THE INFLUENCE OF GENISTEIN IMPLANTATION ON OFFSPRING SEX RATIOS AND THEIR RELATION TO ESTROGEN LEVELS IN THE BLOOD OF IRAQI CHICKENS

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ABSTRACT

This study was carried out at the Poultry Research Station / Agricultural Research Department / Ministry of Agriculture, to investigate the effects of implanting genistein (GE) for the period of 1/February/2021 to 16/August/2021. into Iraqi local chickens at various ages on primary (PSF) and secondary (SSF) sex ratios of female, fertility (FE), and hatchability (HA) traits. At the age of 12 weeks, 100 hens and 20 roosters of Iraqi local chickens from the Poultry Research Station were used in this study. After numbering the hens, the birds were housed in individual cages and divided into four treatments (each with 25 chickens) as follows: T1: none implantation; T2, T3, and T4: implantation of 10 mg GE /kg weight at 14, 18, and 22 weeks of age, respectively. The experiment was divided into three periods, each for 28 weeks, and then rated according to the overall average and all of the traits studied. The results showed that implanting GE into hens had a positive influence on FE, PSF, SSF, and estrogen level (ES), especially at 18 weeks of age. There were also significant correlations between traits and ES in hens' blood. It was also shown that the regression of most traits on ES is first order linear. As a result, it can be concluded that GE has a positive effect on ES, PSF, SSF, with the possibility of predicting sex ratios and sex offspring based on estrogen levels in the blood, and that implantation at 18 weeks of age has produced great results.

Key word: phytoestrogen, primary and secondary sex ratios, Iraqi local chickens, Steroid hormones.

تأثير زرع الجينستين في نسب جنس النسل وعلاقتها بمستوى هرمون الاستروجين في دم الدجاج العراقي

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المستخلص

أجريت هذه الدراسة في محطة أبحاث الدواجن/ دائرة البحوث الزراعية / وزارة الزراعة، للمدة من 1/شباط/2021 ولغاية 16/أب/2021. لبيان تأثير حقن الدجاج العراقي المحلي بالجنسيتين في تحوير النسب الجنسية الأولية والثانوية، وبعض الصفات الإنتاجية. استعمل في هذه الدارسة 100 دجاجة و 20 ديكاً من الدجاج العراقي المحلي، مجهزة من محطة أبحاث الدواجن، 12 بعمر أسبوعاً. ربيت الطيور في اقفاص فردية، ووزعت الطيور على أربعة معاملات (25 دجاجة / معاملة) بعد ترقيم الإناث، و كالأتي: T_1: سيطرة، من غير حقن؛ T_2 و T_3 و T_4 حقنت بجرعة 10 ملغم جينستين / كغم وزن عند الأعمار 14 و 18 و 22 أسبوعاً على التعاقب. قسمت مدة التجربة على ثلاثة مدد، كل مدة 28 أسبوعاً، ثم حسب المعدل العام ولكافة الصفات قيد الدراسة. وقد تبينت تأثيرات إيجابية لحقن الجينستين لاسيما عند 18 أسبوعاً من العمر، في النسبة المئوية للخصوبة، والنسب المئوية الجنسية الأولية والثانوية وتركيز هرمون الاستروجين، كما سجلت علاقات ارتباط معنوية فيما بين أغلب الصفات وتركيز الاستروجين في دم الدجاج. كما تبين أن انحدار أغلب الصفات على تركيز الأستروجين خطياً من الدرجة الأولى. وبذلك يمكن الاستنتاج أن تأثير الجينستين تأثيراً إيجابياً في الأداء الإنتاجي وتركيز هرمون الأستروجين والنسب الجنسية الأولية والثانوية، مع إمكانية توقع النسب الجنسية ونسل الجنس الناتج من مستوى هرمون الاستروجين في الدم، وأن الحقن عند 22 أسبوعاً من العمر قد حقق أفضل النتائج.

