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Effects of Hatching Egg Weight and In Ovo Injection with the Alcoholic Extract of Date Palm Pollen (*Phoenix dactylifera* L.) on Sex Ratio and Growth Uniformity in Broiler Chicks

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Abstract | The study was conducted to evaluate the effects of hatching egg weight and *in ovo* injection of alcoholic extract of date palm pollen (*Phoenix dactylifera* L.) on the sex ratio and growth uniformity of broiler chicks. The hatching eggs were divided into two groups based on weight 61 ± 2 g and 55 ± 2 g, with each group subjected to five treatments. The treatments include T1 (control with no injection), T2 (positive control where animals were injected with normal saline), T3 (injection with alcoholic extract of date palm pollen at a concentration of 125 μg/ml), T4 (injection with alcoholic extract of date palm pollen at 150 μg/ml) and T5 where animals were subjected to injection with alcoholic extract of date palm pollen at 175 μg/ml. A total of 405 chicks obtained from these treatments were divided into three replicates and reared under standard broiler production conditions (Ross 308) for five weeks. The results showed no significant differences between two egg-weight groups in male count, uniformity percentage, or sex ratio. However, males significantly outperformed females in both number and live body weight across both groups. Significant differences were observed among treatments in male count and body weight, with T4 recording the highest male percentage and T2 the highest male body weight. No significant differences were found in uniformity percentage or sex ratio among treatments. The interaction between egg weight and treatment showed significant effects on male count and body weight but not on uniformity percentage or sex ratio. Overall, males consistently outperformed females in both number and body weight, while uniformity values remained within acceptable limits (below 10%). Taken together, the finding highlights a positive impact of alcoholic extract of date palm pollen (*Phoenix dactylifera* L.) on sex ratio and growth uniformity in broiler chicks.

Keywords | Egg weight, Alcoholic extract, Palm pollen, Uniformity, Sex ratio

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INTRODUCTION

Poultry production plays a vital role in achieving food security and economic sustainability, as it is characterized by rapid growth rate, efficient feed conversion, short rearing periods, and high profitability (Zulkifli et al., 2000; Kadhim *et al*., 2021; Ajafar *et al*., 2024). However, these production traits alone are insufficient to address modern challenges in the poultry industry, where biological and environmental factors may significantly influence productivity and profitability. One of the persistent issues facing poultry production is the sex ratio imbalance in broiler chickens. Deviations in sex ratio, whether an excess of males or females, can lead to notable economic losses due to differences in growth performance and carcass yield (Kaleta and Redmann, 2008; Khalil *et al*., 2022; Al-Saeedi *et al*., 2023).

The economic return from broiler production differs markedly between sexes; male broilers exhibit superior performance traits compared to females. Studies have shown that males surpass females by up to 20–30% in final body weight, and also demonstrate higher carcass yield and reduced abdominal fat percentage (Verapeen and Driver, 2000; Engku and Noraziah, 2000; Hassan, 2011; Salman *et al*., 2024). Consequently, manipulating or balancing sex ratio during embryonic development could have substantial implications for production efficiency.

In avian embryos, the gonads possess the early potential to differentiate into either sex. Sexual differentiation depends primarily on the activity of the aromatase enzyme, which converts testosterone into estrogen during embryonic days five to six (Yoshida *et al*., 1996; Shimada, 1998; Zaki *et al*., 2021). Suppression of aromatase activity can inhibit estrogen synthesis, leading to partial or complete masculinization in genetically female embryos (Shimada, 2002). Natural plant-derived compounds (especially flavonoids)are known as natural aromatase inhibitors (Ibrahim and Abul-Hajj, 1990). These bioactive substances can alter steroidogenesis, potentially influencing sexual differentiation in avian embryos.

Recent studies have reported that certain pollen extracts, such as pine pollen, can alter sex ratio in broiler chicks due to their phytoestrogenic and flavonoid contents (Abad *et al*., 2018). Similarly, date palm pollen (*Phoenix dactylifera L.),* rich in flavonoids, sterols, and saponins, has been shown to enhance reproductive and hormonal performance in poultry and mammals (Al-Shaikh and Al-Farsi, 2021; Laghouati *et al*., 2021). However, there is limited scientific evidence on the effect of *in ovo* injection of date palm pollen extract on embryonic development, post-hatch sex ratio, and growth performance in broilers. Additionally, egg weight plays a significant role in chick quality and post-hatch performance. Heavier eggs generally produce larger and stronger chicks at hatch, which can influence early growth rate and uniformity among the flock (Wilson, 1991; Abudabos *et al*., 2017). Yet, there is a lack of research exploring the interactive effects of egg weight and *in ovo* injection with bioactive plant extracts on sex ratio and growth uniformity in broiler chicks.

