

Effects of in ovo *Ocimum gratissimum* leaf extract on hatchability and post-hatch performance in Cobb 500 broilers

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Abstract

Embryonic losses, poor day-old chick quality, and feed inefficiency are major constraints to poultry productivity, highlighting the need for targeted research in these areas. This study investigated the impact of *in ovo* injections of *Ocimum gratissimum* extract (OGE) on hatchability and broiler performance. A total of 600 fertile eggs were randomly assigned to six treatments: T₀⁺ (no injection), T₀⁻ (saline injection), TT (perforation only), and T₂₅, T₅₀, T₇₅ injection of 0.1 mL OGE at 25, 50, or 75 µg/mL, respectively. After hatching, the treatments were evaluated for embryo mortality, hatchability, hatch duration and chick quality. Furthermore, feed intake, body weight, and feed conversion ratio were evaluated in 125 chicks per treatment during six-week trial. Treatments did not significantly affect hatching events, including internal and external pipping, or hatch duration ($P > 0.05$). However, T₅₀ group exhibited significantly higher hatchability and lower embryonic mortality compared to the other groups. No significant difference ($p > 0.05$) was observed between the groups in chick quality. Newly hatched chicks in the T₅₀ group exhibited the highest mean body weight and the lowest yolk weights. Although feed intake was similar across all treatments during the rearing period, final body weights for the T₂₅, T₅₀, and T₇₅ groups were significantly higher than those of other treatments. Additionally, the T₀⁺, T₂₅, T₅₀, and T₇₅ groups exhibited better feed conversion ratios. In conclusion, the *in ovo* administration of *Ocimum gratissimum* extract optimized incubation parameters and promoted enhanced growth performance in broiler chicks.

Introduction

The poultry industry is a vital pillar of global food security, providing an accessible source of essential protein (Risner et al., 2026). Maximizing performance requires excellent breeder management, optimal incubation, and early nutrition. In commercial practice, asynchronous hatching over 36 to 48 hours means that early-hatched chicks may fast for over 48 hours before accessing feed or water (Such et al., 2023; Li et al., 2022). Because embryonic metabolism rapidly depletes limited yolk reserves during this window, these reserves become insufficient to sustain the chicks. Consequently, this delayed feeding impairs growth, reduces slaughter weight, suppresses immunity, and increases mortality (Tona et al., 2023; Li et al., 2022). To mitigate the harmful effects of post-hatch fasting and build up nutrient reserves from birth, the poultry industry evaluates various early nutritional strategies, among them the *in ovo* practice. Considered a highly innovative approach, the *in ovo* injection technique allows essential nutrients and bioactive compounds to be delivered directly into the egg during incubation. Accordingly, numerous studies have evaluated the *in ovo* administration of diverse supplements, ranging from amino acids, carbohydrates, and vitamins to probiotics, nucleotides, peptides, and L-carnitine, to enhance immune responses and growth performance (Das et al., 2021). For example, early supplementation provides multiple benefits: probiotics and prebiotics prevent pathogenic infections (Stadnicka et al., 2020; Arreguin-Nava et al., 202048), specific amino acids enhance growth (Elnesr et al., 2019), vitamins C boosts immunity (Zhu et al., 2020), carbohydrates increase glycogen stores (Uni et al., 2005), and creatine promotes muscle growth (Zhu et al., 2019). In particular, Malheiro et al. (2012) stated that

providing embryos with *in ovo* feeding during incubation enhances biochemical and physiological balances and improves oxidative protection. According to Ipek et al. (2004), *in ovo* nutrient administration can improve hatchling quality and the subsequent growth performance of broiler chickens.

As modern poultry production shifts toward higher productivity and feed efficiency, research highlights the *in ovo* technique as a sustainable approach to improving intestinal health and reducing the industry's reliance on antibiotics. Supporting this trend, El-Kholy et al. (2021) demonstrated that *in-ovo* injection of herbal extracts such as cinnamon, thyme, and clove enhanced broiler body weight, feed conversion ratio, and IgG/IgM levels at 35 days of age, without affecting feed intake or IgA levels. Sulaiman and Al-Gharawi (2025) noted that *Aloe vera* improved hatchability and reduced embryonic deformities, while Mahjar and Al-Salhie (2022) reported that garlic extract enhanced hatchability without adversely affecting liver enzymes.

Ocimum gratissimum is an aromatic plant that possesses various biological activities largely driven by components such as phenolics and terpenoids (Akinmoladun et al., 2007; Prabhu et al., 2009). Research indicates that the antibacterial efficacy of *Ocimum gratissimum* is comparable to that of conventional antibiotics such as gentamicin and streptomycin (Nakamura et al., 1999). While previous studies have analyzed the dietary and oral administration effects of *Ocimum gratissimum* in poultry, the impact of *in ovo* feeding on hatching events and post-hatch performance remains unexplored. Previous studies demonstrate that dietary leaf meal improves growth, carcass characteristics, and meat quality (Olumide and Akintola, 2018), while oral extract administration enhances growth without detrimental effects (Olumide et al., 2022). Furthermore, the extract boosts humoral and cell-mediated immune responses without compromising performance or altering blood metrics (Lin et al., 2022). These benefits are largely driven by the plant's potent antioxidant activity, which is attributed to high concentrations of polyphenols and flavonoids (Akinmoladun et al., 2007). Specifically, eugenol, a key bioactive component, acts as a free radical scavenger that mitigates lipid peroxidation and protects cell membranes from oxidative stress (Zandi et al., 2010). Although *Ocimum gratissimum* possesses highly valuable properties for poultry production, peer-reviewed literature specifically addressing its *in ovo* administration is extremely limited. Therefore, this study was conducted to elucidate the effects of injecting *Ocimum gratissimum* extracts *in ovo* on the hatching parameters and post-hatching performance of Cobb 500 broiler chickens.

