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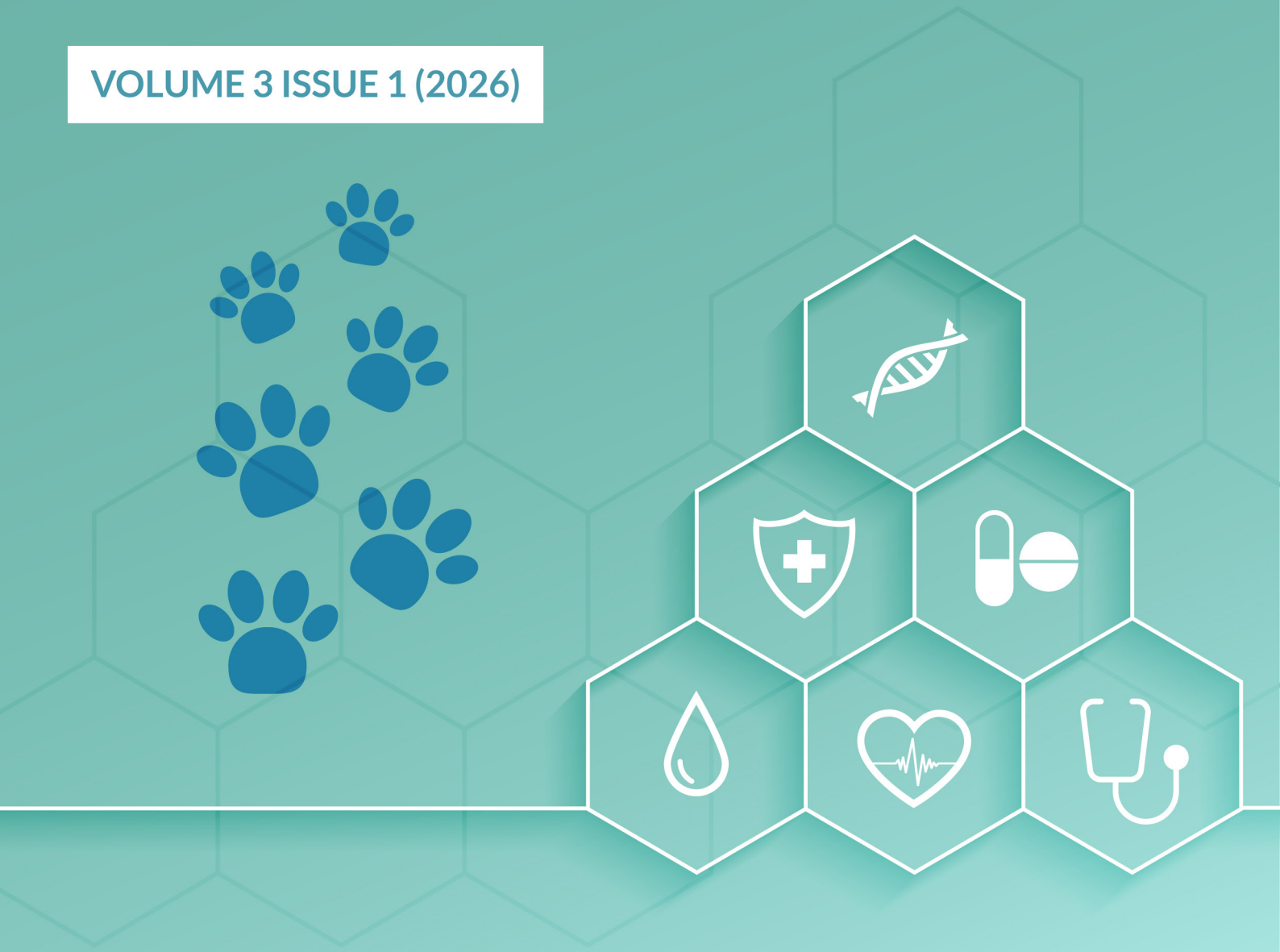

