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Abstract. The aim of this study was to improve the reproductive ability of native Iraqi chickens with the use of glycitein. The Studie was conducted on a of 120 Iraqi native chickens, consisting of 100 hens and 20 roosters. The chickens were 26 weeks old at the time of the study. The chickens were divided into four treatment groups, with each group consisting of 25 chicks. The experimental design consisted of four groups: the first group served as the non-injection control (referred to as T1), while the remaining groups (T2, T3, and T4) were treated with injections of glycitein at concentrations of 5, 10, and 15 mg/kg body weight, respectively. These injections were given subcutaneously in the neck region, with a frequency of once every 28 days across a span of three periods. Subsequently, an examination was conducted on the percentages of fertility and hatchability, as well as the primary and secondary sex ratios pertaining to female subjects. The results of the study showed that the use of glycitein injection had a beneficial impact on fertility, hatching, as well as primary and secondary sex ratios. Therefore, it can be concluded that the impact of glycitein yields a favourable outcome on both the primary and secondary sexual ratios.

Keywords. Phytoestrogens, Estrogen, Aromatase.

1. Introduction

The profitability of the poultry industry is heavily dependent on the specialization in chick production, where males are selected for meat production and females are selected for egg production [1]. This statement highlights the potential for exploring suitable methods to control pre-hatching sex determination [2]. The significance of managing sexual ratios and channelling them towards the production of offspring of a specific sex, based on the type of production, extends beyond the economic dimension. It also contributes to the prevention of various inhumane procedures for disposing of unwanted offspring [3,4]. This statement highlights the potential for exploring suitable methods to control pre-hatching sex determination [2]. The significance of managing sexual ratios and channelling them towards the production of offspring of a specific sex, based on the type of production, extends beyond the economic dimension. It also contributes to the prevention of various inhumane procedures for disposing of unwanted offspring [3,4]

Multiple methods have been used to regulate the sex of offspring, including the manipulation of incubation temperature during the hatching process [5], as well as the administration of steroid

hormones such as estrogen, progesterone [6], testosterone [7], and corticosterone [8], as well as the administration of steroid hormones such as estrogen, progesterone [6], testosterone [7], and corticosterone [8]. The administration of hormonal injections carries inherent hazards, as cautioned by international organizations, hence advising against its widespread distribution. Hence, it is recommended to employ plant chemicals that possess qualities akin to those of hormones, particularly estrogen, to manipulate the developmental pathways of the progeny [9].
Among these chemicals is glycitein, which has a minor estrogenic effect [10] but is otherwise inert. Despite being able to survive in difficult environments, local chickens have been shown to have low productivity indices [11,12].
The aims of this research project are as follows. By injecting glycitein, the researchers hoped to boost chickens' reproductive efficiency and, by extension, increase the number of females in the population.

2. Materials and Methods

2.1. Study Design

Commencing on December 22, 2020, and concluding on March 17, 2021, a team of researchers conducted three distinct 28-day intervals of observation and data collection at Abu Ghraib, a research facility in Iraq. These periods were specifically dedicated to investigating the Poultry Research Station, Livestock Department, and Agricultural Research Department and operation with laboratories in Mosul university Ethical approval No. um.VET.2021.5.
A total of one hundred hens, aged 26 weeks, and twenty male roosters were randomly distributed into four treatment groups, alongside a control group. Each treatment group included of 25 hens and 5 roosters .
The treatments that were included encompassed the following: In the initial treatment (referred to as the control; T1), no injections were administered. Subcutaneous administration of Glycetin was performed in the cervical region, with dosages of 5, 10, and 15 mg/kg body weight for the second, third, and fourth treatments (T2, T3, T4), respectively.
Every avian specimen was allocated an individual enclosure within a multi-level structure consisting of cages constructed from iron mesh. The dimensions of a particular cage were measured to be 80 centimetres in length, 80 centimetres in breadth, and 40 centimetres in height. Each cage is equipped with its own set of longitudinal feeders. To ensure a continuous supply of fresh water for the birds, nipple-operated automatic water drinkers are employed.
The collection of semen in the study was facilitated through the training of male participants [13].