Materials and methods

Ocimum gratissimum leaf extraction

Fresh *Ocimum gratissimum* leaves were harvested from a local farm, air-dried at 20°C in an environmentally controlled room, and milled into a fine powder. Leaf extraction was performed as described by N'nanle et al. (2017). A total of 425 g of the powdered leaves was macerated in 4 L of a hydroalcoholic solution (1:1 v/v ratio of 96% ethanol and distilled water). The mixture was manually homogenized and macerated for 72 hours in a 5 L container, with active stirring every 8 hours. The

macerate was then sequentially filtered through a sieve, a thick layer of cotton, and finally an 11 µm filter paper. The filtrate was concentrated under vacuum using a rotary evaporator until all solvents were removed. The resulting crude extract was frozen at -40°C and subsequently freeze-dried to obtain a dry powder.

Injectable solutions preparation

To prepare the stock solution, 10 g of dry *Ocimum gratissimum* leaf hydroalcoholic extract was dissolved in 20 mL of sterile 0.9% NaCl saline solution. The mixture was homogenized using a vortex mixer to yield a stock solution with a concentration of 500 mg/mL. Three serial dilutions were then prepared according to Table 1 to achieve final concentrations of 25, 50, and 75 µg/mL, following the method previously described by N’nanle et al. (2017).

Table 1
Dilution of *Ocimum gratissimum* extract

Final concentration	Initial stock concentration (500 µg/mL) volume	Required volume of 0.9% NaCl	Final volume
25 µg/mL	2.5 mL	47.5 mL	50 mL
50 µg/mL	5 mL	45 mL	50 mL
75 µg/mL	7.5 mL	42.5 mL	50 mL

Experimental design and in ovo injection

A completely randomized design was used for this trial. A total of 1500 Cobb 500 hatching eggs were obtained from a 46-week-old breeder flock in Lomé. The eggs were weighed, numbered, and incubated in a Petersime incubator at 37.7°C and 60% relative humidity. The incubator was set to automatically turn the eggs hourly.

On day 18 of incubation, all eggs were candled to identify fertility. A total of 1200 fertile eggs were randomly divided into six groups, each with four replicates of 50 eggs, assigned as follows: T0-: Uninjected negative control group; T0+: Positive control group injected with physiological saline (NaCl); TT: Pierced, uninjected control group; T₂₅: Eggs treated with 25 µg/mL of *Ocimum gratissimum* extract; T₅₀: Eggs treated with 50 µg/mL of *Ocimum gratissimum* extract; T₇₅: Eggs treated with 75 µg/mL of *Ocimum gratissimum* extract.

The air sac of the egg was located, disinfected with alcohol, and punctured with two holes using a 21G needle to prevent pressure build-up. Next, 0.1 mL of the respective solution was injected into the inner tube using a 24G automatic syringe. Following inoculation, the injection holes were sealed with sterile adhesive tape, and the eggs were transferred to a Petersime incubator, where the temperature and relative humidity were maintained at 37.5°C and 70%, respectively.

Hatching events and chick quality

From 453 hours of incubation onward, internal pipping (IP), external pipping (EP), and chick emergence were recorded. Eggs were classified as internally pipped when candling revealed the chick's beak penetrating the inner shell membrane into the air cell. External pipping was defined as the first visible cracking of the eggshell. The collected data were used to evaluate cumulative times and durations for key hatching events, including internal pipping (IP), external pipping (EP), and emergence. We also determined the overall hatch duration, hatch window, hatch spread, and the duration of both the IP and EP phases. Also, percentage hatchability was calculated using the following formula:

$$Hatchability (\%) = \frac{\text{Number of hatched chicks}}{\text{Number of eggs containing a viable embryo at day 18}} \times 100$$

Furthermore, unhatched eggs were opened to evaluate their developmental stage and determine embryonic mortality, using the following formula:

$$Mortality (\%) = \frac{\text{Number of dead chicks}}{\text{Number of eggs containing a viable embryo at day 18}} \times 100$$

At the end of the hatch, thirty chicks from each group were weighed and assessed for quality using the Tona score. This scoring system evaluates day-old chick quality based on morphological and functional criteria including activity, down appearance, eyes, legs, navel, and yolk sac resorption (Table 2). Each parameter is assigned points relative to its importance, yielding a maximum score of 100, which classifies chick quality and predicts survival and growth potential during early rearing (Tona et al., 2003)

Table 2
Tona score chart

Parameters	Characteristics	Scores
Activity	Good	6
	Low	0
Down and appearance	Clean and dry	10
	Wet	8
	Dirty and wet	8
Retracted yolk	Normal abdomen	12
	Enlarged and hard abdomen	0
Eyes	Open and bright	16
	Open and dull	8
	Closed	0
Legs	Normal legs and toes	16
	One leg affected	8
	Both legs affected	0
Navel	Clean and closed completely	12
	Partially open and without discoloration	6
	Not completely closed and appears discolored	0
Remaining membrane	No membrane	12
	Small Membrane	8
	Large Membrane	4
	Very large membrane	0
Remaining yolk	No yolk	16
	Small yolk	12
	Large yolk	8
	Very large yolk	0
Source: Tona et al.(2003)		

Relative organ weights

At hatching, 30 chicks per treatment group were euthanized humanely. The yolk sac, liver, heart and gizzard were collected to determine their relative weights, which were expressed as a percentage of body weight using the following formula:

$$Relative\ weight = \frac{\text{Weight of the organ}}{\text{Weight of the chick}} \times 100$$

Post-hatch juvenile growth performance

A total of 600 day-old chicks were randomly assigned to treatment groups (5 replicates of 20 chicks per pen) and reared at the CERSA experimental unit for 6 weeks. Throughout the 6-week period, all groups were fed the same starter-to-finisher diets. The T₂₅, T₅₀, and T₇₅ groups were reared without veterinary products (antibiotics or anticoccidials), except for routine vaccinations. All groups were exposed to an identical lighting schedule, water and feed (Table 3) were provided *ad libitum*. Daily feed intake per pen was calculated by subtracting the refused feed from the offered feed. Birds were weighed each week individually to determine body weight and average daily weight gain. These data were then used to calculate the feed conversion ratio (FCR), defined as feed intake divided by body weight gain.