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In Ovo Surface Application of *Cymbopogon citratus* (Lemongrass) on the Hatching Performance, Post- Hatch Morphometrics, and Spleen-Based Immune Responses in *Gallus Gallus Domesticus* Under Thermal Stress Conditions

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ABSTRACT

Due to the continuous change in climate, thermal stress becomes one of the key factors that interferes with poultry production. This study aims to evaluate the effect of *in ovo* surface application of *Cymbopogon citratus* (lemongrass) on the hatching performance, post-hatch morphometric characteristics, and spleen-based immune responses of *Gallus gallus domesticus* (Rhode Island Red) during thermal stress condition of 39°C. The 90 fertilized eggs were grouped into three: chronic (sprayed with 25%, 50%, 75%, and 100% concentrations), controlled (sprayed with water), and no application groups, which were then assessed using quantitative experimental design. Data was analyzed using one-way ANOVA and post hoc test (Tukey). The study reveals that lemongrass treatment has a varying impact on the hatchability, mortality, physiological traits, and immune response of the chicks. Skeletal characteristics, such as the shrinkage length of the keel, the length of the shank, and wingspan exhibited a great difference between treatments, but body weight, chest circumference, and spleen index exhibited no significant differences. Hence, the surface application of *Cymbopogon citratus* inconsistently affects chick development and immune characteristics across different concentrations.

INTRODUCTION

Heat stress poses an alarming challenge for poultry production that disrupts the fragile embryonic development of *Gallus gallus domesticus* (Rhode Island Red) chicken. Increased heat stress leads to higher mortality and lower hatchability in chicks, thereby impacting profitability of a poultry farm (Saeed *et al.*, 2019). Some studies show that high temperatures during the development stages of an embryo can result in the impairment of physiological traits that are critical to the formation of healthy hatchlings, thus raising reputable concerns for both the welfare of the animal and the safety of food in environments where heat is a factor that is present (Bhanja *et al.*, 2024). Effective management of heat stress can help maintain the viability of the embryo and post-hatch performance, which is especially important in tropical regions where the temperatures are high and the incubation temperatures are elevated. With innovative and affordable temperature control often lacking, the poultry industry urgently seeks safer, more sustainable alternatives to artificial growth promoters that can help address heat stress.

In ovo delivery of bioactive compounds has been extensively considered as a method of manipulating embryonic growth and after hatching in poultry. A number of studies have demonstrated that nutrient or functional compound injection into the embryonic eggs could alter antioxidant status, intestinal development, and overall hatch performance. Indicatively, in ovo injection of soy isoflavones increased the hatchability and antioxidant enzyme activity, and promoted the intestinal morphology of newly hatched chicks, which demonstrated that in ovo supplementation has the potential to regulate the oxidative status and early growth phenotypic characteristic (Abdelghani *et al.*, 2023). Furthermore, studies on in ovo nutrition of amino acids like methionine have proven to have a positive impact on hatching weight and gene expression in relation to growth, which further indicates that early intervention of the embryonic nutrient may influence both developmental and physiological processes (Lugata *et al.*, 2024). These results confirm the idea that bioactive injection during incubation may influence embryonic resilience and performance, but the effects of the process are dependent on the substance, timing, and dosage of the injection.

Other non-invasive procedures like surface application and eggshell sprays have been explored in order to reduce the risk factors that are usually attributed to in ovo injection, but still produce beneficial outcomes. It has been demonstrated that surface delivery of probiotics can also foster desirable microbial colonization and does not adversely impact hatchability, which indicates that alternative delivery methods to the embryo may prevent embryonic injury caused by injections (Oladokun *et al.*, 2023). Also, the use of essential oils and natural compounds has been considered in shell coating and shell preservation; most shell coating studies have found that the addition of lemongrass essential oils to

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shell coatings can increase egg shelf life or preserve egg quality during storage, suggesting that incubation support may be achieved without shell penetration (Minh, 2018). Combined, these studies confirm the reasons behind the study of surface-applied botanical treatments as a less toxic method of directly injecting embryos.