Therefore, the present study was designed to evaluate the effects of hatching egg weight and Injecting with the alcoholic extract of date palm pollen on the sex ratio and growth uniformity of broiler chicks. It is hypothesized that increased egg weight and Injecting with the alcoholic extract of date palm pollen may modulate embryonic sexual differentiation and subsequently enhance post-hatch growth uniformity.

MATERIALS AND METHODS

Experiment design

A total of 1,000 hatching eggs obtained from 38-week-old Ross 308 broiler breeder hens at Al-Baraka Poultry Hatchery, Kut District, Wasit Governorate, Iraq, were used in this study. Eggs were divided into two groups based on weight: The first group (61±2 g) and the second group (55±2 g). Each group was further subdivided into five treatments, with 100 eggs per treatment. The treatments were as follows: T1 (control with no injection); T2 (positive control, injected with 0.2 mL/egg of physiological saline); T3 (injected with 0.2 mL of alcoholic extract of date palm pollen at a concentration of 125 μg/mL); T4 (150 μg/mL); and T5 (175 μg/mL). All injections were administered once into the air cell before incubation.

Preparation of the date palm pollen extract

Palm pollen grains were obtained from a date palm orchard located in Al-Numaniyah 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 pollen was prepared in the laboratories of the Ministry of Science and Technology, Directorate of Environment and Water.

The extraction procedure followed the method described by Basuny *et al*. (2013). Briefly, 100 g of pollen powder was mixed with 500 mL of 80% ethanol in a 1,000 mL glass flask and stirred continuously using a magnetic stirrer for 24 hours at room temperature. The mixture was then filtered through Whatman No. 1 filter paper using a Buchner funnel connected to a vacuum pump. The filtrate was centrifuged at 3,000 rpm for 10 minutes to obtain a clear supernatant, which was subsequently concentrated using a rotary evaporator to remove residual solvent. The resulting dry extract was stored in dark-coloured, airtight glass containers at refrigeration temperature (4 °C) until used for preparing the injection dilutions.

Management of chicks

From each group, 405 chicks were hatched and distributed into three replicates per treatment, with 27 chicks per replicate. The chicks were reared in a poultry house measuring 70 × 11 m, using 30 cages (3 × 1 m). Each cage was labelled with its corresponding treatment and replicate number.

Commercial Feedco pelleted feed was provided *ad libitum* throughout the experiment. A crumbled starter ration was fed from day 1 to 15, a granulated grower ration from day

16 to 28, and a finisher ration from day 29 to 35. During the first three days, circular plastic trays (50 cm diameter) and paper sheets were used for feeding. Paper was removed on day 3, and cylindrical hanging feeders were gradually introduced from day 4 and fully replaced the trays by day 7. Feeders were adjusted weekly to match the birds' back height to minimize feed spillage. Inverted drinkers were used during the first week and replaced with hanging bell drinkers thereafter, with height adjusted weekly to ensure proper access and to prevent litter wetting.

Data collection and statistical analysis

Chicks were weighed weekly throughout the experimental period. The number of males and females, average weight per sex, and uniformity percentage were determined during the fourth and fifth weeks. Uniformity percentage was calculated based on the coefficient of variation (CV).

Data were analyzed using SAS statistical software (SAS, 2012). Differences among means were determined using Duncan's multiple range test (Duncan, 1955), and statistical significance was considered at $P < 0.05$.

RESULTS AND DISCUSSION

Weekly uniformity percentage of broiler chicks

Table 1 show that egg weight had no significant effect on flock uniformity percentage, as no differences were detected between the two egg-weight groups throughout the rearing period. Similarly, the *in ovo* injection with the alcoholic extract of date palm pollen (DPP) did not significantly affect uniformity during the first, third, and fifth weeks compared with the control (T1). However, treatment T3 exhibited a significant improvement over both T1 and T5 during the second week and over T5 during the fourth week.

Table 1: Effect egg weight and injection with alcoholic extract data palm pollen on the weekly uniformity of broiler chicken. (mean ± SE).