Table 3
Gross composition of experimental diets (%)

Feed stuffs	Starter	Growth	Finition
Maize	50.25	67	60
Soya seed	25	20	25
Soy flour	10	0	0
Wheat brand	8	4	9
Concentrate	5	5	3
Oyster shell	1	1.5	1.5
Methionine	0.3	0.3	0.3
Lysine	0.2	20	0.2
Palm oil	0	2	0
Mycotoxin binder (Aflabind)	0.25	0	0.02
Total	100%	100	100
Calculated analysis			
Metabolizable energy (kcal kg ⁻¹)	3085	3342	3153
Crude protein (%)	21.1	15.6	16.8
Crude fibre (%)	4.20	3.16	3.70
Calcium (%)	0.50	0.65	0.70
Available phosphorus (%)	0.35	0.40	0.43
Crude fat (%)	7.10	8.46	7.2
Lysine (%)	1.15	0.95	1.00
Méthionine (%)	0.50	0.50	0.50

Statistical analysis

Data were analyzed using GraphPad Prism 8.0.2. Hatching and mortality rates were compared using the chi-square test. All other parameters were assessed using one-way ANOVA, followed by Tukey's test for multiple comparisons. Differences were considered significant at $p < 0.05$, and data are presented as mean $\pm$ SEM

Results

Effects on hatching events

Ocimum gratissimum in ovo injection did not significantly affect the hatching process (Table 4). Across all treatments, no significant differences were observed in time of internal pipping (TIP), duration of internal pipping (DIP), time of external pipping (TEP), duration of external pipping (DEP), or total hatching duration (p > 0.05).

Table 4
in ovo injection of hydroalcoholic *Ocimum gratissimum* extracts on different stages of hatching.

Parameters(h)	Treatments						p-value
	T0-	T0+	TT	T ₂₅	T ₅₀	T ₇₅	
TIP	476.6 ± 1.12	476.6 ± 1.07	477.2 ± 0.76	478.2 ± 0.60	478.7 ± 0.67	478.2 ± 0.97	0.389
DIP	11.15 ± 0.90	10.97 ± 0.89	8.62 ± 0.74	9.97 ± 0.82	9.53 ± 0.73	9.5 ± 0.970	0.281
EP	487.7 ± 0.75	487.5 ± 0.84	485.9 ± 0.67	488.2 ± 0.67	488.2 ± 0.72	487.7 ± 0.82	0.161
DEP	9.62 ± 0.60	8.294 ± 0.64	9.52 ± 0.57	8.71 ± 0.59	9.176 ± 0.60	8.118 ± 0.49	0.281
THD	20.77 ± 0.716	19.26 ± 0.780	18.14 ± 0.668	8.71 ± 0.712	17.62 ± 0.697	495.8 ± 0.670	0.132

T0-: Uninjected negative control group; T0+: Positive control group injected with physiological saline (NaCl); TT: Pierced, uninjected control group; T₂₅: Eggs treated with 25 µg/mL of *Ocimum gratissimum* extract; T₅₀: Eggs treated with 50 µg/mL of *Ocimum gratissimum* extract; T₇₅: Eggs treated with 75 µg/mL of *Ocimum gratissimum* extract. Time of internal pipping (TIP), duration of internal pipping (DIP), time of external pipping (TEP), duration of external pipping (DEP), and total hatching duration (THD).

Effect on hatching window

Figure 1 shows the distribution of hatched chicks across treatments. Hatching began at approximately 488 hours of incubation in all groups and peaked between 492 and 495 hours. After this peak, the hatching frequency declined until the final chicks emerged at around 505 hours. Overall, the hatching window was consistent across all treatment groups, representing a total duration of approximately 17 hours (488 to 505 hours).

T0-: Uninjected negative control group; T0+: Positive control group injected with physiological saline (NaCl); TT: Pierced, uninjected control group; T₂₅: Eggs treated with 25 µg/mL of *Ocimum gratissimum* extract; T₅₀: Eggs treated with 50 µg/mL of *Ocimum gratissimum* extract; T₇₅: Eggs treated with 75 µg/mL of *Ocimum gratissimum* extract.

Effect on egg hatching rates and embryonic mortality

Table 5 outlines the effects of *in ovo* injection of *Ocimum gratissimum* on hatch rates and embryonic mortality. Treatments significantly influenced both hatch rates ($p = 0.007$) and total embryonic mortality ($p = 0.003$). The T50 group achieved the highest hatch rate and lowest total mortality, while the negative control group (T0-) showed the lowest hatch rate and the highest mortality (Table 5).

Table 5
In ovo injection on egg hatching rates and embryonic mortality

Paramters (%)	Treatments						P-value
	T0-	T0+	TT	T25	T50	T75	
HR	81.67 ± 0.33 ^d	83.33 ± 0.28 ^{cd}	83.33 ± 0.28 ^{cd}	88.70 ± 0.38 ^b	92.63 ± 0.84 ^a	85 ± 0.07 ^{bc}	0.007
MR	18.33 ± 0.42 ^a	16.67 ± 0.88 ^a	16.67 ± 0.79 ^a	11.30 ± 0.60 ^a	7.37 ± 0.08 ^b	15 ± 0.05 ^a	0.003

T0-: Uninjected negative control group; T0+: Positive control group injected with physiological saline (NaCl); TT: Pierced, uninjected control group; T₂₅: Eggs treated with 25 µg/mL of *Ocimum gratissimum* extract; T₅₀: Eggs treated with 50 µg/mL of *Ocimum gratissimum* extract; T₇₅: Eggs treated with 75 µg/mL of *Ocimum gratissimum* extract; HR: Hatching rate; MR, mortality rate. a,b,c,d Means in the same row with different superscripts are significantly different ($p < 0.05$).

