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Research Article

Interactive Effects of Egg Mass and Pre-hatch Injection of Alcoholic Date Palm Pollen Extract on Hatch Traits of Broiler Chickens (Ross 308)

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Abstract | This study aimed to evaluate the effects of egg mass and pre-hatch injection of alcoholic extract of date palm pollen (*Phoenix dactylifera* L.) on hatching performance in Ross 308 broiler chickens. A total of 1,000 fertile eggs (38 weeks of age) were divided into two weight groups: Group 1 (61 ± 2 g) and Group 2 (55 ± 2 g). Each group was further divided into five treatments (100 eggs each): Untreated control (T1), normal saline control (T2), and pollen extract at concentrations of 125, 150, and 175 µg/mL (T3–T5). Injections (0.2 mL) were administered into the air cell prior to incubation. The results showed that egg weight had a significant effect ($P < 0.05$) on some hatch traits. Heavy eggs in Group 1 showed higher chick-to-egg weight percentage (71.43%) compared with light eggs in Group 2 (69.96%), lower egg weight loss during incubation (9.45% vs. 10.21%), and higher chick weight at hatch (43.72 g vs. 38.81 g). However, egg weight had no significant effect ($P > 0.05$) on hatchability percentage (85.59% vs. 87.33%), total embryonic mortality (7.46% vs. 5.82%), or chick length at hatch in Group 1 and Group 2, respectively. Regarding treatments, significant differences ($P < 0.05$) were observed in hatchability percentage, where T3 (125 µg/mL) showed the lowest hatchability (82.50%) compared with T1, which recorded the highest value (90.00%). Lower egg weight loss was observed in T3 (9.37%) compared with T2 (10.22%). In addition, T3 exhibited the highest embryonic mortality (10.00%) compared with T1 and T4, which showed lower values (5.00% and 4.17%, respectively). However, T2 showed a significant reduction ($P < 0.05$) in chick weight at hatch (40.91 g) compared with T1, T4, and T5 (41.38 g, 41.39 g, and 41.43 g, respectively). The interaction between egg weight and treatments showed significant effects ($P < 0.05$), with variations observed in egg weight loss, embryonic mortality, and chick-to-egg weight percentage. However, no consistent improvement was observed that clearly demonstrated a dose-dependent response to the different extract concentrations. The results demonstrated that egg weight significantly affected chick weight and some hatch traits, while alcoholic date palm pollen extract showed no clear beneficial effect. The interaction between egg weight and treatments was inconsistent.

Keywords | Date palm, Pollen extract, Pre-hatch, In Ovo injection, Hatch traits, Egg mass

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INTRODUCTION

Hatchability and productive traits of broiler chickens are influenced by both genetic and environmental factors. Among these, internal and external egg quality traits are particularly important, and egg weight is a key parameter associated with egg quality due to its effect on hatchability and broiler performance (Ulmer-Franco *et* *al*., 2010; Khalil *et al*., 2022). A strong relationship exists between egg weight and hatchability traits, as it influences hatchability percentage, incubation duration, embryonic mortality rate, and chick weight at hatch (Alabi *et al*., 2012; Ajafar *et al*., 2024). Some studies have reported that higher egg weights may reduce fertility and hatchability; however, this effect is often associated with breeder age rather than egg weight itself (Iqbal *et al*., 2016).

Recent research has focused on improving embryo weight and immunity at hatching through in ovo feeding techniques, which enhance embryonic nutrition and compensate for nutrient losses during incubation. Providing essential nutrients directly to the embryo can improve development and hatch performance, contributing to sustainable poultry production (Du *et al*., 2025; Arkan *et al*., 2025). However, a major limitation of this approach is the potential reduction in hatchability due to the injection process, which is generally undesirable in commercial incubation. Therefore, it is important to optimize in ovo supplementation strategies that do not negatively affect hatchability. Several studies have evaluated the effects of injecting nutritional compounds into eggs, but results remain inconsistent, with many reporting reduced hatchability (Al-Shammari, 2025).

Various nutritional components have been shown to enhance embryonic growth and weight (Ma *et al*., 2020; Dos *et al*., 2010). Nevertheless, the exploration of novel in ovo feeding substances remains essential for improving embryonic development. Date palm pollen, the male reproductive structure of the date palm, has traditionally been used in folk medicine and was once referred to as the "Fountain of Youth" due to its reputed health benefits (Okai *et al*., 2025).