Effect factors		UF1	UF2	UF3	UF4	UF5
groups	1	9.28±0.68	8.87±0.44	9.00±0.38	10.15±0.39	11.25±0.42
	2	9.50±0.47	9.31±0.35	9.35±0.28	10.60±0.27	12.37±0.85
Sig.		N.S.	N.S.	N.S.	N.S.	N.S.
Treatments	T1	8.12±0.73	8.16±0.29 b	8.95±0.40	10.34±0.55 ab	13.37±2.13
	T2	10.05±0.91	9.63±0.52 ab	9.19±0.35	10.36±0.45 ab	13.39±0.83
	T3	10.68±1.35	10.40±0.33 a	10.19±0.48	11.35±0.31 a	12.04±0.34
	T4	9.20±0.80	9.33±0.69 ab	9.06±0.75	10.43±0.70 ab	10.81±0.47
	T5	8.92±0.46	7.93±0.69 b	8.48±0.40	9.38±0.41 b	10.43±0.40
Sig.		N.S.	*	N.S.	*	N.S.
group1	T1	7.42±0.97	7.81±0.51 bc	8.60±0.29	9.97±0.94 ab	10.94±0.58 ab
	T2	9.47±1.22	10.18±0.14 ab	9.43±0.67	10.35±0.37 ab	12.16±1.73 ab
	T3	11.36±2.73	10.49±0.70 a	9.80±0.78	11.33±0.63 a	12.38±0.60 ab
	T4	9.20±1.22	8.75±0.95 abc	9.15±1.54	10.41±1.35 ab	10.82±1.00 ab
	T5	8.95±0.99	7.14±0.90 c	8.04±0.71	8.67±0.48 b	9.98±0.71 b
group2	T1	8.82±1.11	8.52±0.17 abc	9.31±0.78	10.71±0.73 ab	15.81±4.04 a
	T2	10.62±1.52	9.07±1.00 abc	8.95±0.34	10.38±0.70 ab	12.62±0.61 ab
	T3	10.00±1.11	10.32±0.19 ab	10.58±0.64	11.38±0.58 a	11.71±0.33 ab
	T4	9.19±1.30	9.92±1.08 ab	8.97±0.67	10.46±0.79 ab	10.79±0.35 ab
	T5	8.89±0.24	8.72±0.97 abc	8.93±0.33	10.09±0.35ab	10.89±0.30 ab
Sig.		N.S.	*	N.S.	*	*

Uniformity (UF)= (Standard Deviation / Mean Body Weight) × 100. Group 1 = Chicks produced from eggs with an average weight of 61 ± 2 g, Group 2 = Chicks produced from eggs with an average weight of 55 ± 2 g, T1 = Control treatment, T2 = Positive control treatment, T3 = Treatment of injection with 125 μg/ml alcoholic extract of palm pollen, T4 = Treatment of injection with 150 μg/ml alcoholic extract of palm pollen, T5 = Treatment of injection with 175 μg/ml alcoholic extract of palm pollen, *Different letters within a column mean significant differences between means (P< 0.05), N.S. means that there are no significant differences between the means within column(P<0.05).

The interaction between egg weight and treatment during the fifth week revealed a significant advantage for T1 of Group 2 compared with T5 of Group 1, whereas no significant differences were found among the remaining combinations. These findings indicate that *in ovo* administration of DPP extract did not substantially influence flock uniformity or body-weight averages in either sex. The hypothesized potential for DPP to enhance female growth performance thereby narrowing the male–female growth gap was not evident, likely due to the relatively low extract concentrations used.

According to Vasdal *et al.* (2019), flock uniformity is influenced by multiple interacting factors, including strain, nutrition, management practices, and environmental stability. In this study, the observed coefficient of variation (CV) exceeded 10% in the fifth week, suggesting low uniformity. A CV below 10% is typically considered indicative of a uniform flock (Feddes *et al.*, 2002; Toudic, 2007).

The lack of a consistent improvement in uniformity following DPP administration aligns with evidence that bioactive plant compounds may not reach effective concentrations in embryonic tissues when applied at low *in ovo* doses (El-Kashlan *et al.*, 2015). Additionally, the physiological effects of DPP in vertebrates are mainly associated with endocrine and antioxidant modulation rather than direct growth enhancement (El-Berry *et al.*, 2011). Likewise, Al-Chalabi (2014) found that DPP supplementation improved reproductive hormones but did not significantly alter growth performance under standard feeding conditions.