Effects on chick quality

The effects of the *in ovo* injection of *Ocimum gratissimum* on chick quality are presented in Table 6. The results show no significant differences ($p > 0.05$) among treatments.

Table 6
in ovo injection of *Ocimum gratissimum* on chick quality

Paramters	Treatments						p-value
	T0-	T0+	TT	T25	T50	T75	
Activity	6.00 ± 0.00	6.00 ± 0.00	6.00 ± 0.00	6.00 ± 0.00	6.00 ± 0.00	6.00 ± 0.00	0.000
Down and appearance	9.67 ± 0.20	9.83 ± 0.20	9.83 ± 0.20	9.667 ± 0.20	9.67 ± 0.20	10.00 ± 0.00	0.700
Retracted yolk	12.00 ± 0.00	12.00 ± 0.00	12.00 ± 0.00	12.00 ± 0.00	12.00 ± 0.00	12.00 ± 0.00	0.000
Eyes	14.67 ± 0.90	16.00 ± 0.00	16.00 ± 0.00	15.33 ± 0.07	16.00 ± 0.00	16.00 ± 0.00	0.210
Leg	15.33 ± 0.67	16.00 ± 0.00	16.00 ± 0.00	16.00 ± 0.00	15.33 ± 0.67	16.00 ± 0.00	0.540
Navel	11.50 ± 0.50	11.50 ± 0.50	11.50 ± 0.50	11.50 ± 0.50	12.00 ± 0.00	12.00 ± 0.00	0.840
Remaining membrane	12.00 ± 0.00	11.67 ± 0.33	11.67 ± 0.33	12.00 ± 0.00	12.00 ± 0.00	11.67 ± 0.33	0.690
Remaining yolk	15.67 ± 0.33	15.67 ± 0.33	15.67 ± 0.33	16.00 ± 0.00	16.00 ± 0.00	16.00 ± 0.00	0.690
Total Score	96.83 ± 1.31	98.67 ± 0.62	98.67 ± 0.62	98.50 ± 0.78	99.00 ± 0.674	99.67 ± 0.33	0.340

T0-: Uninjected negative control group; T0+: Positive control group injected with physiological saline (NaCl); TT: Pierced, uninjected control group; T₂₅: Eggs treated with 25 µg/mL of *Ocimum gratissimum* extract; T₅₀: Eggs treated with 50 µg/mL of *Ocimum gratissimum* extract; T₇₅: Eggs treated with 75 µg/mL of *Ocimum gratissimum* extract.

Effects of in ovo injection of *Ocimum gratissimum* relative organ weights of broiler chicks at hatch.

Table 7 presents the effects of *in ovo* *Ocimum gratissimum* injection on chick morphometric parameters at hatching. Chick weight was significantly influenced, with treatment T₅₀ exhibiting the highest values ($p < 0.05$). Additionally, the T₇₅ group showed a significantly higher yolk sac weight (9.00 ± 0.51 g) but the lowest liver weight (0.43 ± 0.02 g) compared to all other groups ($p < 0.05$). Neither heart nor gizzard weights were significantly affected ($p > 0.05$).

Table 7
in ovo injection of *Ocimum gratissimum* on chick weight and relative visceral organ weights

Parameters (g)	Treatments						p-value
	T0-	T0+	TT	T25	T50	T75	
Chick weight	44.48 ± 0.40 ^b	45.63 ± 0.52 ^b	45.31 ± 0.51 ^b	45.74 ± 0.50 ^b	56.79 ± 10.85 ^a	45.90 ± 0.57 ^b	0.039
Yolk weight	5.63 ± 0.90 ^c	5.53 ± 0.09 ^c	6.50 ± 0.81 ^b	6.46 ± 1.09 ^b	5.00 ± 1.37 ^c	9.00 ± 0.51 ^a	0.013
Heart weight	0.31 ± 0.01	0.27 ± 0.03	0.36 ± 0.07	0.30 ± 0.10	0.30 ± 0.06	0.27 ± 0.10	0.785
Liver weight	1.10 ± 0.10 ^a	1.13 ± 0.09 ^a	1.03 ± 0.06 ^a	1.10 ± 0.05 ^a	0.43 ± 0.02 ^b	1.00 ± 0.17 ^a	0.019
Gizzard weight	2.43 ± 0.18	2.57 ± 0.10	2.47 ± 0.15	3.3 ± 0.50	2.23 ± 0.29	2.70 ± 0.21	0.758
T0-: Uninjected negative control group; T0+: Positive control group injected with physiological saline (NaCl); TT: Pierced, uninjected control group; T ₂₅ : Eggs treated with 25 µg/mL of <i>Ocimum gratissimum</i> extract; T ₅₀ : Eggs treated with 50 µg/mL of <i>Ocimum gratissimum</i> extract; T ₇₅ : Eggs treated with 75 µg/mL of <i>Ocimum gratissimum</i> extract							

Effect of in ovo injection of *Ocimum gratissimum* leaf extracts on post-hatching growth performance

Feed intake

Figure 2 illustrates the daily feed intake of chickens across all treatment groups from the chick stage to week 6. While feed intake increased progressively from week 1 to week 4, it remained relatively constant thereafter. A slight tendency to decrease was noted in group TT; T0⁻ and T0⁺ between week 4 and week 6. Overall, no significant differences were observed throughout the trial ($p > 0.05$).

Figure 2 : Feed intake according to the treatments and the age

T0-: Uninjected negative control group; T0+: Positive control group injected with physiological saline (NaCl); TT: Pierced, uninjected control group; T₂₅: Eggs treated with 25 µg/mL of *Ocimum gratissimum* extract; T₅₀: Eggs treated with 50 µg/mL of *Ocimum gratissimum* extract; T₇₅: Eggs treated with 75 µg/mL of *Ocimum gratissimum* extract

Chickens weight

Table 8 shows the changes in chick weight with age and treatment. During week 1, the T₅₀ and T75 groups had similar live weights, which were significantly ($p < 0.001$) higher than those of the T0⁺, T0⁻, TT, and T₂₅ groups. By week 2, the T₇₅ group was significantly heavier ($p < 0.05$) than all other treatments. At

week 6, the T₂₅, T₅₀, and T₇₅ groups achieved significantly higher ($p < 0.05$) final live weights than the other treatments.