The incubation of embryos in high temperatures has been demonstrated to cause a significant influence on embryonic development, morphometric characteristics of post-hatch, and immune organ development. Heat stress adversely affects the morphology of the intestine, alters the oxidative balance, and disrupts the development of immune-related organs, such as the spleen, which is traditionally considered one of the indicators of immune competence in poultry (Sgavioli *et al.*, 2022). Since incubation stress is very detrimental to the developing embryos and the newly hatched chicks, it is important to optimize the conditions under which eggs are incubated to facilitate normal physiological and structural development. In this regard, antioxidant and immunomodulatory effects of natural phytochemicals, including *Cymbopogon citratus*, could provide some protective value through improved antioxidant and stress-reducing effects, thus increasing hatchability, post-hatch structural development, and immune activity (Faheem *et al.*, 2022). In addition, lemongrass supplement has been linked to better performance and production characteristics in chickens, and the possible application of lemongrass supplement is a functional and sustainable addition to the chicken production system (Konca & Aktug, 2018).

Despite the promising results of these methods, the use of surface application of *Cymbopogon citratus* remains underexplored, particularly in local studies within the SOCCSKSARGEN region. While injection of bioactive compounds has been shown to enhance chick performance, it also increases handling stress and demands specialized training. In contrast, surface application offers a simpler and less invasive strategy to deliver natural compounds that can support embryonic survival and growth. Thus, this study evaluated the effects of applying various concentrations of *Cymbopogon citratus* extract on eggshell surfaces, focusing on embryonic mortality, hatchability, post-hatch physiological traits, and spleen-based immune response under thermal stress conditions, at 39°C.

This study specifically (1) determined the embryonic mortality and hatchability rates by comparing treated and untreated *Gallus gallus domesticus eggs*; (2) assessed post-hatch phenotypic and physiological traits such as body weight, body length, shank length, keel length, chest circumference, wingspan; and blood vessels, (3) determined indirect immunological status by examining and calculating the spleen index. Moreover, it investigated whether *Cymbopogon citratus* applied to the surface of fertilized chicken eggs could improve embryonic survival, hatching performance, early growth traits, and spleen-associated immune responses in chicks incubated under thermal stress conditions. Applying *Cymbopogon citratus* to chicken eggs before they hatch, especially when the eggs are under heat stress, offers a natural and affordable alternative to improve hatch rates and produce stronger chicks. It will help to achieve both SDG 12 (Responsible Consumption and Production) and SDG 15 (Life on Land) because sustainable poultry practices that emphasize animal welfare and increase the resilience of farming systems to climate change will be developed. It also minimizes the waste generated in production because it promotes a green approach towards chicken production.

MATERIALS AND METHODS

Materials

A total of 90 *Gallus gallus domesticus* fertilized eggs were utilized, including 60 treated (sprayed with 25%, 50%, 75%, and 100% of *Cymbopogon citratus* extract), 15 in control group (sprayed with distilled water) and 15 with no application. Two incubators were utilized for incubation, and candling flashlight was used to determine the pre-hatched mortality rates of the embryos while post-hatch development was evaluated by measuring various parameters at hatch and at two (2) weeks old.

Research Design

The study used a quantitative experimental design aimed to determine the developmental effect of in ovo surface application of *Cymbopogon citratus* on embryonic mortality, hatchability, blood vessel density, post-hatch physiological traits, and spleen index of *Gallus gallus domesticus* in normal (37.8°C) and heat-stress (39.0°C) conditions (Creswell, 2018). The fertilized eggs were subjected to *Cymbopogon citratus* extract at (25%, 50%, 75% and 100%0 (Abuoghaba *et al.*, 2021) and distilled water and no-application as controls. 90 eggs were randomly put into treatment groups and incubated for 19-21 days. Three (3) independent trials were used to carry out the experiment in order to maintain consistency and reliability.