الكلمات المفتاحية: الهرمونات النباتية، النسب الجنسية الثانوية والأولية، الدجاج العراقي المحلي، الهرمونات الستيرويدية.

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INTRODUCTION

Steroid hormones, particularly sex hormones, play a key role in determining the sex ratios of the offspring(6) . Aromatase inhibitors cause testicular growth in genetic females, while ES causes left ovary growth and differentiation in genetic males. ES is believed to be one of the most important hormones in determining sex ratios and sex determination (5). ES signals control the selection of sex chromosomes, and ES levels influence the expression of the first sex-determining gene, DMRT1 (14).Despite women's and warnings regarding the use of hormones in foods, phytoestrogens have emerged as a viable alternative to animal hormones in a variety of activities and roles (10). Phytoestrogens are polyphenolic molecules that resemble 17-estradiol in structure and function (15).GE is a widely used plant hormone that has a structure similar to estrogen's, allowing it to perform the same functions as estrogen (40), its ability to fulfill the stimulating and inhibiting roles of ES through its interaction with the alpha and beta ES receptors, as well as its safe use without side effects (28). Additionally, it performs as an antioxidant (8). According to Kasim et al. (20), GE in drinking water caused a significant increase in estrogen levels. When GE was added to the diets of laying hens, egg production increased and egg quality improved (32).All of these suggests that GE has an influence on the hypothalamus-pituitary-ovarian axis. As a basis, the aim of this study was to determine the effect of implanting GE Iraqi local chickens at various ages in order to verify the influence of GE on the offspring's SSF and PSF, ES level in the blood and relationship between ES levels and sex ratios, FE, HAT and HAF are being investigated in Iraqi local chickens.

MATERIALS AND METHODS

Bird management: This study was conducted at the Poultry Research Station by the Agricultural Research Department from February 1, 2021, to August 16, 2021. The Poultry Research Station provided 100 hens and 20 roosters of local Iraqi chickens, all aged 12 weeks, for this study. The birds were raised in single iron cages with a linear feeder, barriers to partition the cages, and automatic fountains with a nipple system to provide water to the birds on a constant basis. The birds were provided one by one (one hen per cage). The birds were fed two diets, according to reports from the Council of American Research: one for pre-production which contained 17.6 percent crude protein and 2763 kilocalories of representative energy per kg of feed, and one for productivity, which contained 18.1 percent crude protein and 2796 kilocalories of representative energy per kg of feed (26). For the duration of the experiment, the birds were kept under a 16 light: 8 dark lighting system. They are distributed symmetrically to maintain equal lighting intensity throughout the hall, using 60-watt electric bulbs. Using an electronic equipment (4-THC) to measure temperature and humidity, the room temperature and relative humidity were recorded four times a day (every six hours). The flock was left untreated fr two weeks to allow the birds to adjust to their new conditions, with the males trained to collect semen.

Implantation of GE

preparation GE 10 mg/mL (PPM): Kuiper's (21) melting GE with sesame oil as follows: To sterilize the oil with an Autoclave, the sesame oil was heated at 121°C for 15 minutes and under 15 lbs. pressure in a heat-resistant glass flask. Allow the oil to cool to 40 – 45° C. 0.6 gm of the axenic company's manufactured Ge is dissolved in a tiny amount of ethyl alcohol (1 ml of alcohol) and mixed to 25 ml of sterile sesame oil. To get rid of the alcohol residues in the solution, we were using a hot plate magnetic stirrer at a temperature of 40-45°C for 30 min. Ge should be kept at -20°C until it is used.

GE is implanted under the skin of the neck

Process and stored at a temperature of 12.2 °C before being incubated in a Belgian Petersen hatchery. After the hatching process is complete, calculation the number of dead embryos there are after breaking the non-hatched eggs. For the purpose of DNA testing, the dead embryos were placed in plastic boxes and frozen. Then, using the following two formulas, determine the FE% and mortality (MO)%: The birds were divided into four treatments, each with 25 hens, the first of which was: T1:no GE treatment; T2,T3,and

FE%= total eggs fertilized X dead embryos 100

T4: GE dissolved in sesame oil implanted under the skin of the neck at a dose of 0.5 ml per kg of hen weight. Using a Chinese-made automated syringe. At 14, 18, and 22 weeks of age, each 0.5 ml of the oil contains 10 mg of GE.

