillus* uses flavoproteins for terminal oxidation and forms hydrogen peroxide, which is a harmful bacterial inhibitor compound in the poultry digestive tract (Azieze et al., 2004). Supplementation of *Eucalyptus globulus* leaves did not affect the biochemical parameters of chicks, including total protein, albumin, triglycerides, total cholesterol, blood glucose, alanine aminotransferase, and aspartate aminotransferase , which did not vary with treatment. The values obtained for biochemical parameters are included in the ranges of the residual values for chicks published by Benlatreche and Lakehal (2013).

CONCLUSION

The present study showed that *Eucalyptus globulus* leaves and the antibiotic had the same effect on the growth performance of *Isa brown* laying hens during the starter phase. The results of the study elucidated that 0.25% of *Eucalyptus globulus* leaf powder may be a suitable substitute for antibiotics because its effects on growth performance, vital organs, and hematological and blood parameters were the same or even better than that of the antibiotic. *Eucalyptus globulus* leaf powder can be suggested as a phytobiotic for use in the diet of *Isa brown* laying hens during the starter phase. It is suggested that this study on the grower and the laying phase be pursued.

DECLARATIONS

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Authors' contribution

Aduayi Akue designed the experiment, collected and analyzed the data, and drafted the manuscript. Essodina Talaki, supervised the work and critically revised the manuscript. Lamboni Lare assisted in data collection and revised the manuscript. All authors read and approved the final version of the manuscript for publication in this journal.

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Availability of data and materials

All data generated or analyzed during this study are included in this published article; supplementary information can be accessed at the reasonable request of the corresponding author.

Ethical consideration

This manuscript does not contain plagiarized sentences and has not been published or accepted for publication elsewhere or under editorial review elsewhere. The data are not fabricated or falsified; hence, the Regional Center of Excellence for Avian Sciences, University of Lomé representative is aware of this submission.

Competing interests

There is no conflict of interest with the authors in this study.

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