Sex ratio, average body weight, and uniformity percentage of male and female broilers during the fourth week

As shown in Table 2, significant differences (P < 0.05) were observed between males and females within each group for both numerical proportion and body weight. Males consistently outperformed females in all measured parameters. In Group 1, males accounted for 66.61% with an average body weight of 1,948.04 g, whereas females represented 33.14% with a lower mean body weight of 1,643.25 g. Similarly, in Group 2, males constituted 63.48% (1,906.82 g), while females comprised 36.52% (1,589.54 g). These findings confirm the general pattern those male broilers grow faster and attain higher body weights due to greater anabolic activity and androgenic responsiveness (Khalil *et al.*, 2021).

No significant differences (P > 0.05) were detected in flock uniformity (UF%) or sex ratio between the two egg-weight groups, indicating that egg weight had no measurable influence on embryonic sex determination or post-hatch uniformity. Regarding the effect of *in ovo* treatments, males again demonstrated significant superiority (P < 0.05) over females in both proportion and body weight across all treatments (T1–T5). Male percentages ranged from 60.90% in the control group (T1) to 70.49% in T4, while male body weights varied between 1,914.55 g (T4) and 1,942.87 g (T2). Although the differences in uniformity and sex ratio were not statistically significant, treatments T3 and T4 exhibited numerically higher UF% values, suggesting a slight but non-significant improvement in flock uniformity.

The interaction between egg weight and treatment also displayed significant differences (P < 0.05) in the numbers of males and females, but had no significant effect on sex ratio or uniformity. The highest male proportions were observed in T3 of Group 1 (20.00%) and T4 of Group 2 (18.67%). Males consistently outweighed females in all treatment combinations, although treatment differences in body weight remained statistically non-significant. Uniformity (UF%) was highest in T3 of Group 2 (7.46%), followed by T3 of Group 1 (7.20%), with all coefficients of variation (CV) below 10%, confirming good flock uniformity (Zuidhof *et al.*, 2019).

The overall results suggest that neither egg weight nor *in ovo* administration of date palm pollen (DPP) extract significantly affected sex ratio or flock uniformity. This indicates that the bioactive components of DPP (including flavonoids, phenolic acids, and steroidal compounds) did not influence embryonic sex differentiation under the tested conditions (Abdallah *et al.*, 2023; Refaie *et al.*, 2022). While DPP has been reported to enhance reproductive performance, semen quality, and hormonal balance in mature birds (Al-Samarrae *et al.*, 2020; Saleh *et al.*, 2021), it's *in ovo* application may not deliver effective concentrations to embryonic tissues during critical stages of gonadal development. The observed variation in male proportion is therefore likely due to random egg allocation rather than hormonal modulation. This observation aligns with previous findings that the avian sex ratio is genetically fixed (ZW/ZZ) and generally resistant to manipulation via phytoestrogenic or steroidal agents (Mokarrami *et al.*, 2020; Zhang et al., 2023).

The slightly improved uniformity observed in certain treatments may be attributed to the antioxidant and metabolic-enhancing effects of moderate DPP doses (Refaie *et al.*, 2019; Al-Sharif *et al.*, 2023). However, since no significant improvements were detected, the extract appears to have limited influence on post-hatch growth uniformity or sex differentiation.

Table 2: Effect egg weight and injection with alcoholic extract data palm pollen on the fourth week uniformity of broiler chicken (mean ± SE).