Measurement ES levels in blood

MO %=100 fertilized eggs multiplied by MO%

After calculating the number of hatched chicks, the hatching percentage (hat ability of total eggs (HAT%))and hatchability of fertilized eggs (HAF%) was computed using the following two formulas:

Hatching chicks collected blood from all females HAT%= total eggs x 100

through the cutaneous ulnar vein (18). Using a centrifuge (3000 revolutions per minute for 10 minutes),HAF% = hatching chicks' fertilized eggs X 100

separate the serum from the cell fraction. The Roche 411 Cobas e device and the Elecsys Estradiol III (Kit) product by Roche Co. are used to measure the level of ES in the blood serum.

FE and HA traits

Collect rooster sperm in a plastic container and dilute with Normal Saline solution (4). A dose of 0.03 ml of semen from a pool sample was injected into females (2). When Artificial insemination was performed for females, it was done at 1.00 pm to verify that all females had deposited eggs and to avoid the existence of a hard-shell egg in the uterus (1, 3). Three people participated in the experiment. the hatchings were carried out every 28 days. The fertilized eggs were collected five days after the second day of the insemination. process and stored at a temperature of 12.2 ° C before being incubated in a Belgian Petersen hatchery. After the hatching process is complete, calculate dead embryos number, there are after breaking the non-hatched eggs. For the purpose of DNA testing, the dead embryos were placed in plastic boxes and frozen. Then, using the following two formula, determine the FE % and mortality (MO) %:

$$\boldsymbol{FE}\ \% = \frac{\boldsymbol{fertilized\ eggs}}{\boldsymbol{total\ eggs}} \times \mathbf{100}$$

$$\boldsymbol{MO}\ \% = \frac{\boldsymbol{dead\ embryos}}{\boldsymbol{fertilized\ eggs}} \times \mathbf{100}$$

After calculating the number of hatched chicks, the hatching percentage (Hatchability of total eggs (HAT%) and Hatchability of fertilized eggs (HAF %) was computed using the following two formula:

$$\boldsymbol{HAT}\ \% = \frac{\boldsymbol{hatching\ chicks}}{\boldsymbol{total\ eggs}} \times \mathbf{100}$$

$$\boldsymbol{HAF}\ \% = \frac{\boldsymbol{hatching\ chicks}}{\boldsymbol{fertilized\ eggs}} \times \mathbf{100}$$

Primary and secondary sex ratios

The hatched chicks were numbered immediately after the hatching process was completed by putting iron numbers in the wing, then sexed at the age of 4 weeks, the number of females was calculated, and the Secondary sex ratio for females (SSF%) was computed using the following formula:

Hatching females:

SSF%=100 hatching chicks multiplied by SSF%

The primary sex ratio for females (PSF%) of females was determined using the formula after determining the sex of the dead embryos using the PCR technique +1:

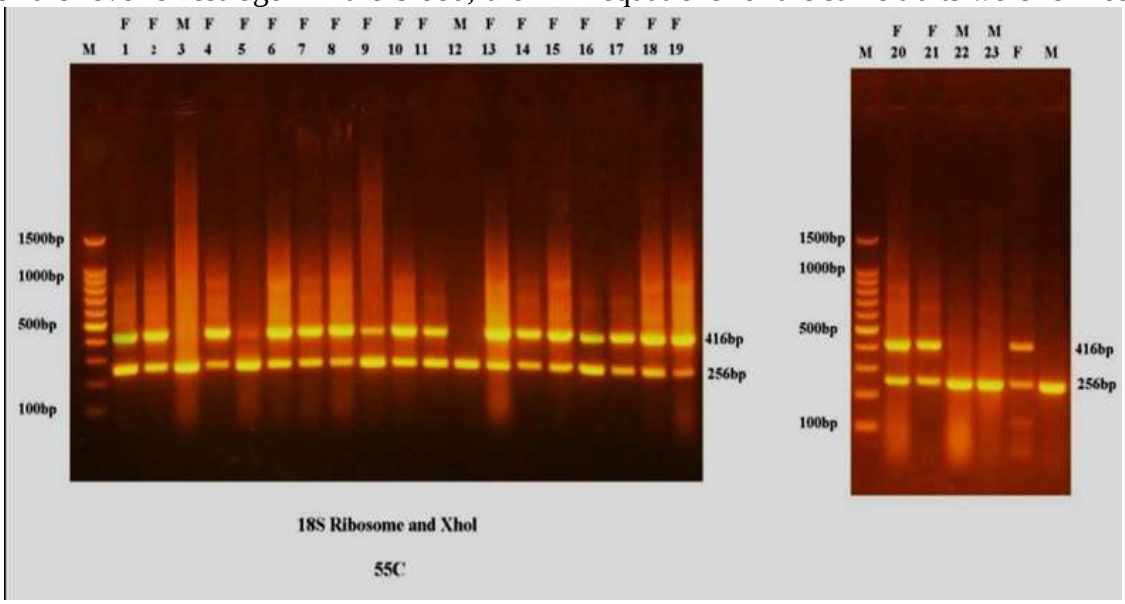
PSF%= hatching females minus dead female embryos multiplied by 100 hatching chicks equals total dead embryos

Polymerase chain reaction (PCR): A sample of the dead embryo' liver was extracted and stored sterile plastic containers, before being frozen at -21 degree Celsius and transported to the lab. In female chickens, the polymerase chain reaction (PCR) technology was applied to amplify a gene NW_001488744.1 on the W chromosome (19). The sex of the dead embryos was determined which use gel images, as the presence of the separated bundle as a result of chain amplification reactions for the separating region of the gene carried on the W chromosome for females was inferred on females, and no separation of that bundle was observed in samples from male embryos (Picture 1).

Statistical analysis

The study data was statistically analyzed using Statistical Analysis System (39), to test the influence of GE implantation on the traits under study. Complete Randomize Design (CRD) was used to analyze the data, and Duncan's Multiple range test was used to compare significant differences across means

(11). The correlation coefficient between the traits under study was then calculated. On the basis of the level of estrogen in the blood, the regression coefficients for the traits under study were calculated, and then the prediction equations for the same traits were formed.



Picture 1. Electrophoresis of extracted DNA and PCR products

RESULTS AND DISCUSSION

Table 1 shows a significant increase ($P<0.05$) in the ES levels (pg/ml) in the blood during the third period of T_2 when compared with T_1.However, there were no significant differences in T_3 and T_4 when compared to T_1 or T_2. The three GE implant treatments (T_2, T_3, and T_4) showed a significant increase ($P<0<$span =""">.O1) in the general average ES level in the blood of chickens when compared to the control treatment (T_1), as indicated in the same table. Table 2 revealed a significant increase ($P<0.05$) in the FE % in T_4 when compared to T_1, with no significant differences between T_2 and T_3 when compared to T_1, throughout the third period. When the overall average of the FE% was computed, it was shown that T_2 and T_4 had a significant increase ($P<0.05$) in FE% when compared to T_1, whereas T_3 had no significant differences when compared to T_1 or T_2 and T_4. During the first and second periods, there are no significant differences in the treatment of genistein implantation compared to the control. Table 3 shows that the HAT (%) in T_3 is significantly lower ($P<0.05$) than in T_1. T_2 and T_4 showed no significant differences when compared with T_1. During the first, third periods, and overall average of HAT (%) did not differ significantly among the GE implantation treatments and the control

Table 1. Effect of genistein implantation (10 mg/kg live weight) date on serum ES (pg/ml) in Iraqi local hens (mean ± SE)