Procedure

Preparation

The preparation for the in ovo surface application began by weighing 90 one-day-old eggs. The eggs were then sanitized by gently rubbing 95% ethanol using a clean cloth (Selim *et al.*, 2025). The eggs were divided into three groups: control, chronic incubation, and no application group. These groups were then labeled with identification tags. Fresh *Cymbopogon citratus* stalks were cleaned under running tap water, cut into 16 cm lengths, and chopped into small pieces. A 490g portion of the sample was evenly spread on aluminum foil placed on trays and air-dried under direct sunlight for approximately 12 hours, followed by room-temperature drying overnight (total drying time of 24 hours). The dried samples were ground into fine powder for 30 seconds using a mill grinder, then stored in sealed polyethylene bags at 3.9–4.1°C (Hashim *et al.*, 2019). The extract was prepared by mixing the powdered

lemongrass with ethanol and concentrating it to 25 mL using rotary evaporation at 60°C for 2–4 hours with a volume setting of 500 mL. Different concentrations of the extract were prepared by dissolving the lemongrass extract with distilled water: 25%(25LG:75H2O), 50% (50LG:50H2O), 75% (75LG:25H2O), and 100% (100LG:0H2O). The prepared extracts were stored in 100 mL spray bottles at a controlled temperature.

Application Timing and Thermal Stress Induction

From day one to five (1-5) of incubation, all eggs were maintained at the standard incubation temperature of 37.8°C. Beginning on day six (6) until day eight (8), the treatment incubator assigned to the chronic incubation group was programmed to gradually increase from 37.8 °C up to 39.0°C. The temperature was held for three hours and was then gradually reduced back to 37.8 °C to simulate thermal stress. After the three-hour heat exposure, eggs in the treatment group were removed from the incubator and left to transition to room temperature for about 30 minutes. After this transition period, the eggs were sprayed with distilled water or *Cymbopogon citratus* solution (pre-warmed to 37.8 °C) until evenly coated, then returned to the same incubator at 37.8 °C. The same process was repeated on days seven and eight. From day nine until the end of the incubation period, the normal temperature (37.8°C) was maintained. The control group undergoes the same spraying process but will receive only distilled water treatment.

Vessel Density

On day 7 of incubation, eggs exposed to thermal stress were candled in a dark room to observe embryonic blood vessel development. The vascular images were obtained and examined with ImageJ software to calculate the vascular area or Region of Interest (ROI). The ratio of vessel to total ROI pixels was determined as vessel density Eq. 1 (National Research Council, Institute for Laboratory Animal Research, 1996):

Embryonic Mortality During Incubation and Hatchability at Hatch

$$\text{Vessel Density} = \frac{\text{Vessel Pixel}}{\text{ROI Pixels}} \times 100 \quad \text{[Eq. 1]}$$

Embryonic mortality was measured on day 18 of the incubation, 3 days before the presumed hatching, through candling using a flashlight underneath each egg. During candling, the presence or absence of blood vessels, embryo movement, and overall development were assessed. Eggs showing no signs of movement were classified as non-viable. To ensure accuracy and maintain ethical standards, unhatched eggs were opened only after the hatching period ended to confirm the developmental stage of the embryo without causing unnecessary harm (Bevans *et al.*, 2024). The mortality rate was calculated as shown in Eq.2.

Hatchability was determined on day 21, representing the expected hatch day, and expressed as the percentage of fertile eggs that successfully produced live chicks. It was computed using the formula Eq.3 (Ahtsu *et al.*, 2015). It was calculated as:

$$\text{Embryonic mortality (\%)} = \frac{\text{number of non-viable embryos}}{\text{total fertile eggs.}} \times 100 \quad \text{[Eq. 2]}$$

$$\text{Hatchability rate (\%)} = \frac{\text{number of hatched chicks}}{\text{total fertile eggs}} \times 100 \quad \text{[Eq. 3]}$$

Post-Hatch Physiological Trait Measurement

A standardized subsampling approach was used to monitor all the viable chicks in each treatment in three separate trials. Healthy hatchlings were chosen randomly and allocated identification numbers, whereas dead hatchlings were counted separately according to ethical procedures to determine late deaths (Ding *et al.*, 2024). The measurements of the physiological traits were taken at three (3) and 17 days after hatching using calibration tools to assess the early life growth impact. The data in the treatment ID, trial, replicate, hatch status, and measurement were carefully entered to be analyzed (Hashim *et al.*, 2019).