Several studies have demonstrated that date palm pollen possesses medicinal properties attributable to its high protein content, which supports muscle and tissue development. It also contains antioxidants and healthy fats that help protect against oxidative stress and maintain physiological health (Salhi *et al*., 2024).

Due to these properties, date palm pollen has been used as a supplement in poultry production. Studies have reported that it can enhance egg production and improve egg quality in laying hens, likely due to its phytoestrogen (estradiol-like) compounds that regulate reproductive functions and improve production efficiency (Mousa *et al*., 2018). Additionally, date palm pollen has been used in in ovo applications to enhance embryonic development, hatchability, and chick weight at hatch (Coşkun *et al*., 2014; Salman *et al*., 2025; Alsudani *et al*., 2025).

Therefore, this study aimed to investigate the effects of egg weight and in ovo injection of alcoholic extract of date palm pollen on hatchability traits of Ross 308 broiler chickens.

MATERIALS AND METHODS

Ethical approval

The present research was performed in compliance with the principles of scientific research ethics and within the rules and under the observation and approval of the Research Ethics Committee of the Animal Production Department, College of Agricultural Engineering Sciences, University of Baghdad. The hatching eggs used in this experiment were incubated in accordance with the official management guide of the Ross 308 strain, with a suitable environmental condition inside the incubator, taking into consideration the safety of the embryo and avoiding any harm not justified by a cause. Official approvals were obtained before the beginning of the experiment.

Experimental design

A total of 1,000 hatching eggs from 38-week-old Ross 308 broiler breeder hens were obtained from Al-Baraka Hatchery in Wasit Province. The eggs were divided into two groups based on egg weight. The first group consisted of large eggs with an average weight of 61 ± 2 g, while the second group consisted of small eggs with an average weight of 55 ± 2 g. Each group contained 500 eggs.

Each weight group was further subdivided into five treatment groups, with 100 eggs per treatment, as follows: T1, control (no injection); T2, positive control (injection with normal saline); T3, injection with alcoholic extract of date palm pollen (125 μg/mL); T4, injection with alcoholic extract of date palm pollen (150 μg/mL); and T5, injection with alcoholic extract of date palm pollen (175 μg/mL).

Preparation of the extract and injection procedure

Palm pollen grains were obtained from a date palm orchard located in Al-Nu'maniyah District, Wasit Province. The pollen was separated from its protective sheath by sieving, and the fine pollen powder was collected. The alcoholic extract of the palm pollen was prepared at the laboratories of the Ministry of Science and Technology/ Directorate of Environment and Water.

The extraction process was carried out following the method described by Basuny *et al*. (2013), using 100 g of palm pollen mixed with 500 mL of 80% ethanol in a 1000 mL glass flask at room temperature. The mixture was stirred thoroughly using a magnetic stirrer for 24 hours. Afterward, the solution was filtered using filter paper and a Buchner funnel connected to a vacuum pump. The filtrate was then transferred to a centrifuge operating at 3000 rpm for 10 minutes to obtain a clear solution.

Table 1: The active compounds present in date palm pollen extract (ppm).

Active compounds (ppm)	B-sterol	Stig-ma-sterol	Gallic acid	Ferulic acid	Rutin	Apigenin	Syringeic acid	Qurcetine	caffeic acid
date palm pollen extract	9.08	17.90	98.0	60.3	87.4	64.9	70.8	80.2	74.9

The resulting supernatant was dried using a rotary evaporator, yielding sufficient quantities of dry extract, which were stored in dark-colored containers at refrigeration temperature until used for preparing injection dilutions.

Sterol and phenolic compounds present in the prepared extract were quantified (Table 1). β-sitosterol and stigmasterol were estimated according to the method of (Maji *et al*., 2014), while phenolic compounds were determined following the procedure of (Mradu *et al*., 2012). Identification of compounds was based on comparing the chromatographic profiles of standard solutions obtained by HPLC with those of the extract, using retention time alignment.