GE Implantation Date (Treatments)	ES levels (pg/ml)													
	1st Period			2nd Period			3rd Period			Overall average				
Control (T_1)	333.78	±	15.66	291.81	±	16.05	341.33	±	26.05	B	322.31	±	14.42	B
At 14 weeks age (T_2)	361.95	±	22.08	311.60	±	13.43	427.98	±	14.96	A	367.18	±	11.64	A
At 18 weeks age (T_3)	381.20	±	22.60	322.41	±	19.40	398.89	±	22.45	AB	367.50	±	14.84	A
At 22 weeks age (T_4)	386.84	±	21.09	330.39	±	17.00	404.91	±	21.46	AB	374.05	±	11.74	A
Sig.	N. S			N.S			0.05				0.01			

At 14, 18, and 22 weeks of age, hens were implanted with 10 mg GE / Kg weight. Durations: The experiment's total duration was divided into three periods (each lasting 28 days), and the overall average of the three periods was calculated for all traits under study. N.S: no significant differences among means. Significant differences among the means are indicated by different letters within the same column

Table 2. Effect of genistein implantation (10 mg/kg live weight) date on FE (%) in Iraqi local hens (mean ± SE)

GE Implantation Date (Treatments)	FE (%)														
	1st Period			2nd Period			3rd Period				Overall average				
Control (T_1)	65.80	±	4.96	53.07	±	5.38	57.80	±	5.02	B	58.89	±	2.72	B	
At 14 weeks age (T_2)	71.00	±	4.45	67.40	±	4.81	66.67	±	4.97	A B	68.36	±	2.89	A	
At 18 weeks age (T_3)	67.00	±	3.83	60.53	±	3.81	62.27	±	4.74	A B	63.27	±	2.72	A B	
At 22 weeks age (T_4)	68.67	±	5.71	64.33	±	5.54	74.69	±	5.34	A	69.23	±	2.59	A	
Sig.	N.S			N.S			0.05				0.05				

At 14, 18, and 22 weeks of age, hens were implanted with 10 mg GE / Kg weight. Durations: The experiment's total duration was divided into three periods (each lasting 28 days), and the overall average of the three periods was calculated for all traits under study. N.S: no significant differences among means. Significant differences among the means are indicated by different letters within the same column

Table 3. Effect of genistein implantation (10 mg/kg live weight) date on FE (%) in Iraqi local hens (mean ± SE)

GE Implantation Date (Treatments)	HAT (%)											
	1st Period			2nd Period			3rd Period			Overall average		
Control (T_1)	55.87	±	5.45	35.73	±	5.05 A	30.27	±	6.42	40.62	±	3.36
At 14 weeks age (T_2)	56.47	±	5.42	37.13	±	3.98 A	31.20	±	5.95	41.60	±	2.61
At 18 weeks age (T_3)	56.80	±	4.54	21.53	±	4.47 B	37.00	±	5.07	38.44	±	2.12
At 22 weeks age (T_4)	56.60	±	6.04	37.67	±	5.24 A	46.34	±	7.02	46.87	±	3.70
Sig.	N.S			0.05			N.S			N.S		

At 14, 18, and 22 weeks of age, hens were implanted with 10 mg GE / Kg weight. Durations: The experiment's total duration was divided into three periods (each lasting 28 days), and the overall average of the three periods was calculated for all traits under study. N.S: no significant differences among means. Significant differences among the means are indicated by different letters within the same column