Effect factors			Sex number%	Weight)gm)	UF%	Sex Ratio
Group 1	M		66.61 ± 2.43 a	1948.04±8.23 a	6.75±0.31	2.25±0.25
	F		33.14± 2.37 b	1643.25±13.53 b	5.44±0.63	
Group2	M		63.48±3.29 a	1906.82±17.03 a	6.41±0.47	2.08±0.31
	F		36.52±3.29 b	1589.54±12.40 c	5.56±0.34	
Sig.			*	*	N.S.	N.S.
T1	M		60.90 ± 5.09 a	1930.09±21.76 a	7.21±0.91	1.76±0.33
	F		39.10 ± 5.09 b	1632.32±17.18 b	5.05±0.69	
T2	M		61.73 ± 4.02 a	1942.87±18.32 a	5.99±0.55	1.80±0.34
	F		37.65 ± 3.88 b	1629.66±21.14 b	5.76±0.63	
T3	M		66.05 ± 6.23 a	1917.25±37.17 a	7.39±0.63	2.37±0.51
	F		33.95 ± 6.23 b	1572.39±24.71 b	6.26±0.29	
T4	M		70.49 ± 3.92 a	1914.55±24.25 a	6.98±0.72	2.78±0.61
	F		29.51 ± 3.92 b	1604.13±29.29 b	5.35±1.10	
T5	M		66.05 ± 3.37 a	1932.41±6.60 a	5.59±0.76	2.11±0.34
	F		33.95 ± 3.37 b	1643.48±14.80 b	4.82±0.34	
Sig.			*	*	N.S.	N.S.
Group 1	T1	M	16.67±1.45 abcd	1938.72±34.08 a	6.69±0.43 ab	1.72±0.38
		F	10.33±1.45abcd	1665.74±17.23 b	6.39±2.54 ab	
	T2	M	16.33±1.86 abcd	1973.69±6.68 a	7.01±0.41 ab	1.77±0.55
		F	10.33±1.76 abcd	1669.28±23.30 b	4.66±0.67 ab	
	T3	M	20.00±1.15 a	1935.60±8.85 a	7.20±0.89 ab	3.09±0.70
		F	7.00±1.15 d	1569.09±24.57 b	6.78±0.53 ab	
	T4	M	18.67±0.88 ab	1961.39±14.71 a	7.06±0.43 ab	2.47±0.47
		F	8.00±1.15 cd	1641.28±34.42 b	5.75±1.65 ab	
	T5	M	18.00±1.53 ab	1930.83±13.57 a	5.79±1.16 ab	2.22±0.65
		F	9.00±1.53 bcd	1670.85±7.58 b	3.62±0.69 b	
Group2	T1	M	16.00±2.56 abcd	1921.46±33.63 a	6.41±0.63 ab	1.80±0.63
		F	10.67±2.37 abcd	1598.90±7.92 b	5.04±0.99 ab	
	T2	M	17.00±1.53 abc	1912.06±26.17 a	5.55±1.01 ab	1.85±0.51
		F	10.00±1.53 abcd	1590.04±11.00 b	6.29±0.59 ab	
	T3	M	15.67±2.85 abcd	1898.90±80.58 a	7.46±1.09 a	1.65±0.54
		F	11.33±2.85 abcd	1575.68±49.38 b	5.85± 0.14 ab	
	T4	M	18.67±2.03 ab	1867.70±22.99 a	6.98±1.89 ab	3.08±1.25
		F	7.67±2.30 d	1566.99±41.53 b	4.86±1.34 ab	
	T5	M	17.67±1.33 abc	1933.98±5.61 a	5.65±0.15 ab	2.00±0.38
		F	9.33±1.33 bcd	1616.11±16.99 b	6.04±0.23 ab	
Sig.			*	*	*	N.S.

Uniformity (UF)= (Standard Deviation / Mean Body Weight) × 100. Sex ratio = number of male/number of female .Group 1 = Chicks produced from eggs with an average weight of 61 ± 2 g, Group 2 = Chicks produced from eggs with an average weight of 55 ± 2 g, T1 = Control treatment, T2 = Positive control treatment, T3 = Treatment of injection with 125 μg/ml alcoholic extract of palm pollen, T4 = Treatment of injection with 150 μg/ml alcoholic extract of palm pollen, T5 = Treatment of injection with 175 μg/ml alcoholic extract of palm pollen, *Different letters within a column mean significant differences between means ($P< 0.05$), N.S. means that there are no significant differences between the means within column($P<0.05$).

Sex ratio, average weight, and uniformity percentage of males and females during the fifth week

The results presented in Table 3 indicate that there were no significant differences between the two egg weight groups (Group 1, Group 2) in the number of males, as Group 1 recorded 17.93% and Group 2 recorded 17.00%. Similarly, male body weight did not differ significantly between the groups (2745.05 g and 2709.63 g, respectively). Female body weight also showed no significant difference

Table 3: Effect egg weight and injection with alcoholic extract data palm pollen on the fifth week uniformity of broiler chicken. (mean ± SE).