The dilutions were prepared at the laboratories of the College of Agricultural Engineering Sciences, University of Baghdad. The required quantities of the extract for each treatment (25, 30, and 35 mg) were weighed using a precision electronic balance. Each quantity was then mixed with 200 mL of normal saline to prepare the target concentrations for the experimental treatments (125, 150, and 175 µg/ml, respectively). The solutions were homogenized using a magnetic stirrer. The prepared solutions were then stored in brown glass bottles under refrigeration until use. The treated eggs were injected of the prepared solution 0.2 ml per egg (Al-Hatheel, 2022) once only into the air cell prior to incubation.

Studied hatching traits

- The hatching percentage of the total eggs (%) = (Number of hatched chicks/ Total number of eggs) × 100 (Hussain *et al*., 2020).
- Embryonic mortality percentage (%) = (Number of embryonic mortalities/ Number of fertile eggs) × 100 (Refaie *et al*., 2022).
- Egg weight loss percentage (%) = [(Egg Weight before incubation – Egg Weight at day 18)/ Egg Weight before incubation] × 100 (Tona *et al*., 2022).

Statistical analysis

The data were analyzed using a Completely Randomized Design (CRD) to study the effect of two factors on the experimental units across various traits, employing the Statistical Analysis System (SAS, 2013). Significant differences between means were determined using Duncan's multiple range test (Duncan, 1955).

RESULTS

Chick-to-egg weight and egg weight loss percentage

As showed in Table 2, incubation period (1-18 days) was significantly influenced by egg weight as well as treatment. Highly significant reduction ($p < 0.01$) was noticed in chick to egg weight percentage in Group 2 over Group 1. At the same time, loss of egg weight during incubation was significantly lower in Group 1 (9.45%) as compared to Group 2 (10.21%) ($p < 0.05$). Among the treatments, the differences that proved to be significant were those in the egg weight loss percentage ($P < 0.05$),

Table 2: Effect of egg weight and injection treatment with alcoholic extract of date palm pollen, and their interaction, on the chick to egg weight percentage and egg loss percentage during the incubation period (1-18 day) (mean ± SE).

Effect factors		Chick weight/ egg weight (%)	Egg loss (1- 18 day, %)
Groups	1	71.43±0.35^{a}	9.45±0.14^{b}
	2	69.96±0.38^{b}	10.21±0.20^{a}
Sig.		**	*
Treatments	T1	70.97±0.36	9.81±0.27^{ab}
	T2	69.58±0.87	10.22±0.37^{a}
	T3	70.51±0.38	9.37±0.30^{b}
	T4	71.02±0.61	9.59±0.21^{ab}
	T5	70.72±0.73	10.14±0.32^{ab}
Sig.		N.S	*
Group1	T1	70.92±0.51^{ab}	9.53±0.47^{bc}
	T2	70.87±0.76^{ab}	9.77±0.20^{abc}
	T3	71.23±0.37^{ab}	9.17±0.48^{c}
	T4	72.08±0.34^{a}	9.28±0.05^{c}
	T5	72.06±0.63^{a}	9.47±0.23^{bc}
Group2	T1	71.02±0.63^{ab}	10.09±0.26^{abc}
	T2	68.29±1.25^{c}	10.68±0.66^{ab}
	T3	69.79±0.27^{abc}	9.57±0.41^{bc}
	T4	69.97±0.79^{abc}	9.90±0.35^{abc}
	T5	69.38±0.70^{bc}	10.80±0.14^{a}
Sig.		*	*

Egg loss percentage during the incubation period from day 1 to day 18. *Different superscript letters within the same column indicate significant differences among means ($P < 0.05$). **Different superscript letters indicate highly significant differences among means ($P < 0.01$). N.S. = not significant (P >

0.05).

with the highest value recorded for T2 (10.22%) and the lowest for T3 (9.37%). Non-significant differences were found for the weight percentage of chicks to eggs. The interaction between egg weight and injection treatments showed that T2 in Group 2 had a significantly lowered percentage of chick-to-egg weight compared to the control of the same group and all treatments in Group 1. On the other hand, T5 in Group 2 recorded a significant increase in egg weight loss when compared with T3 within the same group and all treatments of Group 1 except T2.

Table 3: Effect of egg weight and injection treatment with alcoholic extract of date palm pollen, and their interaction, on embryonic mortality percentage during three incubation stages (mean ± SE).

