EFFECT OF DIETARY SUPPLEMENTATION WITH CLOMIPHENE CITRATE ON SOME PRODUCTIVE AND PHYSIOLOGICAL TRAITS OF LAYING JAPANESE QUAIL

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ABSTRACT

This study was conducted to evaluate the effect of supplementation different levels of Clomiphene citrate to the diet of female quail on productive and some physiological traits. The experiment was conducted in a commercial farm from 2/3/2021 to 24/5/2021 (3 months). Females aged 50 weeks (160 females) were randomly distributed into four treatments, and each treatment included four replicates (10 birds for each replicate). The treatments were divided into a control group (T1; basic diet only), second treatment (T2; basic diet + 0.25 mg / female / day), third treatment (T3; basic diet + 0.50 mg / female / day, fourth treatment (T4; basic diet + 0.75 mg / female / day Clomiphene citrate). The results of the study indicated a significant improvement in favor of the addition treatments compared with the control treatment when calculating the average (%) of eggs production, the average of eggs weight (gm), the average of eggs mass (gm/female/day), and cumulative egg number (egg/female). As for the physiological characteristics, the results shown a high concentration of calcium and phosphorous in the blood serum within the additive treatments compared with the control While there were no significant differences observed among treatments in total protein, albumin, globulin, and cholesterol. It could be conclude from this study that the addition of Clomiphene citrate to the diet improved the reproductive and physiological characteristics of female quail.

Keywords: egg production, egg weight, blood serum, blood proteins, phosphorous concentration.

تأثير إضافة كلومفين ستريت إلى العليقة في بعض الصفات الانتاجية والفسلجية لاناث السمان الياباني

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المستخلص

اجريت الدراسة لتقيم فعالية تاثير اضافة مستويات مختلفة من الكلومفين ستريت الى عليقة اناث طائر السمان في الصفات الانتاجية وبعض الصفات الفسلجية. اجريت التجربه في مزرعة تجارية من 2 / 3 / 2021 الى 24 / 5 / 2021 (3 شهر)، استعمل 160 انثى بعمر 50 اسبوع وزعت عشوائيا الى اربع معاملات كل معاملة شملت اربعة مكررات (10 طير لكل مكرر) وقسمت المعاملات الى معاملة السيطره (T1) عليقة اساسية فقط، المعاملة الثانية (T2) عليقة اساسية +0.25 ملغم / انثى / يوم ، المعاملة الثالثه (T3) عليقة اساسية +0.50 ملغم / انثى / يوم ، المعاملة الرابعة (T4) عليقة اساسية +0.75 ملغم / انثى / يوم كلومفين ستريت. اشارت نتائج الدراسة الى تحسن معنوي لصالح معاملات الاضافة مقارنتا مع معاملة السيطرة عند حساب معدل انتاج البيض (%)، معدل وزن البيض (غم) ، معدل كتلة البيض (غم / انثى / يوم) و عدد البيض التراكمي (بيضة / انثى). اما بالنسبة الى الصفات الفسلجية فاشارت النتائج الى ارتفاع تركيز الكالسيوم والفسفور في مصل الدم لمعاملات الاضافة مقارنتا مع معاملة السيطره ولم يلاحظ وجود فروق معنوية بين المعاملات عند حساب البروتين الكلي، الالبومين، الكلوبيولين والكولسترول. نستنتج من الدراسة ان اضافة الكلومفين ستريت الى العليقة حسن الصفات الانتاجية وبعض الصفات الفسلجية لاناث طائر السمان.

الكلمات المفتاحية: انتاج البيض، وزن البيض، مصل الدم، بروتينات الدم، تركيز الفسفور .