Effect factors			Sex number%	weight(gm)	UF%	Sex Ratio
group 1		M	17.933±0.64 a	2745.05±9.45 a	9.93±0.57	2.25±0.25
		F	8.93±0.64 b	2382.48±23.52 b	7.35±0.85	
group2		M	17.00±0.87 a	2709.63±27.87 a	8.46±0.51	2.08±0.31
		F	9.80±0.89 b	2317.09±17.71 b	8.11±0.73	
Sig			*	*	N.S.	N.S.
T1		M	60.90 ± 5.09 a	2746.78 35.44 ±a	9.55±0.49	1.76±0.33
		F	39.10 ± 5.09 b	2376.56±16.14 b	5.43±0.47	
T2		M	61.73 ± 4.02 a	2761.80± 15.88 a	9.25±1.77	1.80±0.34
		F	37.65 ± 3.88 b	2352.85 ±51.83 b	9.66±1.13	
T3		M	66.05 ± 6.23 a	2673.42±46.74 a	9.58±0.68	2.37±0.51
		F	33.95 ± 6.23 b	2270.08±34.57 b	9.21±1.61	
T4		M	70.49 ± 3.92 a	2705.29±33.40 a	9.25±0.60	2.78±0.61
		F	29.51 ± 3.92 b	2372.78±28.19 b	7.44±1.45	
T5		M	66.05 ± 3.37 a	2749.41 ±21.73 a	8.35±0.44	2.11±0.34
		F	33.95 ± 3.37 b	2376.68±23.08 b	6.93±0.58	
Sig.			*	*	N.S.	N.S.
group 1	T1	M	61.73±5.38 abc	2734.10±8.65 a	10.49±0.44	1.72±0.38
		F	38.27 ± 5.38 def	2401.93±24.80 b	4.74±0.53	
	T2	M	60.49 ±6.87 abcd	2770.80±19.51 a	11.69±2.64	1.77±0.55
		F	38.27 ± 6.53 def	2449.39±32.01 b	8.66±0.42	
	T3	M	74.07 ± 4.28 a	2704.72±13.17 a	9.82±0.03	3.09±0.70
		F	25.93 ± 4.27 f	2257.42±66.32 b	10.31±3.39	
	T4	M	70.09 ± 4.03 a	2758.79±14.34 a	9.56±0.71	2.47±0.47
		F	29.92 ± 4.03 f	2392.97±37.37 b	5.77±1.49	
	T5	M	66.67 ± 5.66 a	2755.94±30.70 a	8.11±0.45	2.22±0.65
		F	33.33 ± 5.65 f	2410.72±34.95 b	7.29±1.02	
group2	T1	M	60.07 ±9.98 abcd	2758.57±77.88 a	8.62±0.33	1.80±0.63
		F	39.93 ± 9.98 cdef	2351.19±6.60 b	6.12±0.60	
	T2	M	62.96 ± 5.66 ab	2752.79±28.28 a	6.81±1.65	1.85±0.51
		F	37.04 ± 5.65 df	2256.32±55.60 b	10.65±2.29	
	T3	M	58.02 ± 10.55 abcde	2642.12±98.85 a	9.35±1.50	1.65±0.54
		F	41.97 ± 10.54 bcdef	2282.74±37.65 b	8.11±0.55	
	T4	M	70.89 ± 7.77 a	2651.78±50.42 a	8.94±1.11	3.08±1.25
		F	29.11 ± 7.78 f	2352.58±46.58 b	9.11±2.33	
	T5	M	65.43 ± 4.94 a	2742.89±37.11 a	8.60±0.85	2.00±0.38
		F	34.57 ± 4.93 f	2342.64±16.85 b	6.57±0.70	
Sig.			*	*	N.S.	N.S.

Uniformity= (Standard Deviation / Mean Body Weight) × 100. Sex ratio = number of male/number of female. Group 1 = Chicks produced from eggs with an average weight of 61 ± 2 g, Group 2 = Chicks produced from eggs with an average weight of 55 ± 2 g, T1 = Control treatment, T2 = Positive control treatment, T3 = Treatment of injection with 125 µg/ml alcoholic extract of palm pollen, T4 = Treatment of injection with 150 µg/ml alcoholic extract of palm pollen, T5 = Treatment of injection with 175 µg/ml alcoholic extract of palm pollen, *Different letters within a column mean significant differences between means ($P < 0.05$), N.S. means that there are no significant differences between the means within column($P<0.05$).