Table 8

The effect of *in ovo* injection of *Ocimum gratissimum* extract on the growth performance and body weight of broiler chickens

Age (week)	Treatments						p-value
	T0-	T0+	TT	T ₂₅	T ₅₀	T ₇₅	
1	156.3 ± 284 ^c	167.7 ± 3.74 ^b	159.5 ± 3.06 ^{bc}	166.1 ± 2.95 ^b	170 ± 2.75 ^a	177.3 ± 3.925 ^a	0.0003
2	374.1 ± 6,08 ^c	402.7 ± 9.17 ^b	380.8 ± 6.43 ^c	391.1 ± 6.80 ^{bc}	411.9 ± 6.83 ^b	433.5 ± 10.61 ^a	0.0001
3	679.8 ± 11.47 ^b	694.1 ± 15.97 ^a	692.8 ± 11.72 ^a	672.5 ± 1.28 ^b	701.8 ± 12.53 ^a	694.7 ± 18.21 ^a	0.049
4	1051 ± 18.6 ^{bc}	1060 ± 24.89 ^b	1062 ± 22.91 ^b	1071 ± 23.41 ^b	1094 ± 17.5 ^a	1066 ± 28.64 ^b	0.042
5	1475 ± 28.21 ^b	1416 ± 30.5 ^d	1459 ± 31.34 ^c	1466 ± 31.81 ^c	1497 ± 26.66 ^a	1485 ± 23.77 ^{ab}	0.048
6	1784 ± 47.9 ^c	1851 ± 39.21 ^b	1819 ± 39.29 ^c	1899 ± 37.58 ^a	1913 ± 35.28 ^a	1908 ± 38.75 ^a	0.023

a,b,c,d Means in the same row with different superscripts are significantly different ($p < 0.05$).

T0-: Uninjected negative control group; T0+: Positive control group injected with physiological saline (NaCl); TT: Pierced, uninjected control group; T₂₅: Eggs treated with 25 µg/mL of *Ocimum gratissimum* extract; T₅₀: Eggs treated with 50 µg/mL of *Ocimum gratissimum* extract; T₇₅: Eggs treated with 75 µg/mL of *Ocimum gratissimum* extract.

Feed conversion ratio

Figure 3 shows the effect of *in ovo* injection of *Ocimum gratissimum* on the feed conversion ratio (FCR). The FCR was statistically similar across the T0⁺, T₂₅, T₅₀, and T₇₅ treatment groups, but was significantly higher compared to the T0⁻ and TT groups ($p < 0.05$).

a,b,c,d Means in the same row with different superscripts are significantly different ($p < 0.05$).

T0⁻: Uninjected negative control group; T0⁺: Positive control group injected with physiological saline (NaCl); TT: Pierced, uninjected control group; T₂₅: Eggs treated with 25 µg/mL of *Ocimum gratissimum*

extract; T_{50} : Eggs treated with 50 $\mu\text{g/mL}$ of *Ocimum gratissimum* extract; T_{75} : Eggs treated with 75 $\mu\text{g/mL}$ of *Ocimum gratissimum* extract.

Discussion

In this study, hatching events, including the timing and duration of both internal and external pipping, did not differ significantly among the treatment groups. Besides, the hatching of the first and last chicks occurred after 488 and 505 hours of incubation, respectively, corresponding to a span of approximately 17 hours in all treatments. The absence of significant differences between the treated and control groups indicates that the extract was safe and did not adversely affect hatching uniformity. Similarly, N'nanle et al. (2019) observed that *in ovo* administration of *Moringa oleifera* extract did not affect external pipping, but did reduce the total incubation duration. In contrast, Kpossou et al. (2024) reported that injecting soursop leaf extract significantly accelerated hatching, reducing both the overall incubation period and the timing of internal and external pipping. The 50 $\mu\text{g/mL}$ treatment group demonstrated the highest hatchability and greatest chick weight compared to all other groups. This indicates that *in ovo* injection of *Ocimum gratissimum* at this concentration optimizes hatching outcomes more effectively than the other tested concentrations. Al-Shammari et al. (2026) reported that embryonic metabolic rates increase significantly during the final phase of incubation to meet the demands of hatching and the shift from chorioallantoic to pulmonary respiration. This metabolic surge triggers an overproduction of reactive oxygen species (ROS), causing oxidative stress that compromises both embryonic viability and hatch weight. Although antioxidant capacity was not directly measured in this trial, previous research confirms that *Ocimum gratissimum* is rich in thymol and eugenol. These bioactive compounds exhibit potent antioxidant effects, protecting developing embryonic tissues by scavenging free radicals and mitigating lipid peroxidation (Akinmoladun et al., 2007; Zandi et al., 2010). Therefore, we hypothesize that the enhanced hatching performance observed in the 50 $\mu\text{g/mL}$ group results from an adequate concentration of these phytochemicals.