2.2. Injectable Glycetin Preparation

In the study conducted by [14], it was reported that Glycetin was solubilized in sesame oil.

- To achieve sterilization of the oil, it is recommended to employ an autoclave. This involves subjecting the sesame oil to a temperature of 121 degrees Celsius (155 degrees Fahrenheit) within a heat-resistant glass flask for a duration of 15 minutes.
- It is to allow the oil to cool within the temperature range of 40 to 45 degrees Celsius.
- To begin the experiment, carefully measure 25 millilitres of sesame oil and transfer it into a distinct vessel. Subsequently, introduce a precise amount of dissolved cholinesterase, specifically 0.3, 0.6, or 0.9 grams, into the same container.
- The solution should be vigorously agitated with a hotplate magnetic stirrer.
- Maintain a temperature of -20 degrees Celsius till the substance is prepared for utilization.

The experiment involved the utilization of Glycetin, a product manufactured by Shandong Green Chemical Co Limited in China. Three different quantities of Glycetin, specifically 0.3 g, 0.6 g, and 0.9 g, were measured out and afterwards dissolved in 25 ml of sesame oil. This was done to achieve final concentrations of 5, 15, and 10 mg / Kg of live body weight, respectively.
The hens received Glycetin injections in the neck region every 28 days, in conjunction with the artificial insemination process. The injections were administered using a syringe with a capacity of 10 ml [15].

According to [13], a sample of rooster semen was collected and pooled. The insemination-ready semen had been diluted with Normal Saline [16]. To confirm that all the females had lain their eggs and to prevent the placing of a hard-shelled egg during artificial insemination, 0.03 ml of semen from the joint sample was injected into each of the females at 1:00 p.m. [17].

2.3. Fertility and Hatching Traits

Every 28 days, hatchings would take place. Five days following the second day of insemination, the fertilized eggs were collected and kept at a temperature of 12.2 ° C until they were sent to a Petersen hatchery in Belgium for incubation.

The number of deceased embryos can be estimated when hatching is complete, and the unhatched eggs have been broken apart. The frozen embryos were stored in plastic containers for later DNA analysis. Then, calculate fertility and mortality using the following two formulas:

$$fertility\% = \frac{fertilized\ eggs}{total\ eggs} \times 100$$

$$\text{mortality } \% = \frac{dead\ embryos}{fertilized\ eggs} \times 100$$

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

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

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

2.4. Primary and Secondary Sex Ratios

Upon the completion of the hatching procedure, the newly hatched chicks were promptly assigned numerical identifiers by affixing iron numbers onto their wings. After doing sex determination at the age of 4 weeks, the investigators proceeded to calculate the quantity of female individuals. Subsequently, the Secondary Sex Ratio for females was computed utilizing the subsequent formula:

Secondary sex ratio %=100 hatching chicks multiplied by secondary sex ratio%

The primary sex ratio for female individuals was ascertained by employing a formula subsequent to detecting the sex of deceased embryos through the utilization of the polymerase chain reaction (PCR) technology.

Primary sex ratios%= hatching females minus dead female embryos multiplied by 100 hatching chicks equals total dead embryos.

2.5. Polymerase Chain Reaction (PCR)

A specimen of the deceased embryo's liver was collected and preserved in sterile plastic containers, thereafter, subjected to freezing at a temperature of -21 degrees Celsius, and subsequently delivered to the laboratory. The application of polymerase chain reaction (PCR) technology was utilized to amplify a specific gene, NW_001488744.1, located on the W chromosome in female chickens [18].

The determination of the sex of the deceased embryos was achieved through the utilization of gel pictures, wherein the identification of the separated bundle was indicative of the chain's outcome.

