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EFFECT OF THE LEVEL OF CORTICOSTERON HORMONE IN THE BLOOD OF LOCAL IRAQI CHICKEN MOTHERS ON THE SEX RATIO OF THE PRODUCED OFFSPRINGS

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Abstract

This study was conducted to investigate the relationship between corticosterone hormone in the blood of local Iraqi white chicken mothers and the sexual ratio of the hatching chicks. A field experiment located at the Poultry Research Station, Livestock Department, Agricultural Research Department, Ministry of Agriculture for the period from 16 of September 2018 to 25 of December 2018. One hundred and forty birds of Iraqi local chicken mothers (100 females + 40 males) at the age of 28 weeks were provided from the poultry research station. The birds were raised in individual cages and distributed sequentially to the cages after the numbering of the females. Data were recorded in three periods, each period was of 28 days, and then the general average of each studied character was calculated. Blood was withdrawn at the end of each period to measure the concentration of corticosterone in maternal blood, and then the birds were distributed on the basis of hormone concentration to three groups: low, medium, and high to compare the performance of mothers. The results showed a significant difference in the concentration of corticosterone hormone in the blood of mothers (Ng/100 ml) according to the level of the class, percentage of hatching chicks and the secondary and primary sex ratios. A linear relationship was observed between the studied traits and corticosterone concentration levels through calculation the regression and correlation as well as the adoption of the method of estimating the results of the prediction to reach the best predictive values that bring closer to reality. It can be concluded that the second period of the experiment was the best duration and gave distinct results and that the corticosterone hormone at the variance of the level was working to modify the sexual ratio in birds by its direct effect on the disruption of sex hormones activity in the mothers of Iraqi local chickens.

Keywords **:** Corticosterone, hatching, sex hormones.

Introduction

One of the major problems facing the poultry industry was the increase in the ratio of male to female in chickens that produce eggs and hatcheries (Kaleta and Redman, 2008). This is similar to the increase in the ratio of females to males in broilers, which is a potential economic loss. The profits of naturalization of the broiler before the hatching was 1.6% per dinar, or 160%, and these are very encouraging profits to develop the process of naturalization, and investment in the poultry industry (Jubiar *et al*., 2018). Therefore, the specialists in the poultry industry is of great importance in its development through the introduction of modern technologies that increase production and its resources, especially as this industry represents a large part of the human need for food from animal sources (Zulkifli *et al*., 2010). Thus, limited studies have begun recently to address the conversion and identification of the sex ratio in birds (Engelhardt *et al*., 2006). The identification of sex in birds is a complex and mysterious process, whose elements have not yet been identified. It cannot be determined as a documented method to follow. It is also difficult to identify factors affecting sex and sexual ratio (Navara *et al*., 2006). Sex determination was defined as the process of naturalization of chicks before hatching during the embryonic stage to convert the sex ratio according to the purpose of rearing, to produce eggs or to produce meat (Piálková, 2011) as well as to increase economic returns commercially, increasing the accuracy of scientific research, studying the nutritional requirements of each sex, avoiding the methods used to eliminate the undesired sex, and contrary to the international agreements (Animal welfare organization, 2018).

The primary sex ratio is defined as the percentage determined by the initial filament division of the fertilized egg through which the sex of the egg is determined, while the secondary sex ratio is defined as the ratio that is determined in the subsequent stages for animals that give birth and when hatching for birds (Aslam, 2014). One of the scientific facts, males are superior in growth rates, food conversion coefficients, excretion rates, and cuttings (Fayyad and Nagy, 1989). Studies have indicated that this superiority was due to the roles of testosterone in regulating the work of the nitrogen balance, increasing the retention and stimulation of further protein synthesis (Squires, 2003). The male offspring of the egg-producing mothers are a major problem since there was no economic significance of these males, as they are characterized by low growth rates that cannot be used for meat production and ultimately kill (Bukhard *et al*., 2011; Ayers *et al*., 2013). More than 2.5 billion chicks have been killed, and were often used to produce animal protein concentrates or to feed animals in gardens (Aslam, 2014).

Materials and Methods

This study was carried out at the Poultry Research Station / Animal Resources Department / Agricultural Research Department / Ministry of Agriculture for the period from 16 of September 2018 to 25 of December 2018 to study the relationship of corticosteron concentration in the mothers of domestic white chickens with the sex ratio of the fertilized eggs. In this study, one hundred hen and forty rooster of local white chicken mothers were used at the age of 28 weeks, provided from the poultry research station. The birds were housed in a single metal cages with three layers, one cage per bird. The dimensions of the cage were 80 x 80 x 40 cm in length, width and height, respectively. Equipped with longitudinal feeders divided into each cage and automatic nipple system for continuous water supply.