After incubation, a total of five randomly selected chicks per treatment were used for morphometric measurements, including: (1) body weight, measured by placing each chick individually on a calibrated digital scale; (2) body length, measured from the top of the head to the tip of the tail using a measuring tape; (3) chest circumference, obtained by wrapping a measuring tape around the chest at the top of the pectus, just behind the wings and around the neck; (4) keel length, measured from the base of the sternum to the tip of the keel bone along the center of the chest using a flexible measuring tape; (5) shank length, measured from the top of the hock joint down to the foot pad; and (6) wingspan, measured by gently restraining the chicks and extending the wings carefully to ensure accurate measurement using a flexible measuring tape without causing stress or injury.

Immune Organ Index

The spleen index analysis was done using the spleen of three (3)-week-old chicks per treatment group that were collected at the City Slaughterhouse of Koronadal. The spleen of every animal was removed and stripped of connective tissue, dried on a towel, and weighed on a digital scale (Fuseini *et al.*, 2023). The weight of the spleen was then computed by the use of the equation shown below:

$$\text{Immune organ index (g/kg)} = \frac{\text{weight of immune organ (g)}}{\text{live body weight (kg)}} \quad \text{[Eq. 4]}$$

Data Analysis

The research study examined embryonic mortality, hatchability, and post-hatch morphometric data using one-way analysis of variance (ANOVA) in JASP statistical software. ANOVA was used at the $\alpha = 0.05$ level of

significance to assess whether there was a significant difference between the treatment groups. In case a high F-value was observed, the Tukey Honestly Significant Difference (HSD) post hoc test was used to determine specific group differences, controlling the risk of Type I error of multiple comparison (McHugh, 2011) This statistical methodology provided a good and stable assessment of the impact of *Cymbopogon citratus* when subjected to chronic incubation and thermal stress. Findings were tabulated, and windows of comparative findings were made between the chronic incubation and control groups.

Ethical Consideration

The welfare of eggs and chicks was given priority in this study through the ethical practice to reduce stress and discomfort. The research adviser and school principal were informed of the research and the 3Rs (Replacement, Reduction, Refinement) principle helped to use minimal and humane treatment to animals. Euthanasia was performed by trained professionals through a certified procedure and biosafety and animal welfare principles were upheld in all laboratory procedures. The accuracy and ethicality of data collection were ensured, and the waste management was adequate and no conflicts of interest were announced.

RESULTS AND DISCUSSION

Embryonic Mortality and Hatchability Under Thermal Stress

Table 1: Embryonic Mortality and Hatchability of Chicken Eggs Treated with Lemongrass Under Thermal Stress Conditions

Treatment	Fertile Eggs, n=15			
	Embryonic Mortality (n)	Mortality Rate (%)	Hatched Chicks (n)	Hatchability Rate (%)
No Application	7	46.67%	8	53.33%
Water Control	5	33.33%	10	66.67%
25%	8	53.33%	7	46.67%
50%	7	46.67%	8	53.33%
75%	5	33.33%	10	66.67%
100%	1	6.67%	14	93.33%

Embryonic death rates and hatching success of chicken eggs subjected to heat stress differed among treatment groups. As shown in Table 1, the 100% treatment group recorded the highest embryonic death rate of 6.67% and the highest hatching success of 93.33%, suggesting improved incubation results, while the 25% treatment group showed the worst embryonic mortality rate of 53.33% and the lowest hatching rate of 46.67%, indicating low resistance to stress. These findings indicate that embryo survival could be enhanced with higher doses of treatment, as minimal doses may be inadequate to provide protection during incubation, whereas higher treatment levels may improve survival and hatchability by strengthening resistance to heat stress (Brannan *et al.*, 2026).