2.6. Statistical Analysis

The study data was statistically analyzed using SPSS [19], to assess the influence of Glycetin injection 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 [20].

3. Results

According to the data presented in Table 1, there was a significant increase in the percentage of fertilized eggs in T4 compared to T1 across all three experimental periods as well as in the overall mean. During the first period, T2 exhibited a significant increase in the percentage of fertilized eggs in comparison to T1. There were no statistically significant changes seen between T3 and T1 over the three periods and their overall mean.

Table 1. Effect of injecting native chickens with Glycetin on fertilized eggs (%; mean ± standard error).

Treatments	Percentage of fertilized eggs %			
	1st period	2nd period	3rd period	Overall mean
T1	68.33 ± 1.50^{B}	68.83 ± 1.92^{B}	69.71 ± 1.55^{B}	68.96 ± 1.23^{B}
T2	71.76 ± 1.35^{A}	72.79 ± 1.55^{AB}	70.67 ± 1.42^{B}	71.74 ± 1.24^{AB}
T3	67.52 ± 1.60^{B}	69.90 ± 1.46^{B}	70.84 ± 1.68^{B}	69.42 ± 1.22^{B}
T4	72.07 ± 1.42^{A}	75.20 ± 1.85^{A}	74.07 ± 1.72^{A}	73.78 ± 1.34^{A}
Sig.	0.035	0.001	0.007	0.041

Different letters in the same column indicate statistically significant differences between the means.

The data presented in Table 2 indicate a statistically significant increase in the Hatchability of total eggs (%) in treatments T2, T3, and T4, when compared to treatment T1, over all three experimental periods as well as the overall duration of the study.

Table 2. Effect of injecting native chickens with Glycetin on Hatchability of total eggs (%; mean ± standard error).

Treatments	Hatchability of total eggs (%)			
	1st period	2nd period	3rd period	Overall mean
T1	57.47 ± 1.65^{B}	53.00 ± 1.42^{B}	53.53 ± 4.08^{C}	54.67 ± 2.25^{B}
T2	56.47 ± 1.42^{B}	54.52 ± 1.01^{B}	57.53 ± 2.74^{B}	56.17 ± 2.42^{B}
T3	56.80 ± 1.54^{B}	53.18 ± 0.89^{B}	58.67 ± 2.92^{B}	56.22 ± 2.17^{B}
T4	60.95 ± 1.42^{A}	58.60 ± 1.93^{A}	62.12 ± 5.20^{A}	60.55 ± 2.22^{A}
Sig.	0.034	0.029	0.001	0.006

Different letters in the same column indicate statistically significant differences between the means.

According to the data shown in Table 3, there is a significant increase in the Hatchability of fertilized eggs (%) in the T4 treatment group as compared to the T1 group, both during the first and third terms as well as in the overall average .

It is worth mentioning that T3 exhibited an important increase in the Hatchability of fertilized eggs (%) in comparison to T1, but this trend was observed exclusively during the initial period.

Table 3. Effect of injecting native chickens with Glycetin on Hatchability of fertilized eggs (%; mean ± standard error).

Treatments	Hatchability of fertilized eggs (%)			
	1st period	2nd period	3rd period	Overall mean
T1	82.65 ±1.90^{B}	70.62 ±4.89	67.13 ± 1.70^{B}	73.47 ±1.59^{B}
T2	83.12 ±1.98^{B}	70.08 ±4.40	69.93 ± 1.08^{B}	74.38 ±1.36^{B}
T3	85.65 ±1.37^{AB}	68.32 ±3.84	76.11 ± 1.81^{A}	76.69 ±1.50^{AB}
T4	86.52 ±1.07^{A}	70.56 ±4.32	76.12 ± 1.80^{A}	77.73 ±0.72^{A}
Sig.	0.034	N. S	0.001	0.006

Different letters in the same column indicate statistically significant differences between the means.

According to the data presented in Table 4, there is a significant decrease in the mortality % associated with the T4 treatment when compared to the control group throughout periods 1 and 3, as well as in the overall mean.