The herd was left for two weeks without any treatment to adapt the birds to their new location with male training on sperm collection to facilitate the collection process during the experiment. Then the birds were distributed on the cages after numbering the females in each bird's leg. The lighting system applied 16: 8 hours/day for the duration of the

experiment, and 60 watt power lamps were used, and distributed uniformly to ensure that the lighting intensity was equal throughout the hall. Three air fans (3Vs) were used to ventilate the hall and renovate the air conditioning, with the desert cooling system of the hall. Hall temperature and relative humidity were measured using an electronic thermometer (THC - 4) for each hour of the duration of the experiment. Birds were fed to meet the needs of birds for feed, minerals and vitamins recommended in NRC (1994) reports, the feed was manufactured at the manure factory at the Poultry Research Station. It contained 17% crude protein and 2793.52 kcal/energy metabolized and allocated 85 g/hen/day, and 100 g/rooster/day, throughout the trial period. Table 1 shows the percentage of feed material in the composition of the feed with its chemical composition.

Table 1 : Percentages of feed component and its chemical composition in the experimental diet

Feed Substances%	Percentage %
Yellow corn	40
Wheat	29.7
Soybeans (48% protein)	17
Animal protein concentrate	5
Calcium Phosphate	0.5
Lime Stone Powder	7.5
NaCl	0.3
Total	100
Calculated chemical analysis *	
Crude protein	17.0
Metabolic Energy (kcal/ kg)	2793.52
Methionine (%)	0.37
Lysine (%)	0.886
Calcium (%)	3.20
Phosphorus (%)	0.40

* According to chemical analysis of NRC (1994)

The total duration of the experiment was divided by three periods (each period 28-day) and all studied traits were calculated. These attributes include each of the following:

The concentration of corticosteron hormone in the blood: The ELISA device was used to measure the concentration of corticosteron hormone in the serum using the kit (Chicken Corticosterone (CORT) ELISA Kit) produced by SunLong Biotech Co., LTD, according to the bulletin attached with the kit.

Fertility rates and hatching

Collection of semen: The semen was collected from the cocks as indicated by Draghi (2013), as the method requires two people, the first holding bird by making the head back and the pool forward with both hands. The second person will massage the abdominal dorsal area (the bird's back to the base of the tail) quietly until the papilla is inflated and the sperm flow and then collect the semen using a plastic box as shown in (Fig. 3), then extend the semen with Normal Saline 9%.

Artificial insemination: Local female chickens were vaccinated according to the method described by Daraji *et al*. (2011) and Darraji (2013), which was summarized as follows: The first person to carry the female between his left arm and chest. Holding the legs of the female with both hands, so that the head under the arm. The second person presses on the abdomen and above the hole of the complex, to the heart of the complex and the emergence of the vaginal opening, the second person insert the injection syringe deep 4-6 cm inside the vagina, then the first person relieve the pressure on the abdomen to return the vagina to its normal, then injected semen found in Plastic Injection Vaginal Injection Syringe (Al-Daraji and Al-Janabi, 2005). A dose of 0.03 mL of semen was used to vaccinate each female. The IVF procedure was performed at 2 pm to ensure that all females had laid eggs and to avoid having an egg with a hard shell in the uterus when female IVF was performed.

Fertility rate: The incubation process was carried out three times throughout the experiment period with one bubble per 28 days of the experiment. The fertilized eggs were collected in the five days following the second day of the fertilization process, the eggs were stored in the at 15.5 °C and were incubated in a hatched type of the Belgian-type Petersime origin of the hatchery of the poultry research station of the Department of Agricultural Research / Abu Ghraib. After the completion of the hatching process, the number of dead embryos was registered by cracking the non-fouled eggs and placing them in plastic containers and freezing them for the purpose of conducting the analyzes, and then calculating the fertility rate and the percentage of the dead embryos according to the following equations:

Fertility ratio=(fertilized egg number)/(total egg number) x 100

Dead embryos ratio % = (number of dead embryos)/

(fertilized egg number) x 100

Hatching rate: The rate of hatching was calculated after counting the number of chicks, according to the following equations:

Hatching ratio of total eggs %= (Hatches number)/ (total egg number) X 100

Hatching ratio of Fertilized eggs of % = (Hatches number) / (fertilized egg number) X100

Sex ratio of chicks

Secondary sexual rates: After the hatching process was completed, the broiler chicks were counted by placing iron numbers in the wing and raising them for 4 weeks until the secondary sex signs appeared. Then, the number of females was calculated and applied the equation of the secondary sexual ratio of the chicks as follows:

Secondary female percentage = Hatches number of female/ Hatches number of chicks x 100%