Embryonic Vascular Development

Vessel density varied across treatment groups but

Table 2: Effects of Lemongrass Surface Application on Embryonic Vessel Density Under Thermal Stress Conditions

Parameter	**No Application**	**Water (Control)**	**Treatments (Chronic)**				**F**	**P**	**p2**
			25%	**50%**	**75%**	**100%**			
Vessel Density	72.28 ± 2.988	80.17 ± 11.87	80.54 ± 5.910	67.14 ± 10.31	73.55 ± 9.902	85.65 ± 4.122	2.018	0.148	0.457

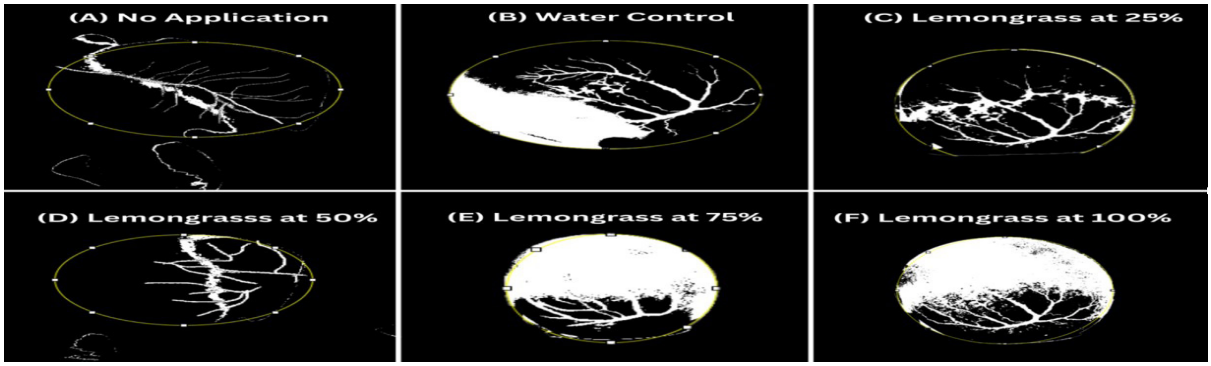

Figure 1: Representative Embryonic Vascular Development Under Different Treatments
Note. Images were captured on day 7 of incubation and analyzed using ImageJ to quantify vessel density

remained within normal developmental ranges, as shown in Table 2. The 100% had the highest vessel density of 85.65 ± 4.12, while the 50% had the lowest mean of 67.14 ± 10.31. These findings indicate that the resulting p-value of 0.148 is not significant. These findings are inversely related to previous studies that reported lemongrass supports healthy vessel formation because it protects endothelial cells, which are cells involved in forming blood vessels (Silva & Barbara, 2022). Moreover, essential oils used in embryonic treatments do not induce vascular abnormalities when applied at appropriate concentrations (Oliveira *et al.*, 2023). Hence, the presented results do not support the hypothesis that lemongrass extract facilitates normal and healthy blood vessel formation in developing Rhode Island Red embryos; however, it also does not induce negative effects.

Table 3: Early Post-Hatch Morphometric Traits of Chicks at Day 3

Morpho metrics	No Application	Water (Control)	Treatments (Chronic)				F	P	p2
			25%	50%	75%	100%			
Body Weight (g)	40.10 ± 4.095a	40.44 ± 2.287a	39.43 ± 2.299a	38.89 ± 3.756a	39.70 ± 1.767a	39.80 ± 2.898a	0.289	0.917	0.029
Body Length (cm)	11.00 ± 0.667a	11.89 ± 0.333ab	12.43 ± 1.397b	12.11 ± 0.782b	10.80 ± 0.789a	11.80 ± 0.919ab	5.167	<0.05	0.345
Chest Circumfe rence (cm)	10.50 ± 0.850a	9.889 ± 0.928a	10.14 ± 1.345a	9.778 ± 1.202a	10.70 ± 1.160a	10.20 ± 0.632a	1.119	0.363	0.102
Keel Length (cm)	4.000 ± 0.000a	4.000 ± 0.000a	5.286 ± 1.113b	5.444 ± 1.333b	4.700 ± 0.483ab	4.600 ± 0.516ab	6.212	<0.05	0.388
Shank Length (cm)	4.550 ± 0.599a	5.065 ± 0.464ab	5.571 ± 0.607b	5.778 ± 0.916b	5.050 ± 0.956ab	4.350 ± 0.474a	5.693	<0.05	0.367
Wingspan (cm)	5.150 ± 1.203a	4.889 ± 0.601a	6.786 ± 1.075b	6.378 ± 1.150b	5.950 ± 0.438ab	5.300 ± 1.059a	5.101	<0.05	0.342