Table 4. Effect of injecting native chickens with Glycetin on Mortality (%; mean ± standard error).

Treatments	Mortality %			
	1st period	2nd period	3rd period	Overall mean
T1	17.35 ± 1.90^{A}	29.38 ± 4.89	32.87 ± 1.70^{A}	26.53 ± 1.59^{A}
T2	16.88 ± 1.98^{A}	29.92 ± 4.40	30.07 ± 1.08^{A}	25.62 ± 1.36^{A}
T3	14.35 ± 1.37^{AB}	31.68 ± 3.84	23.89 ± 1.81^{B}	23.31 ± 1.50^{AB}
T4	13.48 ± 1.07^{B}	29.44 ± 4.32	23.88 ± 1.80^{B}	22.27 ± 0.72^{B}
Sig.	0.034	N. S	0.001	0.006

Different letters in the same column indicate statistically significant differences between the means.

The results shown in Table 5 indicate a significant increase in the primary sexual ratio of female in the fourth treatment group as compared to the control group throughout all three time periods as well as the overall average.

Table 5. Effect of injecting native chickens with Glycetin on primary sex ratios (%; mean ± standard error).

Treatments	Primary sex ratios (%)			
	1st period	2nd period	3rd period	Overall mean
T1	55.01 ± 1.68^{B}	45.28 ± 1.07^{B}	61.40 ± 1.41^{B}	53.90 ± 1.01^{B}
T2	56.80 ± 1.31^{B}	44.31 ± 1.36^{B}	62.07 ± 1.03^{B}	54.39 ± 1.80^{B}
T3	56.40 ± 1.61^{B}	47.00 ± 1.61^{B}	68.03 ± 1.71^{A}	57.14 ± 1.44^{AB}
T4	63.60 ± 1.86^{A}	55.47 ± 1.60^{A}	69.64 ± 1.43^{A}	62.90 ± 1.83^{A}
Sig.	0.006	0.018	0.002	0.001

Different letters in the same column indicate statistically significant differences between the means.

The results presented in Table 5 indicate a significant increase in the secondary sex ratio among females at T2 in comparison to T1, but only during the initial period. During the first and third terms, as well as the overall mean. T3 exhibited a significant increase in the secondary sex ratio in comparison to T1. The fourth treatment exhibited a significant increase in the characteristic as compared to the control group, across the second and third intervals as well as the overall duration.

Table 6. Effect of injecting native chickens with Glycetin on secondary sex ratios (%; mean ± standard error).

Treatments	Secondary sex ratios (%)			
	1st period	2nd period	3rd period	Overall mean
T1	56.80 ± 1.70^{B}	50.05 ± 1.75^{B}	55.24 ± 1.65^{B}	54.03 ± 1.27^{B}
T2	68.00 ± 1.10^{A}	48.11 ± 7.91^{B}	53.47 ± 1.54^{B}	56.52 ± 1.68^{B}
T3	69.33 ± 1.57^{A}	45.08 ± 1.19^{B}	67.28 ± 1.25^{A}	60.56 ± 1.09^{A}
T4	62.00 ± 1.79^{AB}	57.20 ± 1.10^{A}	64.04 ± 1.98^{A}	61.08 ± 1.36^{A}
Sig.	0.006	0.018	0.002	0.001

Different letters in the same column indicate statistically significant differences between the means.

4. Discussion

The initiation of estrogen expression occurs during the initial stages of embryonic development, preceding the production of aromatase and estrogens by the gonads [21]. According to [22], variations in steroid hormone levels have a crucial role in the elevation of estrogen levels. The primary functions of estrogen are associated with the growth and development of the reproductive system, as indicated by studies conducted [23,24]. The present discussion focuses on the pivotal stage of embryonic development in relation to sex determination, specifically examining the influence of estrogen in regulating sex ratios, according to the findings of [25], it has been observed that estrogen undergoes metabolism into estrone within the egg during the initial 48 hours of incubation, prior to the process of sexual differentiation.