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INTRODUCTION

Quail eggs (*Coturnix coturnix*) production is the main goal of chicken breeders, as it is used either for human consumption or for the purpose of other industries such as glue or to produce chicks in the fields of laying hens and broiler breeder. Through age, egg production begins to decrease, and then the herd must be replaced with another one (6). Quail egg is a rich source of nutrients beneficial to human health and high in nutritional value, which is 3-4 times more than chicken eggs (16), so it became highly desirable by the consumer. There are many medicines and drugs were used to treat infertility including Clomiphene citrate (11), which has been used since 1962 (25), and stimulates the process of ova and sperm formation (4). Clomiphene citrate helps pregnancy process by stimulating the body and pituitary hormones as well as stimulating the ovaries to produce mature eggs that are suitable for fertilization, Clomiphene mechanism is by influencing the hormonal chain that regulates ovulation process. At the beginning of each menstrual cycle, the hypothalamus gland sends gonadotropin hormones to the pituitary gland, and this gland secretes follicular stimulating hormone (FSH) and luteinizing hormone (LH) to stimulate the ovary to produce the estrogen (13,19,33). Clomiphene also used to solve anovulation and irregularity cycle problems because there is a drop in ovulation and cases of Polycystic ovaries (7,20), Clomiphene may be useful in pregnancy because it helps the ovaries to produce an egg and helps the female to get pregnant by stimulating sex hormones. As for ovulation, it usually occurs 10-5 days after the date of the last dose of Clomiphene taken. The effectiveness of Clomiphene is through stimulating the secretion of gonadotropin-releasing hormone (GnRH), which in turn releases luteinizing hormone (LH) and follicle-stimulating hormone (FSH) (11) These hormones have the function of controlling the gonads in females and males (28). As a result of the lack of research on the use of this drug on quail, this study aimed to know the effect of using different levels of Clomiphene citrate on productive traits and some physiological traits, and to determine the best concentration.

MATERIALS AND METHODS

In this experiment, 160 females 50 weeks age, raised in cages, were used, and they were randomly distributed to 4 treatments Each treatment had 4 replicates (10 females/replicate) for a period of 12 weeks. They were fed by balanced diets of energy and protein (Table 1). Water and feed were available throughout the experiment period (*ad libitum*) freely. The birds were equipped with 16 hours of light per day. Clomiphene citrate was added as an addition to the diet as follows:

T1: The first treatment (control) was without adding clomiphene citrate

T2: The second treatment was with addition of 0.25 mg clomiphene citrate / female /day

T3: The third treatment was with adding 0.50 mg clomiphene citrate / female /day

T4: Fourth treatment was with adding 0.75 mg clomiphene citrate / female /day

Table 1. The chemical compositions of the experiment diet

Ingredients	%
Soybean	25
Wheat	31.9
Corn	30
Concentrated Protein	5
Corn Oil	2
D.C.P.	0.3
Limestone	5.5
NaCl	0.3
Total	100
Calculated chemical analysis	
Energy (Kcal/ Kg)	2894
Protein (%)	19.5
Lysine (%)	1.2
Methionine (%)	0.57
Ca (%)	2.5
Available P (%)	0.49

The feed format was designed according to 24

Productivity traits were calculated (egg weight, egg production %, egg mass, and cumulative number of eggs) as indicated by (23) and (15). As for the physiological characteristics, blood samples were collected (4 birds/treatment) at the age of 55 and 61 (week) by Venipuncture of the cutaneous ulnar vein (wing or brachial vein). A 10 ml glass tube without anticoagulant was used to collect the blood. the centrifugation of blood was conducted at 3000r/min cycles for 14 min, then the serum was stored at - 18°C for further analysis. Total protein, albumin, globulin, cholesterol, calcium and phosphorous concentration were estimated following the kit instructions.

Statistical analysis

Complete random design (CRD) was used to analyze the effect of different treatments on the studied traits and the significant differences between the means were compared with test (5), and the statistical program (27) was used to analyze the data.