Effect factors		M1 (%)	M2 (%)	M3 (%)	TM (%)
Groups	1	1.53±0.51	1.53±0.51^{a}	2.88±0.69	7.46±1.08
	2	0.86±0.38	0.17±0.17^{b}	3.42±0.75	5.82±0.97
Sig.		N.S	*	N.S	N.S
Treat-ments	T1	0.00±0.00^{b}	0.42±0.42^{b}	2.50±1.01^{b}	5.00±1.41^{b}
	T2	2.23±0.99^{a}	0.00±0.00^{b}	3.13±1.17^{ab}	6.25±1.62^{ab}
	T3	1.25±0.72^{ab}	1.25±0.72^{ab}	5.42±1.46^{a}	10.00±1.94^{a}
	T4	1.25±0.72^{ab}	0.42±0.42^{b}	1.25±0.72^{b}	4.17±1.29^{b}
	T5	1.30±0.75^{ab}	2.17±0.96^{a}	3.48±1.21^{ab}	7.83±1.77^{ab}
Sig.		*	*	*	*
Group1	T1	0.00±0.00^{b}	0.83±0.83^{b}	1.67±1.17^{b}	5.00±2.00^{b}
	T2	3.33±1.65^{a}	0.00±0.00^{b}	3.33±1.65^{ab}	7.50±2.41^{ab}
	T3	0.83±0.83^{ab}	2.50±1.43^{ab}	6.67±2.29^{a}	12.50±3.03^{a}
	T4	2.50±1.43^{ab}	0.00±0.00^{b}	0.83±0.83^{b}	4.17±1.83^{b}
	T5	0.91±0.91^{ab}	4.55±2.00^{a}	1.82±1.82^{ab}	8.18±2.63^{ab}
Group2	T1	0.00±0.00^{b}	0.00±0.00^{b}	3.33±1.65^{ab}	5.00±2.00^{b}
	T2	0.96±0.96^{ab}	0.00±0.00^{b}	2.88±1.65^{b}	4.81±2.11^{b}
	T3	1.67±1.17^{ab}	0.00±0.00^{b}	4.17±1.83^{ab}	7.50±2.41^{ab}
	T4	0.00±0.00^{b}	0.83±0.83^{b}	1.67±1.17^{ab}	4.17±1.83^{b}
	T5	1.67±1.17^{ab}	0.00±0.00^{b}	5.00±2.00^{ab}	7.50±2.41^{ab}
Sig.		*	*	*	*

Embryonic mortalities

Embryonic mortality was determined at three levels of incubation: Early incubation (M1: 1-7 days), mid-incubation (M2: 7-14 days), and late incubation (M3: 14-21 days). Analysis of the results presented in Table 3 indicates that egg weight did not significantly influence embryonic mortality in the early (M1) and late (M3) incubation periods; total embryonic mortalities in the two weight categories were not significantly different. But in the mid-incubation stage (M2), there was a significant ($P < 0.05$) reduction in embryonic mortality in Group 2 relative to that in Group 1. Concerning the impact of treatment, there were significant differences ($P < 0.05$) among the variables regarding total embryonic mortality (TM). A higher rate was recorded under treatment T3 (10.00 %), and this was significantly different from treatments T1 and T4, with values of 5.00 % and 4.17 %, respectively. Interaction analysis showed that T3 in Group 1 recorded the significantly higher rate of mortality for stages compared with T1 and T4 within the same group, and also compared with T1, T2, and T4 in Group 2. There were no significant differences among other treatment levels.

Dead and live pipped eggs

As presented in Table 4, no significant differences were observed between the two groups in the percentages of dead pipped eggs (PD) or live pipped eggs (PL) ($P > 0.05$). This indicates that egg weight did not influence the proportion of embryos that failed to emerge or those that successfully pipped but failed to hatch. Similarly, no significant differences were detected among treatments for either PD or PL, suggesting that in ovo injection with alcoholic extract of date palm pollen did not affect hatchability during the pipping stage. The interaction between egg weight and treatment was also not significant, indicating that neither egg size nor extract concentration had a measurable effect on the proportion of dead or live pipped chicks. Overall, the consistently low PD and PL values across all treatments reflect uniform incubation performance. This may be attributed to environmental and genetic uniformity, which likely minimized late-stage embryonic losses.

Table 4: Effect of egg weight and treatment with alcoholic extract of date palm pollen, and their interaction, on the percentages of dead and live pipped eggs (mean ± SE).