According to [26], the intricate mechanism of estrogen transfer from the mother to the egg and its impact on embryonic sex is overly complex. The temporal alignment between estrogen metabolism and the onset of undifferentiated gonads' secretion of estrogen raises the possibility of foetal sex

reversal, as proposed by [27]. This phenomenon may be attributed to the inhibition of aromatase activity and its impact on the DMRT1 gene, as suggested by [28]. The application of RNA interference (RNAi) technology in the initial stages of embryonic development has demonstrated a decrease in DMTR1 expression, resulting in the feminization of the gonads [29].

The chicken embryo exhibits bipotential gonads, encompassing both female and male reproductive structures. Specifically, the embryo possesses the rudimentary forms of the oviduct, known as the Müllerian ducts, and the ducts, referred to as the Wolffian ducts. Hence, the ontogenetic development of the reproductive system is primarily determined by the process of gonadal differentiation [30]. The implementation of this approach has the potential to result in an augmentation in fertility and hatchability rates, while concurrently reducing the incidence of embryo mortality, as evidenced by the data presented in Tables 1, 2, 3, and 4.

The formation of primordial follicles in the ovarian cortex occurs shortly after hatching, often around day 3-4 of age [31]. According to [31], the formation of primary follicles occurs at the age of 4 weeks. The results obtained from this study, particularly in relation to proportions, fertility, and hatching (Tables 1, 2, 3, and 4), can be explained by logical reasoning.

The determination of yolk formation and its deposition in the follicles, as well as the rate at which this process occurs, are influenced by the functions of luteinizing hormone and estrogen, as indicated by [32]. This phenomenon was apparent in the observed impact on the modification of sex ratios, as indicated by the data presented in Tables 5 and 6. This is due to the influence of the yolk deposition rate, which is known to contribute to the process of sex chromosomal selection. According to [33] study, there was observed variation in the susceptibility of ovarian follicles to hormones during rapid yolk deposition, which was dependent on the sex chromosome they retained. The phenomenon of accelerated sedimentation occurs concurrently with the biological process of vitellogenesis, as observed in the study conducted by [34].

The arguments about the influence of estrogen on enhancing fertility and hatching ratios, as well as modifying sex ratios, could be attributed to the impact of glycetin in elevating estrogen levels within the bloodstream [35].

Conclusion

Subcutaneous neck injections were given every 28 days for three periods. Next, female fertility, hatchability, and primary and secondary sex ratios were examined. The study found that glycitein injection improved fertility, hatching, and primary and secondary sex ratios. Thus, glycitein improves primary and secondary sexual ratios.

References

[1] Afriani, T., Udin, Z., Jaswandi, J., & Asmairicen, S. (2011). Pengaruh waktu pelapisan spermatozoa sapi pada media talp yang disuplementasi Bovine Serum Albumin (BSA) terhadap jenis kelamin embrio in vitro. Jurnal Peternakan Indonesia (Indonesian Journal of Animal Science), 13(2), 141-148 .

[2] Iswati, Natsir, M. H., Ciptadi, G., & Susilawati, T. (2021). Exogenous Progesterone for Offspring Sex Ratio Manipulation in Arabic Chickens (Gallus turcicus) Leading to Increasing Female Chicks Proportion. American Journal of Animal and Veterinary Sciences, 16(1), 48-55. https://doi.org/10.3844/ajavsp.2021.48.55

[3] Aslam, M. A., Hulst, M., Hoving-Bolink, R. A., Smits, M. A., de Vries, B., Weites, I., Groothuis, T. G., & Woelders, H. (2013). Yolk concentrations of hormones and glucose and egg weight and egg dimensions in unincubated chicken eggs, in relation to egg sex and hen body weight. General and comparative endocrinology, 187, 15-22 .