Table 4 shows that implanting hens with GE at 18 weeks of age (T_3) resulted in a significant decrease ($p<0.05$) in HAF (%) during the second period when compared to the T_1, but no significant differences were shown in T_2 or T_4 when compared to the T_1. The same table also shows that during the first and third periods, there were no significant differences in HAF (%) among the three genistein implanting treatments and the control treatment, as well as the overall average of hatching percentage from fertilized eggs. Table 5 indicates that different GE implanting times had no effect on MO% in T_2, T_3, and T_4 if compared to T_1, at the first and third periods, or the overall average of the same trait. T_3, on the other side, had seen a significant increase ($P<0.05$) in MO% as compared to T_1, in the second period. Table 6 reveals that the T_2 and T_3 had a significant increase ($P<0.05$) in SSF (%) when compared to the T1, however the T4 had no significant differences from the T_1 or the T_2 and T_3 during the first period. In the second period, no significant differences in SSF (%) among GE implantation treatments at different ages were observed if compared to T_1, with significant differences ($P<0.05$) in favor of T_4 when compared to T_3. While the SSF (%) during the third period significant increased ($P<0.05$) in T_3 compared to T_1, there were no significant differences in T_2 and T_3 when compared to T_1, and a significant decrease ($P<0.05$) in T_2 compared to T_3. In terms of the overall average of study durations, table 6 shows a significant increase ($P<0.05$) in the SSF (%) in T_3 and T_4 as compared to T_1. T_2 did not differ significantly from T_1, although it did decrease significantly ($P<0.05$) when compared to T_3 and T_4.

Table 4. Effect of genistein implantation (10 mg/kg live weight) date on HAF (%) in Iraqi local hens (mean ± SE)

GE Implantation Date (Treatments)	HAF (%)													
	1st Period			2nd Period				3rd Period			Overall average			
Control (T_1)	85.33	±	4.55	68.00	±	6.95	A	51.13	±	8.83	68.16	±	4.87	
At 14 weeks age (T_2)	79.33	±	4.93	61.67	±	6.53	A	50.53	±	8.61	63.84	±	3.27	
At 18 weeks age (T_3)	84.67	±	4.71	37.33	±	7.64	B	66.13	±	7.39	62.71	±	3.54	
At 22 weeks age (T_4)	83.33	±	5.00	55.07	±	7.33	A B	66.13	±	7.55	68.18	±	4.71	
Sig.	N.S			0.05				N.S			N.S			

At 14, 18, and 22 weeks of age, hens were implanted with 10 mg GE / Kg weight. Durations: The experiment's total duration was divided into three periods (each lasting 28 days), and the overall average of the three periods was calculated for all traits under study. N.S: no significant differences among means. Significant differences among the means are indicated by different letters within the same column

Table 5. Effect of genistein implantation (10 mg/kg live weight) date on MO (%) in Iraqi local hens (mean ± SE)

GE Implantation Date (Treatments)	MO%													
	1st Period			2nd Period				3rd Period			Overall average			
Control (T_1)	14.67	±	4.55	28.00	±	6.45	B	48.87	±	8.83	30.51	±	4.87	
At 14 weeks age (T_2)	20.67	±	4.93	38.33	±	6.53	B A	49.47	±	8.61	36.16	±	3.27	
At 18 weeks age (T_3)	15.33	±	4.71	62.67	±	7.64	A	33.87	±	7.39	37.29	±	3.54	
At 22 weeks age (T_4)	16.67	±	5.00	40.93	±	7.17	A B	33.87	±	7.55	30.49	±	4.71	
Sig.	N.S			0.05				N.S			N.S			

At 14, 18, and 22 weeks of age, hens were implanted with 10 mg GE / Kg weight. Durations: The experiment's total duration was divided into three periods (each lasting 28 days), and the overall average of the three periods was calculated for all traits under study. N.S: no significant differences among means. Significant differences among the means are indicated by different letters within the same column

Table 6. Effect of genistein implantation (10 mg/kg live weight) date on SSF (%) in Iraqi local hens (mean ± SE)

GE Implantation Date (Treatments)	SSF (%)													
	1st Period			2nd Period				3rd Period			Overall average			
Control (T_1)	56.80	±	1.70	B	50.05	±	0.75	A B	55.24	±	1.65	B	54.03	±	0.27	B
At 14 weeks age (T_2)	68.00	±	1.10	A	48.11	±	1.91	A B	53.47	±	1.54	B	56.52	±	0.68	B
At 18 weeks age (T_3)	69.33	±	0.57	A	45.08	±	2.19	B	67.28	±	1.25	A	60.56	±	1.09	A
At 22 weeks age (T_4)	62.00	±	0.79	A B	57.20	±	1.10	A	64.04	±	0.98	A B	61.08	±	0.36	A
Sig.	0.05				0.05				0.05				0.05			