Effect factors		PD	PL
Groups	1	1.02±0.41	0.51±0.29
	2	0.86±0.38	0.51±0.30
Sig.		N.S	N.S
Treatments	T1	0.38±0.59	1.25±0.72
	T2	0.45±0.45	0.45±0.45
	T3	1.67±0.83	0.42±0.42
	T4	1.25±0.72	0.00±0.00
	T5	0.43±0.43	0.43±0.43
Sig.		N.S	N.S
Group1	T1	0.83±0.83	1.67±1.17
	T2	0.83±0.83	0.00±0.00
	T3	2.50±1.43	0.00±0.00
	T4	0.83±0.83	0.00±0.00
	T5	0.00±0.00	0.91±0.91
Group2	T1	0.83±0.83	0.83±0.83
	T2	0.00±0.00	0.96±0.96
	T3	0.83±0.83	0.83±0.83
	T4	1.67±1.17	0.00±0.00
	T5	0.83±0.83	0.00±0.00
Sig.		N.S	N.S

PD = Percentage of dead pecking chicks, PL = Percentage of live pecking chicks, N.S = no significant differences between means

(P> 0.05) within one column.

Hatchability percentage and chick's weight and length at hatch

As shown in Table 5, egg weight had no significant effect on hatchability percentage (P > 0.05), as similar hatch rates were observed in heavier eggs of Group 1 (85.59%) and lighter eggs of Group 2 (87.33%). However, a significant difference was observed between groups in chick weight at hatch (P < 0.05), with heavier chicks recorded in Group 1 (43.72 g) compared with Group 2 (38.81 g). Chick length at hatch was not significantly influenced by egg weight.

Regarding treatments, hatchability was significantly higher in T1 (90.00%) compared with T3 (82.50%), while no significant differences were observed among the remaining treatments. Chick weight was also significantly affected by treatment (P < 0.05), with T2 showing a noticeable reduction compared with most other treatments, except T3.

Table 5: Effect of egg weight and treatment with alcoholic extract of date palm pollen, and their interaction, on hatching percentage, chick weight, and chick length at hatch (mean ± SE).

Effect factors		Hatchability(%)	Chick's weight at hatch)gm)	Chick's length at hatch(cm)
Groups	1	85.59± 1.45	43.72±0.05 ^{a}	18.36±0.05
	2	87.33±1.38	38.81±0.10^{b}	18.21±0.05
sig		N.S	*	N.S
Treatments	T1	90.00± 1.94^{a}	41.38±1.07^{a}	18.40±0.05
	T2	84.82 ±2.40^{ab}	40.91±1.13^{b}	18.21±0.07
	T3	82.50± 2.46^{b}	41.22±1.09^{ab}	18.28±0.06
	T4	89.17 ± 2.01^{ab}	41.39±1.10^{a}	18.30±0.09
	T5	85.65 ± 2.23^{ab}	41.43±1.12^{a}	18.25±0.13
Sig.		*	*	N.S
Group1	T1	90.83± 2.65^{a}	43.76±0.05^{ab}	18.43±0.07
	T2	81.67±3.55^{bc}	43.43±0.01^{b}	18.28±0.09
	T3	77.50±3.83^{c}	43.65±0.05^{ab}	18.34±0.05
	T4	90.00±2.75^{ab}	43.84±0.09^{ab}	18.48±0.04
	T5	88.18±3.09^{ab}	43.93±0.12^{a}	18.29±0.22
Group2	T1	89.17±2.85^{ab}	39.00±0.30^{c}	18.37±0.09
	T2	88.46±3.15^{ab}	38.39±0.20^{d}	18.14±0.12
	T3	87.50±3.03^{ab}	38.79±0.08^{cd}	18.21±0.09
	T4	88.33±2.94^{ab}	38.95±0.22^{c}	18.12±0.09
	T5	83.33±3.42^{abc}	38.92±0.12^{c}	18.21±0.17
Sig.		*	*	N.S

*Different superscript letters within the same column indicate significant differences among means (P < 0.05). N.S. = not significant (P > 0.05).

No significant differences were observed in chick length among treatments (P > 0.05). The interaction between treatment and egg weight showed that T3 in Group 1 had lower hatchability compared with most other treatment combinations, except for T2 in Group 1 and T5 in Group 2. For chick weight, treatments within Group 1 exhibited higher values than those in Group 2, indicating a strong association between egg size and chick weight at hatch. In contrast, chick length was not affected by either factor or their interaction, showing no observable interaction effect on this trait.