Note. Means within the same row with different superscript letters differ significantly ($p < 0.05$).

Post - Hatch Growth and Morphometric Traits

Post-hatch morphometric measurements shown in Table 3 revealed that the application of *Cymbopogon citratus* during days 6–8 under 39°C did not significantly affect chick body weight and chest circumference on day 3, with the highest body weight in the water control group (40.44 g) and the lowest in the 50% treatment group (38.89 g). Body length and wingspan were highest in the 25% treatment group (12.43 cm and 6.786 cm, respectively) and lowest in the no-application control, while keel and shank lengths were longer in treated groups than in controls ($p < 0.05$), indicating a response in skeletal development. Large effect sizes were observed for keel length (38.8%), shank length (36.7%), body length (34.5%), and wingspan (34.2%), whereas chest circumference showed a medium-to-large

Table 4: Post-Hatch Growth and Morphometric Traits of Chicks at Day 17

Morpho metrics	No Application	Water (Control)	Treatments (Chronic)				F	P	p2
			25%	50%	75%	100%			
Body Weight (g)	129.0 ± 15.84c	117.2 ± 14.47c	171.3 ± 30.03a	163.5 ± 23.23a	65.0 ± 26.66d	130.4 ± 18.77b	9.765	<0.05	0.494
Body Length (cm)	20.50 ± 1.439a	20.33 ± 1.369a	20.94 ± 1.474a	19.79 ± 1.912a	21.15 ± 1.794a	19.50 ± 1.544a	1.726	0.146	0.147
Chest Circumfe rence (cm)	16.00 ± 0.707a	15.83 ± 1.677a	16.64 ± 1.887a	17.00 ± 1.753a	17.40 ± 1.174a	17.09 ± 1.181a	1.85	0.120	0.156
Keel Length (cm)	10.75 ± 0.886c	10.78 ± 0.667c	11.79 ± 1.468b	12.25 ± 0.707a	12.15 ± 1.292a	10.71 ± 1.267c	4.186	<0.05	0.295
Shank Length (cm)	7.125 ± 0.443c	7.444 ± 0.527b	8.071 ± 1.018a	8.688 ± 0.594a	7.740 ± 0.959b	7.714 ± 0.752b	4.192	<0.05	0.295
Wingspan (cm)	16.88 ± 0.680b	16.41 ± 1.283c	17.13 ± 1.538b	17.88 ± 1.382a	17.52 ± 1.322ab	16.18 ± 1.310c	2.59	0.037	0.206

Note. Means within the same row with different superscript letters differ significantly ($p < 0.05$).

effect (10.2%) and body weight a small effect (2.9%). Post-hoc results in Table 3 showed that body weight and chest circumference did not differ significantly among treatments, while body length, wingspan, keel length, and shank length were generally higher in the 25% and 50% treatment groups, lowest in the no-application and 100% groups, and intermediate in the water and 75% treatments, suggesting that moderate levels of *Cymbopogon citratus* may enhance early skeletal development without affecting overall body weight.

As shown in Table 4, on day 17 post-hatch, the effects of treatment became more evident. Body weight differed significantly among treatments ($p < 0.05$), with the highest observed in the 25% group (171.3 g) and the lowest in the 75% group (65.0 g), possibly due to reduced food intake and dietary aversion over the 2 weeks. In contrast, body length ($p = 0.146$) and chest circumference ($p = 0.120$) did not differ significantly, while keel ($p = 0.003$), shank ($p = 0.003$), and wingspan ($p = 0.037$) varied significantly, indicating a response to spray exposure. The 50% treatment showed the largest effect sizes for skeletal traits ($\eta^2 = 20.6\%–29.5\%$).