RESULTS AND DISCUSSION

It was noticed from the data shown in Table (2) that there were a significant differences among the treatments (P<0.01) in the percentage of egg production. The fourth treatment (T4) had higher increasing than T1, T2 and T3, and the third treatment (T3) increased than T1 and T2 through the first period of the experiment. In the second and third periods of the experiment, it was noticed that the fourth treatment (T4) was significantly superior than T1, T2 and T3 (P<0.01), while the third treatment was significantly higher (P<0.01) than T1 and T2. However, the second treatment (T2) was higher than the control for the average of egg production, the results indicated that there were significant differences between treatments (P<0.01). The fourth treatment (T4) outperformed than T1, T2 and T3. Also, T3 outperformed than T1 and T2

Table 2. Effect of adding clomiphene citrate to the diet on the percentage of egg production HD (%) of quail (mean ± standard error)

Treatments	Periods			Total Average
	1st	2nd	3rd	
T1	64.64±1.08^{c}	65.89±0.66^{d}	63.21±0.64^{d}	64.58±0.72^{c}
T2	66.71±0.77^{c}	68.03±0.69^{c}	65.62±0.61^{c}	66.79±0.60^{c}
T3	69.73±0.45^{b}	71.25±0.45^{b}	68.40±0.68^{b}	69.79±0.37^{b}
T4	73.66±0.47^{a}	75.18±0.37^{a}	73.31±0.98^{a}	75.36±1.18^{a}
Significant Level	**	**	**	**

****; refer to the different significantly (P <0.01) between the experiment treatments**

T1; Control , T2,T3 and T4 supplemented with 0.25, 0.50 and 0.75 mg clomiphene citrate /female /day respectively

When the average egg weight was calculated (Table 3), there were no significant differences among the treatments through the three periods of the experiment, but the general average had significant differences among treatments (P<0.05), as T2 and T4 outperformed treatments T1 and T3.

Table 3. Effect of adding clomiphene citrate to the diet on average egg weight (g) of quail (mean ± standard error)

Treatments	Periods			Total Average
	1st	2nd	3rd	
T1	10.59±0.35	10.88±0.24	10.79±0.42	10.75±0.16^{b}
T2	11.38±0.24	10.95±0.20	10.96±0.27	11.10±0.10^{a}
T3	11.05±0.29	11.12±0.19	11.02±0.27	11.06±0.70^{ab}
T4	11.44±0.19	11.44±0.17	10.82±0.18	11.24±0.06^{a}
Significant Level	N.S	N.S	N.S	*

***: - mean significant differences among the treatments at the level (P <0.05).**

N.S: - No significant differences between treatments

T1; Control , T2,T3 and T4 supplemented with 0.25, 0.50 and 0.75 mg clomiphene citrate /female /day respectively

The results shown in Table (4) indicated that there were a significant differences among treatments (P<0.01) in the egg mass and general average of egg mass. In the first period of the experiment, the fourth treatment (T4) outperformed than treatments T1, T2 and T3.

Also, treatments T2 and T3 outperformed than the control treatment. Similarly, in the second and third period of the experiment, results indicated that the fourth treatment (T4) was significantly superior to treatments T1, T2 and T3, and treatment T3 was higher than the control. When calculating the general average of egg mass, the results indicated that there were a significant differences (P<0.01) among the experimental groups, were T4 was superior to the rest of the treatments, and treatments T2 and T3 outperformed the control treatment.

Table 4. Effect of adding clomiphene citrate to the diet on average egg mass (gm/ female /28day) of quail (mean ± standard error)

Treatments	Periods 1st	2nd	3rd	Total Average
T1	191.76±8.24^{c}	200.64±3.37^{c}	190.85±6.08^{c}	583.17±7.72^{c}
T2	212.65±5.77^{b}	208.52±3.09^{bc}	201.08±5.34^{bc}	622.65±5.62^{b}
T3	215.79±5.37^{b}	221.76±4.57^{b}	210.74±4.13^{ab}	648.69±5.70^{b}
T4	235.97±4.76^{a}	240.83±6.65^{a}	222.19±5.55^{a}	711.29±14.36^{a}
Significant Level	**	**	**	**

****; refer to the different significantly (P <0.01) between the experiment treatments**

T1; Control , T2,T3 and T4 supplemented with 0.25, 0.50 and 0.75 mg clomiphene citrate /female /day respectively

It was noticed when calculating the average number of eggs and the general average in Table (5) that there were significant differences among the groups (P<0.01), as the fourth treatment (T4) outperformed the other groups, and T3 outperformed the other two treatments T1 and T2 in the first period of the experiment, while in the second and third periods of the experiment. The experiment shows that the fourth treatment (T4) was superior to the rest of the treatments, treatment T3 was superior to treatments T1 and T2, and the second treatment was superior to the control treatment (T1). When calculating the general average, the results indicated that there were differences significantly (P<0.01) among the experiment groups, were T4 outperformed the other groups, treatment T3 outperformed treatments T1 and T2, and the second treatment outperformed the control treatment (T1).