[4] Krautwald-Junghanns, M.-E., Cramer, K., Fischer, B., Förster, A., Galli, R., Kremer, F., Mapesa, E., Meissner, S ،.Preisinger, R., & Preusse, G. (2018). Current approaches to avoid the culling of day-old male chicks in the layer industry, with special reference to spectroscopic methods. Poultry Science, 97(3), 749-757 .

[5] Kallimanis, A. (2010). Temperature dependent sex determination and climate change. Oikos, 119(1), 197-200.

[6] Correa, S. M., Adkins-Regan, E., & Johnson, P. A. (2005). High progesterone during avian meiosis biases sex ratios toward females. Biology Letters, 1(2), 215-218.

[7] Goerlich, V. C., Dijkstra, C., Schaafsma, S. M., & Groothuis, T. G. (2009). Testosterone has a long-term effect on primary sex ratio of first eggs in pigeons—in search of a mechanism. General and comparative endocrinology, 163(1-2), 184-192 .
[8] Gam, A. E., Mendonça, M. T., & Navara, K. J. (2011). Acute corticosterone treatment prior to ovulation biases offspring sex ratios towards males in zebra finches Taeniopygia guttata .Journal of Avian Biology, 42(3), 253-258 .
[9] Çiftci, H. B. (2022). Effects of the injection of chicken gonadotropin-releasing hormone-ı on egg fertility and hatching traits. Journal of Poultry Research, 19(2), 30-37 .
[10] Xiang, T., & Jin, W. (2023). Mechanism of glycitein in the treatment of colon cancer based on network pharmacology and molecular docking. Lifestyle Genomics, 16(1), 1-10 .
[11] Abdulwahid, H., Al-Hassani, D., & Razuki, W. (2019). Associations of very low-density lipoprotein receptor (VLDLR) gene polymorphisms with egg production traits in Iraqi local brown chickens. Iraqi Journal of Agricultural Sciences, 50 .(2)
[12] Sahib, A. M., Al-Khalisy, A. F., & Abdulwahid, M. T. (2021). Association of TGF-β2 gene polymorphism with growth rate in local chickens. The Iraqi Journal of Veterinary Medicine, 45(1), 9-16 .
[13] Al-Hayani, W., & Al-Daraji, H. (2013). Effect of supplementing male guinea fowls diet with different levels of L-carnitine on the characteristics of their semen. Scientific Journal of King Faisal University (Basic and Applied Sciences), 14(1), 23-47 .
[14] Kupiec, T. C., Matthews, P., & Ahmad, R .(2000) .Dry-heat sterilization of parenteral oil vehicles. International Journal of Pharmaceutical Compounding, 4(3), 223-224 .
[15] Al-Hamdani, S. A. J., & Al–Hayani, W. K. (2019a). Effect of melatonin administration with different light colors on fertility traits and hatching of local chickens. Plant Archives, 19(2), 1495-1498 .
[16] Al-Hamdani, S. A. J., & Al–Hayani, W. K. (2019b). Effect of melatonin administration with different light colors on the production of local chickens. Plant Arch, 19, 1489-1494 .
[17] Al-Mhanawi, A. A., & Al-Hayani, W. K. (2020). The effect of adding nano concentrations of hydrogen peroxide to the diluted semen of iraqi local roosters on some laboratory characteristics after cooling and cryopreservation. Plant Archives, 20(2), 2697-2703 .
[18] Kalina, J ،.Mucksová, J., Yan, H., & Trefil, P. (2012). Rapid sexing of selected Galliformes by polymerase chain reaction. Czech J. Anim. Sci, 57(4), 187-192 .
[19] Wagner III, W. E. (2019). Using IBM® SPSS® statistics for research methods and social science statistics. Sage Publications .
[20] Duncan, D. B .(1955) .Multiple range and multiple F tests. biometrics, 11(1), 1-42 .
[21] Hanlon, C., Ziezold, C. J., & Bédécarrats, G. Y. (2022). The diverse roles of 17β-estradiol in non-gonadal tissues and its consequential impact on reproduction in laying and broiler breeder hens. Frontiers in physiology, 13, 1 .338
[22] Al-Maksousi, S. K., Al-Hayani, W. K., & Hussein, F. M. (2019). Effect of the level of corticosteron hormone in the blood of local iraqi chicken mothers on the sex ratio of the produced offsprings. Plant Archives, 19(2), 1411-1415 .
[23] Abu-Rekaiba, R ،.Al-Anbari, E., & Razuki, W. (2021). Polimorphic characterization of esr1 and foxl2 genes and the association of their interaction with productive traits of brown local iraqi chickens. Iraqi Journal of Agricultural Sciences, 52(6), 1391-1400.
[24] Al-Ghurairi, R. A. S., W. Kh. A. Al – Hayani & Y. M. A. Maaeni. 2023. the influence of genistein implantation on offspring sex ratios and their relation to estrogen levels in the blood of iraqi chickens. Iraqi Journal of Agricultural Sciences, 54 (4), 1016 – 1025.
[25] Paitz, R. T., Angles, R., & Cagney, E. (2020). In ovo metabolism of estradiol to estrone sulfate in chicken eggs: implications for how yolk estradiol influences embryonic development. General and comparative endocrinology, 287, 113320 .
[26] Groothuis, T. G., Hsu, B.-Y., Kumar, N., & Tschirren, B. (2019). Revisiting mechanisms and functions of prenatal hormone-mediated maternal effects using avian species as a model. Philosophical Transactions of the Royal Society B, 374(1770), 20180115 .
[27] Smith, C. A., & Sinclair, A. H. (2004). Sex determination: insights from the chicken. Bioessays, 26(2), 120-132 .
[28] Yan, N., Hu, J., Li, J., Dong, J., Sun, C., & Ye, X. (2019). Genomic organization and sexually dimorphic expression of the Dmrt1 gene in largemouth bass (Micropterus salmoides). Comparative Biochemistry and Physiology Part B: Biochemistry and Molecular Biology, 234, 68-77 .
[29] Lee, H. J., Seo, M., Choi, H. J., Rengaraj, D., Jung, K. M., Park, J. S., Lee, K. Y., Kim, Y. M., Park, K. J., & Han, S. T. (2021). DMRT1 gene disruption alone induces incomplete gonad feminization in chicken.