(2382.48 g in Group 1 vs. 2317.09 g in Group 2). These results suggest that egg weight alone did not exert a significant influence on chick sex ratio or flock uniformity, but males consistently exhibited higher body weights and counts than females across both egg weight groups. This finding aligns with previous studies reporting that larger eggs tend to produce slightly heavier chicks, though not necessarily with altered sex ratios (Ulmer-Franco *et al*., 2010; Abiola *et al*., 2008; Al-Sudani *et al*., 2025).

Regarding treatment effects, significant differences were observed among the five treatment groups (T1–T5) in both male count and body weight, while no significant differences were found in flock uniformity (UF%) or sex ratio. Treatment T4 (150 μg/ml) recorded the highest male percentage (70.49%), followed by T3 and T5 (66.05%), whereas the control (T1) showed the lowest (60.90%). For body weight, males in T2 (positive control, saline injection) recorded the highest mean value (2761.80 g), while females in T3 exhibited the lowest (2270.08 g). The uniformity percentage ranged from 5.43% to 9.66% with no significant differences among treatments, indicating a consistent flock structure. These results agree with the findings of Feedes *et al*. (2002) and Toudic (2007), who noted that uniformity values below 10% reflect good management and stable growth performance in broilers.

The interaction between egg weight and treatment revealed significant effects on male count and average body weight, but no significant influence on uniformity percentage or sex ratio. In Group 1, T3 (125 μg/ml) recorded the highest male percentage (74.07%), followed by T4 (70.09%) and T5 (66.67%), while T1 had the lowest (61.73%). Similarly, in Group 2, T4 achieved the highest male proportion (70.89%), followed by T5 and T2, whereas T3 showed the lowest (58.02%).

Regarding body weight, males in Group 1 treated with T2 recorded the highest average (2770.80 g), while females in Group 2 under the same treatment showed the lowest (2256.32 g). These results collectively suggest that the combination of egg weight and injection treatment affected male chick numbers and growth performance, but not flock uniformity or sex ratio.

The absence of treatment effects on sex ratio implies that date palm pollen extract (DPPE) does not induce hormonal imbalance or alter embryonic sex differentiation. This observation supports earlier studies suggesting that natural bioactive compounds in date palm pollen, such as flavonoids, alkaloids, and sterols, primarily enhance growth and reproductive performance through improved antioxidant and endocrine modulation rather than sex determination (Elgasim *et al*., 1995; Hassan *et al*., 2012; Khalifa *et al*., 2016). The observed improvement in male performance may therefore be linked to the anabolic and androgenic-like properties of DPPE components, which can enhance testosterone levels and protein synthesis in growing chicks (Mansour *et al*., 2018; Al-Daraji *et al*., 2019). Overall, uniformity remained within the optimal threshold (< 10%), suggesting stable flock performance and efficient management during rearing, consistent with commercial broiler standards (Yassin *et al*., 2009; Aviagen, 2022).

CONCLUSIONS

The uniformity percentage in Ross 308 broilers was not significantly affected by either egg weight or *in ovo* injection with the alcoholic extract of date palm pollen, as all treatments maintained acceptable uniformity levels (CV < 10%). Egg weight and *in ovo* injection with the alcoholic extract of date palm pollen had no significant effect on the sex ratio or on the proportion of males and females during the fourth and fifth weeks. Female growth performance remained unaffected across treatments, whereas males consistently exhibited higher body weights in all groups. These findings suggest that the date palm pollen extract is a safe natural additive that does not interfere with embryonic sex differentiation or post-hatch growth uniformity. However, the extract (particularly at moderate concentrations) may support slight improvements in male growth performance, warranting further investigation into its bioactive and hormonal mechanisms.

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NOVELTY STATEMENT

This study presents a novel approach to evaluate the effects of hatching egg weight and Injecting with the alcoholic extract of date palm pollen on the sex ratio and growth uniformity of broiler chicks. The results highlight the positive effects of these alcoholic extract. It is hypothesized that increased egg weight and Injecting with the alcoholic extract of date palm pollen may modulate embryonic sexual differentiation and subsequently enhance post-hatch growth uniformity, suggesting their potential benefit in practical breeding practices.

AUTHOR'S CONTRIBUTION

BSK conceptualized and designed the experiments, and carried out the experimental work. BSK and BMI analysed the data and drafted the manuscript. All authors reviewed and approved the final version of the manuscript.

Generative AI and AI-assisted technology statement

The authors declare that no Genrative AI was used in the creation of this manuscript.

Conflict of interest

Authors declared no conflict of interest.

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