At hatch, chicks from the group injected with 75 $\mu\text{g/mL}$ of *Ocimum gratissimum* extract had significantly heavier relative yolk sac weights than all other groups. The yolk sac provides vital nutrients for the developing embryo and newly hatched chick, and the assimilation of these reserves fuels metabolism during the bird's critical first hours of life (Noy and Sklan, 1999). Our yolk sac weight data suggest that high extract concentrations impair this energy utilization during late embryonic development, likely contributing to the elevated mortality observed in the 75 $\mu\text{g/mL}$ group. Conversely, the 50 $\mu\text{g/mL}$ treatment group exhibited the most efficient yolk sac absorption, resulting in the highest hatchability, the greatest body weight at hatch, and the lowest embryo mortality. This indicates enhanced mobilization of yolk reserves for embryonic growth. Therefore, we hypothesize that *in ovo* administration of *Ocimum gratissimum* modulates yolk metabolism in a dose-dependent manner. Previous studies indicate that plant extracts rich in bioactive compounds can enhance avian embryonic development when administered at optimal concentrations (Kuka et al., 2024). On the other hand, the high embryo mortality rate in T_{75} suggests that higher dosages of the *Ocimum gratissimum* extract are associated with adverse

effects in the embryo. The precise mechanisms responsible for the high embryonic mortality observed in the T₇₅ require further investigation. Numerous studies demonstrate that *in ovo* administration of plant extracts improves hatchability and chick weight while reducing embryonic mortality (Kpossou et al., 2024; Al-Shammari et al., 2025; Bilalissi et al., 2019; Ngueda et al., 2021; Kuka et al., 2024).

The morphometric assessment of organs is essential for evaluating metabolic status. Our results showed that *in ovo* injection of *Ocimum gratissimum* extract 50 µg/mL significantly reduced liver weight among treatments. Because *O. gratissimum* is rich in phytochemicals with strong antioxidant properties (Akinmoladun et al., 2007), this dosage likely protected the embryos from oxidative stress during the metabolically demanding final stages of embryogenesis. Consequently, the reduced oxidative stress lowered the liver's demand to produce endogenous antioxidant enzymes, resulting in a smaller organ weight. Furthermore, alleviated hepatic stress enabled the liver to mobilize yolk lipids more efficiently, which ultimately explains the improved hatch rate and higher weight of chicks in the 50 µg/mL group.

By the end of the 6-week trial, birds hatched from extract-treated eggs had significantly greater final body weights than those in the control groups, despite receiving no conventional antibiotic or anticoccidial treatment. This result is likely the cumulative effect of the *Ocimum gratissimum* extract modulating the physiological and health status of the chicks during incubation. *In-ovo* administration of 50 µg/mL extract most effectively stimulated embryonic metabolism, positively impacting hatchability and chick weight. During rearing, birds from the 25 µg/mL and 75 µg/mL groups compensated for lower initial weights by utilizing heavier yolk reserves, ultimately achieving final body weights comparable to the µg/mL group. This suggests that the 50 µg/mL *Ocimum gratissimum* extract was absorbed more rapidly during incubation, which may explain why its effects were not sustained throughout the rearing period. Although improved gut health offers a plausible explanation for post-hatch performance, the specific effects of the extract on gut microbiota require further study. Previous studies have established that administering up to 0.6 ml of *Ocimum gratissimum* extract improves growth performance in poultry without compromising carcass traits. Similarly, Olumide and Akintola (2018) reported that supplementing broiler diets with *Ocimum gratissimum* leaf meal further enhances meat quality and overall performance

Conclusion

Our findings demonstrate that *in ovo* administration of *Ocimum gratissimum* extract significantly influenced both incubation and post-hatch phases. The 50 µg/mL treatment group showed the highest hatchability, heaviest chick weight, and lowest embryo mortality, correlating with highly efficient yolk sac absorption. Conversely, the 75 µg/mL dose resulted in heavier relative yolk sac weights and increased embryonic mortality. By week 6, birds from treated eggs exhibited significantly higher final body weights than the control group, despite the absence of conventional antibiotics or anticoccidials. Therefore, *in ovo* injection of 50 µg/mL *Ocimum gratissimum* extract is a promising strategy to improve hatchability and post-hatch broiler performance, although the specific mechanisms influencing gut microbiota and histomorphological changes require further investigation.

Declarations

Competing Interests

The authors declare no conflicts of interest

Ethics approval

All experimental procedures were reviewed and approved by the Ethics Committee of the Regional Centre of Excellence in Poultry Science (CERSA), University of Lomé, Togo (approval number: CERSA/ANI/2025/014; date of approval: April 12, 2026), operating under the National Ethics Committee for the Control and Supervision of Experiments on Animals. This article provides details on the housing conditions, care, and pain management for the animals, ensuring that the impact on the animals is minimized during the experiment.

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None

Author contributions

Voemesse, and Benawo: conceptualization, data analysis, and visualization. Voemesse, Benawo and Seme : writing manuscript preparation, review, and editing. Oke and Pitala have read and agreed to the published version of the manuscript.