At 14, 18, and 22 weeks of age, hens were implanted with 10 mg GE / Kg weight. Durations: The experiment's total duration was divided into three periods (each lasting 28 days), and the overall average of the three periods was calculated for all traits under study. N.S: no significant differences among means. Significant differences among the means are indicated by different letters within the same column

During the first and second periods of the study, Table 7 reveals a significant increase ($P<0.05$) in the PSF (%) in T_4 compared to T_1, but no significant differences in T_2 and T_3 compared to T_1. T_3 and T_4 had a significant increase ($P<0.05$) in PSF (%) during the third period when compared to T_1, which did not differ significantly from T_2. When compared to T_3 and T_4, T_2 showed a significant decrease ($P<0.05$). Table 7 shows a significant increase ($P<0.05$) in the overall average of the PSF (%) for T_4 when compared to T_1 on the one hand, and T_2 on the other. While there were no significant differences between T_4 and T_1. It's worth noting that the differences between treatments T_3 and T_4 aren't significant. The correlation coefficients of FE (%) with HAT (%) and MO (%) are positive and significant. HAT (%) had a positive and significant correlation coefficient with HAF (%) and SSF (%), but a significant negative correlation coefficient with MO (%). HAF (%) was also significantly correlated with SSF (%) and negatively correlated with MO (%). The MO (%) and SSF (%) have a significantly negative correlation coefficient. The correlation coefficient of SSF (%) with PSF (%) and ES was significantly positive, as was the correlation coefficient of ES with PSF (%), HTF (%) and HAF (%) as shown in Table 8. The regression coefficients of HAT (%), HAF (%), SSF (%), and PSF (%) on ES LEVEL were significant and positive, as shown in Table 9

Table 7. Effect of genistein implantation (10 mg/kg live weight) date on PSF (%) in Iraqi local hens (mean ± SE)

GE Implantation Date (Treatments)	PSF (%)															
	1st Period				2nd Period				3rd Period				Overall average			
Control (T_1)	55.01	±	0.68	B	45.28	±	0.47	B	61.40	±	1.41	B	53.90	±	0.31	B
At 14 weeks age (T_2)	56.80	±	1.31	B	44.31	±	0.66	B	62.07	±	1.03	B	54.39	±	0.80	B
At 18 weeks age (T_3)	56.40	±	0.61	B	47.00	±	0.61	B	68.03	±	0.71	A	57.14	±	0.44	A B
At 22 weeks age (T_4)	63.60	±	0.86	A	55.47	±	0.60	A	69.64	±	0.43	A	62.90	±	0.83	A
Sig.	0.05				0.05				0.05				0.05			

At 14, 18, and 22 weeks of age, hens were implanted with 10 mg GE / Kg weight. Durations: The experiment's total duration was divided into three periods (each lasting 28 days), and the overall average of the three periods was calculated for all traits under study. N.S: no significant differences among means. Significant differences among the means are indicated by different letters within the same column

Table 8. Correlation coefficients of the studied traits of Iraqi local chickens

Traits	FE%	HAT%	HAF%	MO%	SSF%	PSF%
HAT%	0.482**					
HAF%	-0.088	0.758**				
MO%	0.137*	-0.737**	-0.975**			
SSF%	-0.029	0.214**	0.206**	-0.202**		
PSF%	0.095	0.139*	0.089	-0.073	0.478**	
ES (pg/ml)	0.034	0.179*	0.145*	-0.043	0.191*	0.172*

Table 9. Regression coefficients for the FE (%), HAT (%), HAF (%), SSF (%) and PSF (%) on ES levels of Iraqi local chickens