Table 5. Effect of adding clomiphene citrate to the diet on the average cumulative number of eggs (egg/ female) of a quail (mean ± standard error)

Treatments	Periods 1st	2nd	3rd	Total Average
T1	18.10±0.30 ^{c}	18.45±0.18^{d}	17.70±0.18^{d}	54.25±0.61^{d}
T2	18.65±0.22^{c}	19.05±0.19^{c}	18.38±0.17^{c}	56.08±0.52^{c}
T3	19.53±0.13^{b}	19.95±0.13^{b}	19.15±0.19^{b}	58.63±0.31^{b}
T4	20.63±0.13^{a}	21.05±0.10^{a}	20.53±0.28^{a}	62.20±0.47^{a}
Significant Level	**	**	**	**

****; refer to the different significantly (P <0.01) between the experiment treatments**

T1; Control , T2,T3 and T4 supplemented with 0.25, 0.50 and 0.75 mg clomiphene citrate /female /day respectively

The results of the statistical analysis in Table (6) indicated that there were no significant differences between treatments when calculating total protein, albumin and globulin at 55 and 61week of age

Table 6. Effect of adding clomiphene citrate to the diet on blood proteins (g / 100 ml) of quail at 55 and 61 week of age (mean ± standard error)

Treatments	55 week			61 week		
	Total protein	Albumen	Globulin	Total protein	Albumen	Globulin
T1	6.86± 0.15	3.95 ± 0.27	2.91 ± 0.12	6.94 ± 0.18	4.13 ± 0.31	2.81 ± 0.14
T2	6.89 ± 0.16	3.96 ± 0.47	2.93 ± 0.36	6.91 ± 0.19	4.00 ± 0.39	2.91 ± 0.22
T3	6.87 ± 0.32	3.94 ± 0.16	2.94 ± 0.24	6.93 ± 0.36	4.14 ± 0.23	2.79 ± 0.21
T4	6.88 ± 0.29	3.95 ± 0.10	2.92 ± 0.25	6.91 ± 0.31	4.18 ± 0.15	2.74 ± 0.20
Significant Level	N.S	N.S	N.S	N.S	N.S	N.S

N.S: - No significant differences between treatments

T1; Control , T2,T3 and T4 supplemented with 0.25, 0.50 and 0.75 mg clomiphene citrate /female /day respectively

No significant differences were observed between the groups for the blood cholesterol concentration at the age of 55 and 61 week, but not for calcium and phosphorous concentrations (P<0.05) in additive treatments (T2, T3 and T4) was significantly superior to the control (T1)

Table 7. Effect of adding clomiphene citrate to the diet on cholesterol level and calcium and phosphorous concentration (mg/100ml) for quail at 55 and 61 week of age (mean ± standard error)

Treatments	55 week			61 week		
	Cholesterol	Ca	P	Cholesterol	Ca	P
T1	226.75 ± 18.72	9.22 ± 0.66[b]	6.7 ± 0.15[b]	233.00 ± 17.24	9.34 ± 0.73[b]	6.66 ± 0.25[b]
T2	226.00 ± 17.37	10.88 ± 0.53[a]	7.06 ± 0.72[ab]	232.25 ± 17.92	11.13 ±0.28[a]	7.44 ± 0.31[a]
T3	226.5 ± 22.72	10.99 ± 0.10[a]	6.92 ± 0.26[ab]	234.00 ± 27.77	11.55 ± 0.44[a]	7.43 ± 0.51[a]
T4	226.63 ± 16.87	11.19 ± 0.19[a]	7.09 ± 0.15[a]	232.00 ± 21.75	11.44 ± 0.15[a]	7.40 ± 0.36[a]
Significant Level	N.S	*	*	N.S	*	*

***: - mean significant differences among the treatments at the level (P <0.05).**

N.S: - No significant differences between treatments

T1; Control , T2,T3 and T4 supplemented with 0.25, 0.50 and 0.75 mg clomiphene citrate /female /day respectively

When calculating the general average of total protein, albumin and globulin in serum (50-61 week) presented in Figure (1), found out no significant differences among the groups, and when calculating the general average of cholesterol, calcium and phosphorous. Figure (2) indicated that the cholesterol level was not affected by treatment, while a significant differences were noted in calcium and phosphorous concentrates among the groups, as the addition treatments recorded a significant increase (P<0.05) compared with the control treatment.