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Data Availability

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

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Figures

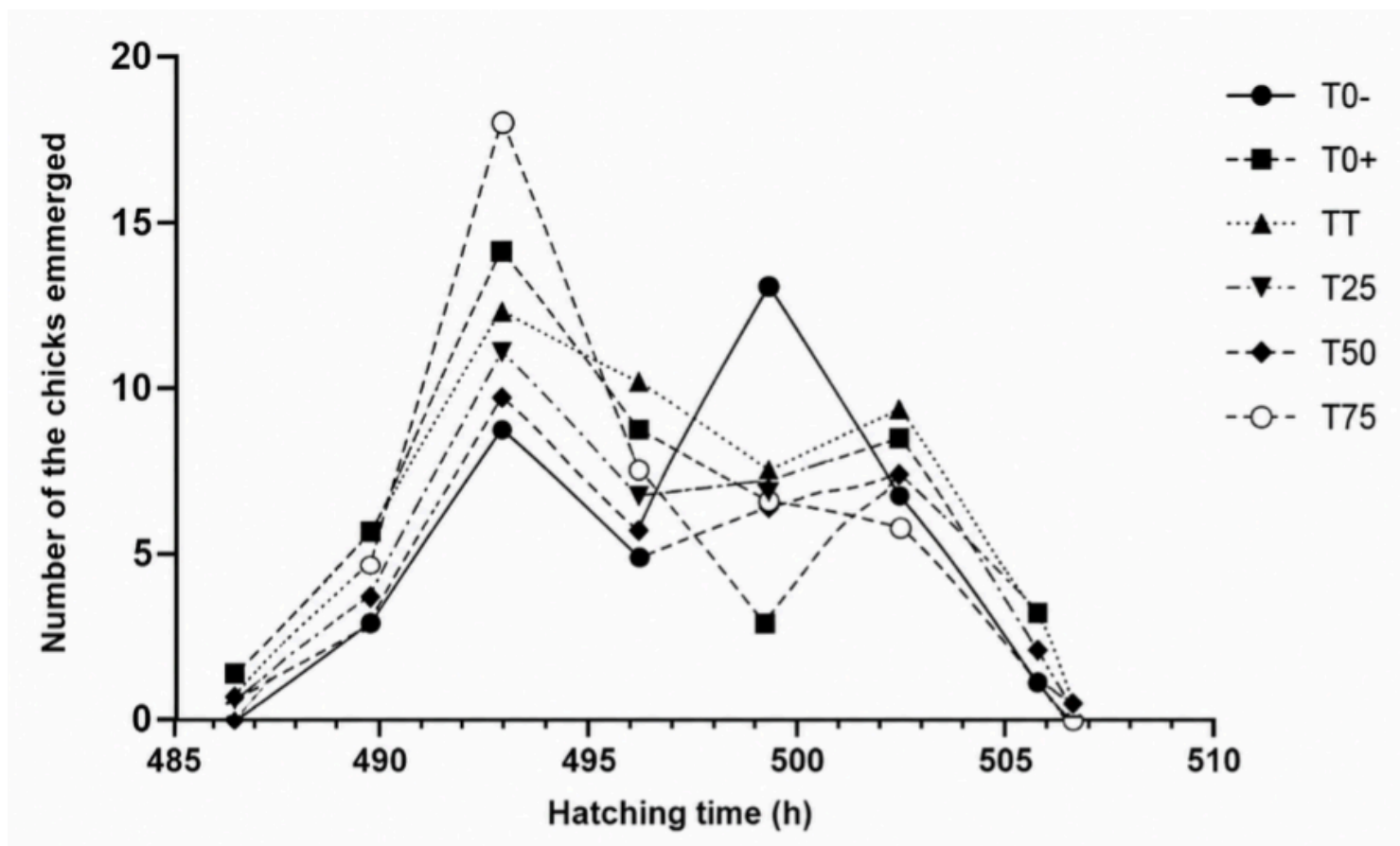


Figure 1

Effects of *in ovo* injection of *Ocimum gratissimum* on hatching window

T0-: Uninjected negative control group; *T0+*: Positive control group injected with physiological saline (NaCl); *TT*: Pierced, uninjected control group; *T₂₅*: Eggs treated with 25 µg/mL of *Ocimum gratissimum* extract; *T₅₀*: Eggs treated with 50 µg/mL of *Ocimum gratissimum* extract; *T₇₅*: Eggs treated with 75 µg/mL of *Ocimum gratissimum* extract.