Initial sexual Ratio: The primary sex ratio of fertilized eggs was calculated as in the following equation:

Primary sex ratio% = [(Hatches number of female + number of dead embryos) / (Hatches number of chicks + dead embryos)] x 100

Polymerase chain reaction (PCR): Dead embryos were naturalized during embryonic growth by cracking non hatched eggs. Sample of the dead fetus (liver) was taken and stored in 10 ml plastic containers and frozen at a temperature of -20 ° C and transferred to the laboratory for polymerase chain reaction (PCR). The Polymerase Chain amplification process was performed using the Primer initiator, which targets the specific sequence of a gene found on the W chromosome in the female chicks, to amplify the chain reaction PCR (Kalina *et al*., 2012). The sex of the dead fetus was determined through the gel images, as the females were

determined by the presence of the separated bundle as a result of the amplification of the chain of the gene-dependent area of the embryo on the W chromosome of the female. This package was not observed in the male embryo models.

Distribution of corticosteron: Corticosteron levels in maternal blood were divided into three categories during the three periods and their overall rate, and included:

Class I: Low concentration is smaller than 14 ng /100 ml.
Class II: Medium concentration between 14-19 ng /100 ml.
The third category: the high is greater than 19 ng /100 ml.

On the basis of categories, the data were statistically analyzed to show the effect of the corticosteron on the studied traits. Table 2 shows the numbers of birds by category and duration.

Table 2 : Distribution of corticosteron by duration

Category	Duration			
	Low	Medium	High	Total
First period	32	21	47	100
Second period	54	12	34	100
Third period	43	29	28	100
Average	38	30	32	100

The data of this study were analyzed according to complete randomize design (CRD) and the differences between the averages were compared with the Duncan (1955) multidimensional test. The statistical program SPSS (2010) was used in statistical analysis.

Results and Discussion

Corticosteron concentration: Table 3 shows sigificant difference ($p < \mathbf{0.0001}$) in the concentration of corticosterone (ng/100 ml) in maternal blood by groups in the first, second, third, and general periods. The first group (greater than 19 ng/100 ml) recorded the highest rate of corticosteron in maternal blood, followed by the medium group (14-19 ng/100 ml) and then low (smaller than 14 ng/100 ml).

Hatching rate: The results of Table 4 showed a significant ($P<0.05$) superiority in the percentage of hatching broiler chicks (%). The lower corticosteron group (ng <14) had the highest hatching rate compared to the medium and high categories of corticosteron during the first period. A significant increase ($P <0.05$) was observed for the high concentration (greater than 19 ng/100 ml) compared to the medium concentration (14-19 ng/100 ml), with insignificant difference with the low concentration (less than 14 ng/100) during the second period. The same table showed that the effect of corticosteron concentration was not significant in the percentage of hatching chicks in the third period. In terms of the effect of the general rate of corticosteron concentration in the overall rate of hatch percentage, the same table showed a significant decrease in the high group compared to the medium category, with no significant differences between the two groups compared to the low category.

Secondary sexual ratio of hatching female: Table 5 shows a significant decrease ($P <0.05$) in the secondary sex ratio of female (%) of medium category mothers (14-19 ng/100 ml) compared to the low and high corticosteron levels during the first period. The same table shows a significant increase ($P <0.05$) in the secondary sexual ratio of females in the higher group of corticosteron compared to the low group, with no significant differences between the two groups, compared to the average during the second period. The same table showed that the concentration of corticosteron in the medium group (14-19 ng/100 ml) was significantly higher than the high percentage of secondary sex ratio of hatching female and did not differ from the low group during the third period.

Regarding the total average, there was a significant increase ($P <0.05$) in the secondary sex ratio of the female (%) at the low concentration of the corticosteron compared with the medium concentration (14-19 ng/100 ml) Comparison with high concentration, which did not differ significantly from the medium concentration.

The primary sex ratio of the hatching females: Table 6 shows a significant ($P <0.05$) increase in the primary sex ratio of hatching females (%) for the high group of corticosteron concentration (greater than 19 ng/ml) compared to the medium group, while no significant differences were observed between the high and low groups (Smaller than 14 ng/100 ml) and between medium and low during the first and second periods. However, a significant superiority was observed for low and medium groups compared to the high group of corticosteron concentration during the third period. The low group also had a significant effect on the other two groups in the total rate of corticosteron concentration in the primary sex ratio of the hatching female.

The decline in the percentage of hatching may be due to a high concentration of corticosteron in the blood of local chicken mothers. Glucocorticoids disrupt fetal growth during incubation and inhibit fetal cell function (Kaltner *et al*., 1993). The high concentrations of corticosteron in the fetus reduce the percentage of the hatch sharply, due to corticosteron receptors in the faces and limbs of the fetus, which cause deformities in those parts of the embryo's body, resulting in high mortality and low hatching rate (Pavlik *et al*., 1986). Mashaly (1991) showed that the low concentration of corticosteron increases the percentage of hatching. This study showed that there was an inverse relationship between corticosteron concentration in mothers and the percentage of hatching.