Regression traits on estrogen	Regression coefficient (b)	Straight-line equation (expectation)	Sig.	Coefficient of determination (R^2)
FE (%)	0.008	Y^ = 62.04 + 0.008 (X)	N.S	0.001
HAT (%)	0.022	Y^ = 33.99 + 0.022 (X)	0.05	0.026
HAF (%)	0.016	Y^ = 60.06 + 0.016 (X)	0.05	0.044
MO (%)	-0.015	Y^ = 38.92 - 0.015 (X)	N.S	0.002
SSF (%)	0.034	Y^ = 45.81 + 0.034 (X)	0.05	0.022
PSF (%)	0.024	Y^ = 48.40 + 0.024 (X)	0.05	0.019

Because GE has the same structure as ES, it can perform the same roles as ES, including such binding to and estrogen receptors (31, 35), This supports the results of this study (Table 1). As ES secretion is a response to the hypothalamic-pituitary-gonadal axis' (HPG) mechanism of action (30), ES regulates and stimulates ovulation (36). It is suggested that GE stimulates this axis because it increases GnRH transcription, ES levels in the blood, and activates apolipoprotein (APO) receptors in the ovary, all of which stimulate the direction of increased egg production (23). The significant increase in FE (%) might be related to the impacts of ES caused by implanting GE under the skin of hens, as this ES affects the female genital tract's growth and development, increasing its size and efficacy, ES also improves the sperm-storage activities of the uterine-vaginal glands by increasing their activity and capacity to store sperm, allowing the sperm to become plentiful for binding to the egg and fertilization (17). The sex ratios of the offspring produced during the mitotic stage are influenced by the physiological mother's state and the concentrations of her hormones that are passed to the egg and then to the embryo (33). During rapid yolk deposition, steroid hormones may modify sex, and the sensitivity of ovarian follicles to these effects differs depending on which chromosome they retain (27) (Navara, 2013). This lends support to the role of GE and its effects on ES levels, as well as their structural and functional similarities (41). Love and Williams (22) reported that hormone levels in the egg yolk have an effect on the sex of the offspring, and that modifying these hormones and their levels could modify the offspring's sex ratio. In birds, steroid hormones play a vital role in regulating sex ratios (38). Variations in corticosterone, progesterone, and testosterone levels, for example, can modify the primary and

secondary sex ratios before a certain period of time after ovulation (12, 27). The transmission of ES from the mother to the egg, as well as its impact on the embryo' sex is a complicated process (13). ES was metabolized to estrone during the first 48 hours of incubation, which precedes the beginning of embryonic sexual differentiation (29). The synchronicity of ES metabolism with the initiation of ES synthesis by undifferentiated gonads (16), supports the hypothesis of sex reversal in the embryo during the 4.5–5.5 day of embryo life by inhibiting the activities of aromatase inhibitors and their effects in the gene DMRT1 (24, 34). Using RNA interference (RNAi) technique to restrict DMART1 protein expression in early male embryos lead in gonad feminization (9, 25). Gonadal differentiation is a vital stage in the development of the reproductive system. The embryo contains bipotential gonads and the rudiments of oviducts and deferent ducts in the form of the Müllerian and Wolffian ducts, respectively, prior to this point in time (37). The process of sex reversal during embryonic development, as well as the physiological changes that follow it, may lead to an increase in dead embryos, which explains the large percentage of MO% in this study, and the decrease HAT%, HAF% (Tables 3, 4 and 5). The results of this study support the positive relation among ES, SSF, PSF, HAT and HAF, as the correlation coefficient is positive and significant (Table 8), and the regression coefficient is significant and positive (Table 9), implying that these traits are influenced by different levels of ES in the blood of Iraqi local chicken. That is, there is a linear relationship between estrogen levels and sex ratio (7). Based on the results, it could be concluded that implanting GE in hens at 22 weeks of age has a long-term positive effect on SSF and PSF. The sex ratios of the offspring can be predicted by measuring estrogen levels in the blood.

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