Post-hoc data showed that body weight, keel length, and shank length were higher in the 25% and 50% treatments, lowest in the 75% group, while wingspan was widest in 50%, lowest in the controls and 100%, and intermediate in 25% and 75%; body length and chest circumference showed no significant differences. These results indicate that moderate lemongrass exposure enhances skeletal and appendicular growth, while control groups shows lower values due to stress vulnerability. This aligns with recent studies demonstrating that early-life interventions, such as incubation therapies, can influence post-hatch skeletal and growth outcomes, with bone and muscle responses being dose- and time-dependent (Such *et al.*, 2023). This finding shows that lemongrass strengthens skeletal development without significantly affecting body weight, consistent with studies showing herbal additives modulate bone and tissue growth more than overall mass (Iraqi *et al.*, 2023 & Mukarram *et al.*, 2022).

Table 5: Effect of Different Treatment Concentrations on Relative Spleen Weight

Parameter	No Application	Water (Control)	Treatments (Chronic)				F	P	p2
			25%	50%	75%	100%			
Spleen Weight	0.918 ± 0.054	1.379 ± 0.575	1.237 ± 0.073	0.777 ± 0.252	1.462 ± 0.013	1.785 ± 1.125	1.471	0.27	0.38

Table 5 shows that the relative spleen weight was different across treatments, with the lowest at 50% *Cymbopogon citratus* group (0.777 ± 0.252) and highest at 100% (1.785± 0.575), with the no-application group (0.918 ± 0.054) being the baseline development and the water control group (1.379 ± 0.575) showing a moderate increase. Although the differences were not significant ($p = 0.27$), the large effect size ($\eta2 = 0.38$) indicates that treatment concentration was a significant source of variance in spleen weight. The increased spleen weights in the 75% and 100% groups may indicate that this primary lymphoid organ is more developed, which might indicate physiological but not pathological hypertrophy, and agrees with earlier findings that the organ weight can be used as a preliminary morphometric measure of immune status (DeChant *et al.*, 2021). On the other hand, the smaller weight at 50% could be the normal biological variation or less development of the lymphoid tissue. These findings suggest that increased levels of lemongrass could stimulate immune organ growth in chicks, which could increase early immune capacity and resilience, although the effect can change with species and dose.

CONCLUSION

Surface application of *Cymbopogon citratus* in ovo during thermal stress produces varied effects on hatching performance and post-hatch morphometric characteristics. Eggs treated with lemongrass exhibit lower embryonic mortality and higher hatchability compared with untreated eggs, which shows increased mortality and reduced hatchability. Phenotypic analysis indicates that although the extract does not significantly affect body weight, chest circumference, or blood vessel density, it has notable effects on skeletal development, resulting in longer keel, shank, and wingspan. While the treatment effect on spleen index is not statistically significant, the observed tendency toward higher spleen mass at greater concentrations may suggest potential enhancement of immune organ development. These findings indicate that in ovo application of *Cymbopogon citratus* may improve embryonic survival while selectively supporting post-hatch skeletal growth and immune organ development.

Recommendations

Physiological measures of stress, corticosterone, and oxidative stress biomarkers should be used in future studies to comprehend embryonic and post-hatch responses to stress. Other interventions such as non-invasive egg treatment, egg incubation management, or post-hatch nutrition ought to be assessed in relation to their impacts on hatchability, immune system performance, and development performance. Varying levels of exposure, timing, and duration are also encouraged through experimental designs in order to determine the best conditions for development. Immune

organs and physiological responses to various stressors, including humidity, stocking density, and nutritional stress, should also be studied. Also, it is advisable to consider botanical or nutritional interventions to improve skeletal development, vascular stability, and tolerance to stress. Lastly, evaluation of alternative chicken breeds has the potential to determine genetic effects on growth and development, which will assist in supporting livelier and sustainable poultry farming.

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