The difference in the levels of corticosteron in the blood of the mother works to confuse the activity of sex hormones, which in turn leads to the modification of the sexual ratio during the stage of division of the first thread, as the high concentration of corticosteron hormone with the moderation of the concentration of progesterone and testosterone before a specific period of ovulation can work on the deviation of the sexual ratio by affecting sexual chromosomes (DuRant *et al*., 2016). Corticosteron, progesterone and testosterone can also affect sexual ratios at high or low levels before ovulation by hours (4 or 5 hours) through influencing the reduction process itself (Correa *et al*., 2005; Gam *et al*., 2011; Pinson *et al*. 2011). It was observed that the high concentration of corticosteron hormone leads to a high concentration of progesterone in the blood (Etches and Croze, 1983), also showed that the sharp increase in the concentration of corticosteron hormone during the approximate time of mayo se division affects the secondary sexual ratio (Gam and Navara, 2016). In conclusion, the difference in the level of concentration of corticosteron in blood of Iraqi local chicken mothers directly affects the secondary and primary sex ratios, in different directions. Additionally, it have a clear effect on the offspring produced and the relationship between the level of corticosteron hormone and hatching rate was inverse relationship which means that the stress leads to a decrease in the percentage of hatch.

Table 3 : Level of corticosterone hormone (ng/100 ml) in maternal blood according to hormone categories (mean + standard error)

Concentration of corticosteron (ng/100 ml)	Concentration of corticosteron hormone (ng/100 ml)			
	Periods (day)			Average
	1	2	3	
Low (less than 14)	0.42± 9.31 C	6.87± 0.48 C	9.96± 0.39 C	11.30± 0.35 C
Medium (14-19)	0.31± 16.85 B	16.87± 0.51 B	16.11± 0.26 B	15.98± 0.21 B
High (more than 19)	0.74± 25.72 A	26.27± 0.99 A	23.82± 1.01 A	22.62± 0.50 A
Significance level	0.0001	0.0001	0.0001	0.0001

Variable letters within one column indicate significant differences between averages

Table 4 : Effect of corticosteron concentration in the blood of Iraqi local chicken mothers on hatching rate (mean + standard error)

Concentration of corticosteron (ng/100 ml)	Hatching chicks percentage %			
	Periods (day)			Average
	1	2	3	
Low (less than 14)	A 88.03± 5.41	AB 59.13± 6.08	66.73± 7.25	AB 68.05± 4.28
Medium (14-19)	B 76.19± 4.52	B 53.40± 5.08	64.83± 8.91	A 73.89± 4.81
High (more than 19)	B 73.96± 3.27	A 66.77± 7.19	61.61± 9.05	B 63.31± 4.17
Significance level	0.05	0.05	N.S	0.05

Variable letters within one column indicate significant differences between averages.
N.S: There are no significant differences

Table 5 : Effect of corticosteron concentration in maternal blood on secondary sexual percentage of hatching female (%; mean + standard error)

Concentration of corticosteron (ng/100 ml)	Secondary sexual percentage of hatching female			
	Periods (day)			Average
	1	2	3	
Low (less than 14)	48.28±3.40 A	41.25±4.42 B	50.27±3.54 AB	51.43±2.80 A
Medium (14-19)	35.90±4.96 B	43.90±2.57 AB	58.14±3.98 A	42.83± 3.03 B
High (more than 19)	49.42±2.90 A	51.14±3.63 A	46.39±3.66 B	47.24±2.85 AB
Significance level	0.05	0.05	0.05	0.05

Variable letters within one column indicate significant differences between averages

Table 6 : Effect of corticosteron levels in the blood of local chicken mothers on the primary sexual percentage of hatching females (%; mean + standard error)

Concentration of corticosteron (ng/100 ml)	Primary sexual percentage of hatching females (%)			
	Periods (day)			Average
	1	2	3	
Low (less than 14)	52.72 ± 2.91AB	51.43 ± 3.84 AB	57.61± 2.16 A	58.52± 2.63 A
Medium (14-19)	48.52± 3.82 B	44.00 ± 2.60 B	56.38 ± 3.86 A	49.32± 3.30 B
High (more than 19)	54.85± 4.36 A	54.60 ± 3.17 A	50.00± 2.44 B	50.56± 2.37 B
Significance level	0.05	0.05	0.05	0.05

Variable letters within one column indicate significant differences between averages

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