DISCUSSION

The current study demonstrated that egg weight was the primary factor influencing chick weight and egg weight loss, whereas the effect of in ovo injection of date palm pollen (DPP) extract on hatchability and embryonic mortality was relatively limited. This finding is consistent with previous reports indicating that inherent egg characteristics play a fundamental role in determining embryonic development and chick quality. Larger eggs contain greater nutrient reserves and a larger surface area, which may enhance gas exchange and support water balance during incubation (Grochowska *et al*., 2019). In contrast, smaller eggs, characterized by reduced yolk content and relatively higher rates of water loss, produced chicks with lower chick-to-egg weight percentage, in agreement with Yousaf *et al*. (2024). Differences in egg weight loss between groups may also be associated with variations in eggshell conductance, as suggested by Al-Jebory and Ibrahim (2021), Tona *et al*. (2022), and Al-Saeedi *et al*. (2023).

Although DPP extract is rich in bioactive compounds such as flavonoids, phenolics, and phytosterols, which possess antioxidant and hormonal regulatory properties (Hussain *et al*., 2020), the tested concentrations (125–175 µg/ml) did not significantly improve hatchability. This may be attributed to the sensitivity of avian embryos to the plant-derived compounds. Such exposure may induce osmotic stress, where differences in solute concentration disrupt water balance across embryonic membranes, potentially impairing cellular function. In addition, chemical stress may occur, as excessive bioactive compounds could interfere with metabolic processes (Refaie *et al*., 2022).

The slight increase in embryonic mortality observed at the concentration (T3) suggests that even moderate doses may influence metabolic homeostasis or gas exchange, consistent with findings by El-Kholy *et al*. (2021). Embryonic mortality may also be linked to reduced embryonic efficiency, as embryos with limited physiological capacity are less able to utilize nutrients and tolerate incubation stress, particularly during the late stages of development (Druyan and Levi, 2012). However, the minor reduction in hatchability was not associated with percentages of dead or live pipped eggs or deformities in hatched chicks, indicating that DPP extract did not adversely affect late embryogenesis or neuromuscular coordination during hatching. These findings agree with previous studies reported that embryos developing under optimal incubation conditions generally hatch normally despite minor treatment effects (Yalcin *et al*., 2022; Akosile *et al*., 2023; Oke *et al*., 2021), and that normal chick morphology reflects the physiological safety of the tested concentrations.

CONCLUSIONS

From the results of this research, the fact that egg weight strongly determines the weight of the chicks as well as the egg weight loss can now be affirmed. Despite the fact that the hatchability percentage of eggs was not influenced by the weights of the eggs, the heaviest eggs produced the heaviest chicks and more favorable chick-to-egg weight percentage. The in ovo injection with alcoholic extract of date palm pollen (DPP) did not bring remarkable improvements regarding hatchability. Nevertheless, differences among treatments and control by mortality percentage imply that embryo sensitivity varies with regards to concentration and egg size. Lack of harm caused to chick length and hatched success excludes negative impacts and reveals that none of the DPP doses used had positive effects. Overall, the study proves that the natural properties of the egg, especially weight and the biochemical content, play a more significant role in embryo development and chickes weight at hatched than the external addition of phytochemicals. The selection of eggs and the incubation conditions continue to play the most important critical factors in the production of quality chicks in the broiler production systems. Future studies are required to optimize in ovo injection techniques, establish optimal concentrations of natural bioactive compounds, and determine their molecular effects on embryonic metabolism to improve hatchability and post-hatch performance.

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NOVELTY STATEMENT

This study provides a novel evaluation of the combined effects of egg mass and in ovo injection of alcoholic date palm pollen extract on broiler hatch traits. It reveals that egg weight remains the primary determinant of chick quality, while the tested phytochemical intervention shows no added benefit, offering new insight into the limited efficacy of such applications and guiding future optimization of in ovo strategies.

AUTHOR'S CONTRIBUTION

BSK: Conducted the experiment and collected the data. BMI: Performed the statistical analysis and critically reviewed the article.

AI and AI-assisted technologies use declaration

No generative AI or AI-assisted technology was used in the preparation of this manuscript.

Conflict of interest

The authors have declared no conflict of interest.

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