FASEB journal: official publication of the Federation of American Societies for Experimental Biology, 35(9), e21876 .
[30] Vizcarra, J. A., Cerón-Romero, N., Taofeek, N., & Kirby, J. (2022). Reproduction in male birds. In Sturkie's avian physiology (pp. 987-1014). Elsevier .
[31] Johnson, A. L. (2014). The avian ovary and follicle development: some comparative and practical insights. Turkish Journal of Veterinary & Animal Sciences, 38(6), 660-669 .
[32] Cui, Z., Amevor, F. K., Feng, Q., Kang, X., Song, W., Zhu, Q., Wang, Y., Li, D., & Zhao, X. (2020). Sexual maturity promotes yolk precursor synthesis and follicle development in hens via liver-blood-ovary signal axis. Animals, 10(12), 2348 .
[33] Navara, K. J. (2013). The role of steroid hormones in the adjustment of primary sex ratio in birds: compiling the pieces of the puzzle. In: Oxford University Press.
[34] Ruan, Y., Wong, N.-K., Zhang, X., Zhu, C., Wu, X., Ren, C., Luo, P., Jiang, X., Ji, J., & Wu, X. (2020). Vitellogenin receptor (VgR) mediates oocyte maturation and ovarian development in the Pacific white shrimp (Litopenaeus vannamei). Frontiers in physiology, 11, 485 .
[35] Kiyama, R. (2023). Estrogenic flavonoids and their molecular mechanisms of action. The Journal of Nutritional Biochemistry, 114, 109250 .