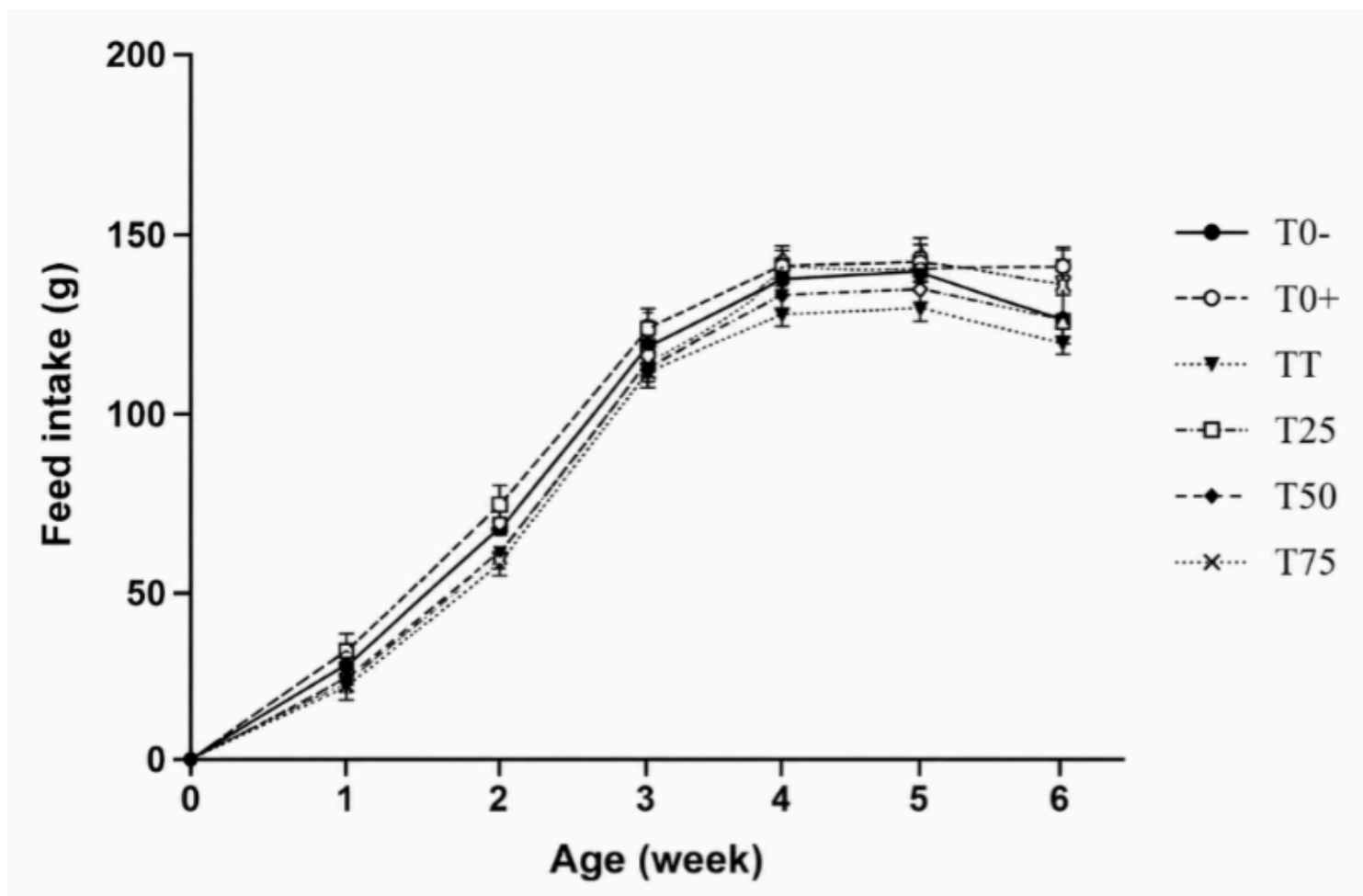


Figure 2

Feed intake according to the treatments and the age

T0-: Uninjected negative control group; *T0+*: Positive control group injected with physiological saline (NaCl); *TT*: Pierced, uninjected control group; *T₂₅*: Eggs treated with 25 µg/mL of *Ocimum gratissimum* extract; *T₅₀*: Eggs treated with 50 µg/mL of *Ocimum gratissimum* extract; *T₇₅*: Eggs treated with 75 µg/mL of *Ocimum gratissimum* extract

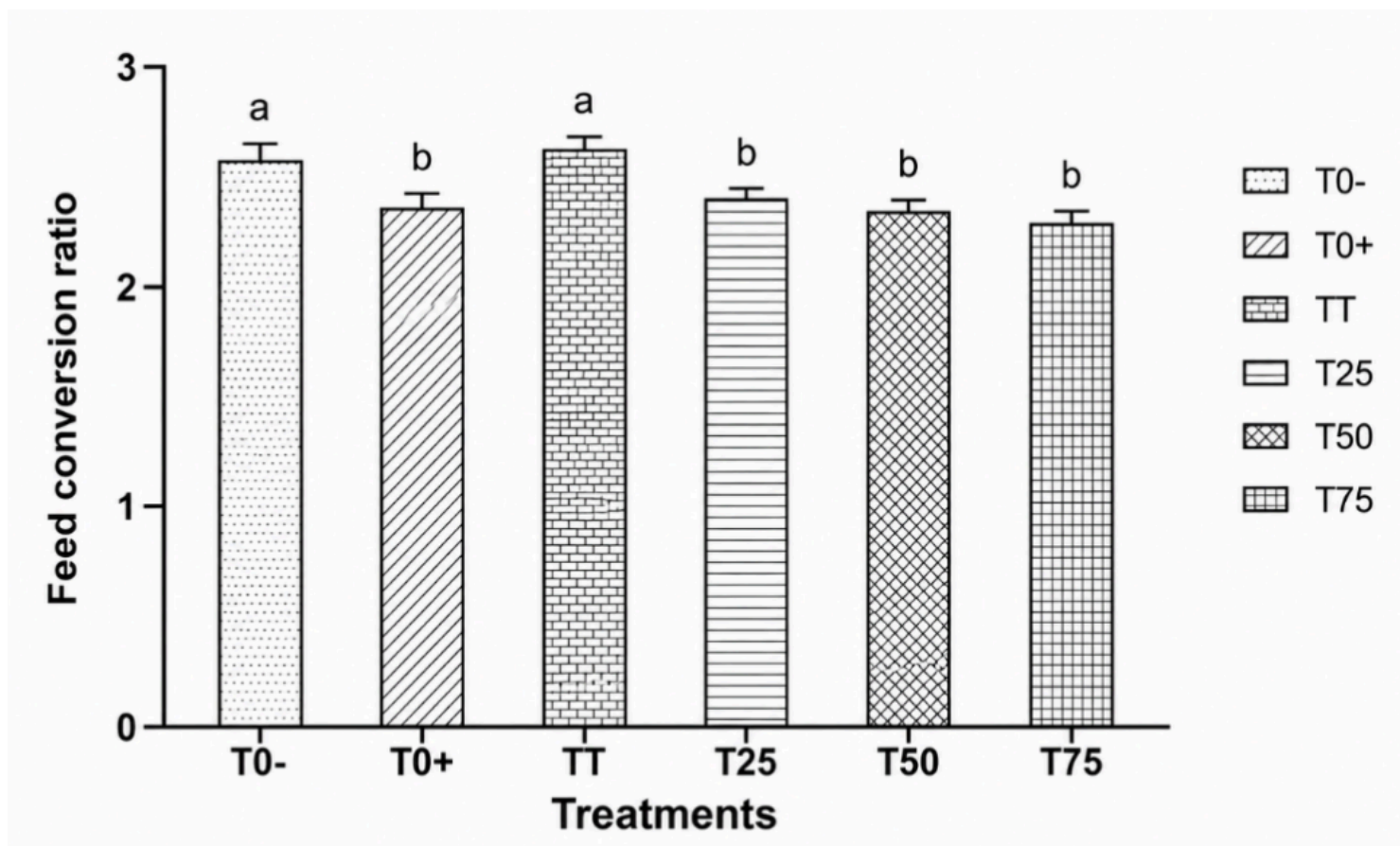


Figure 3

Feed conversion ratio according to the treatments

a,b,c,d Means in the same row with different superscripts are significantly different ($p < 0.05$).

T0⁻: Uninjected negative control group; T0⁺: Positive control group injected with physiological saline (NaCl); TT: Pierced, uninjected control group; T₂₅: Eggs treated with 25 µg/mL of Ocimum gratissimum extract; T₅₀: Eggs treated with 50 µg/mL of Ocimum gratissimum extract; T₇₅: Eggs treated with 75 µg/mL of Ocimum gratissimum extract.