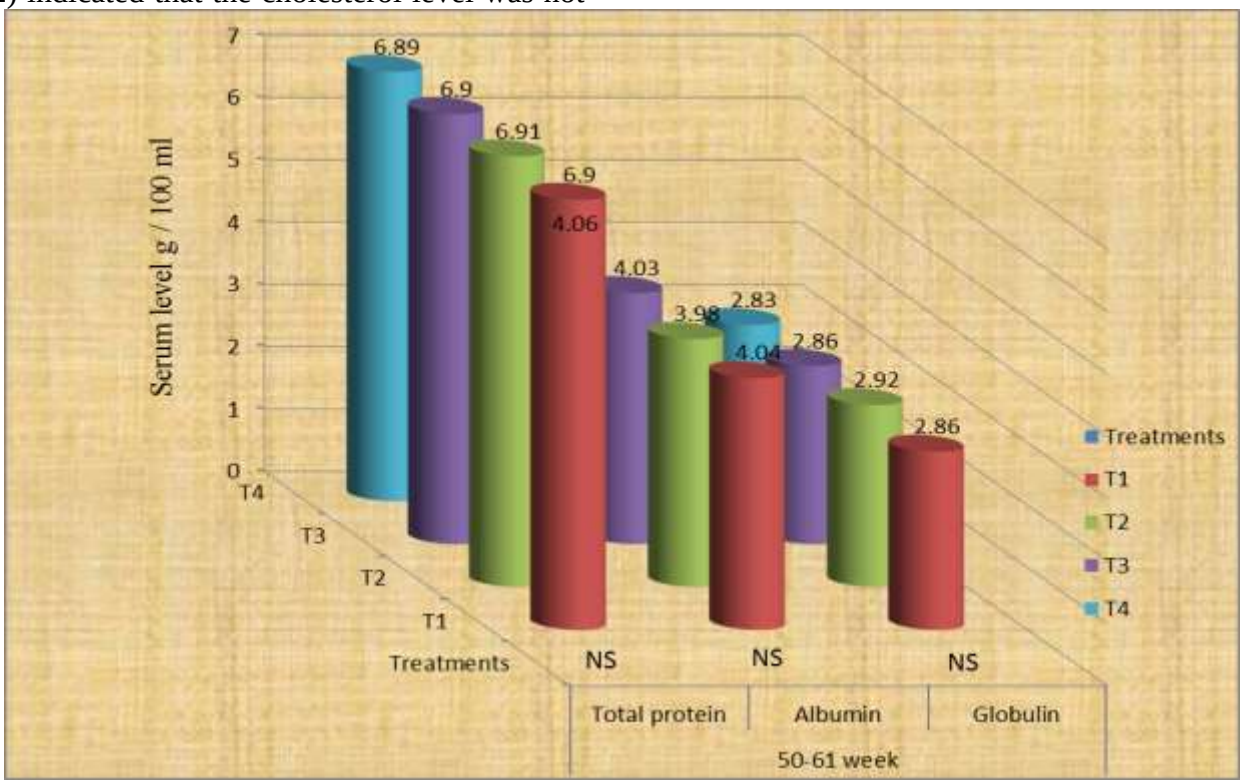

Figure 1. Effect of adding clomiphene citrate to the diet on the general average of blood proteins (g / 100 ml) of quail at 50-61 week of age

N.S: - No significant differences between treatments.

T1; Control , T2,T3 and T4 supplemented with 0.25, 0.50 and 0.75 mg clomiphene citrate /female /day respectively

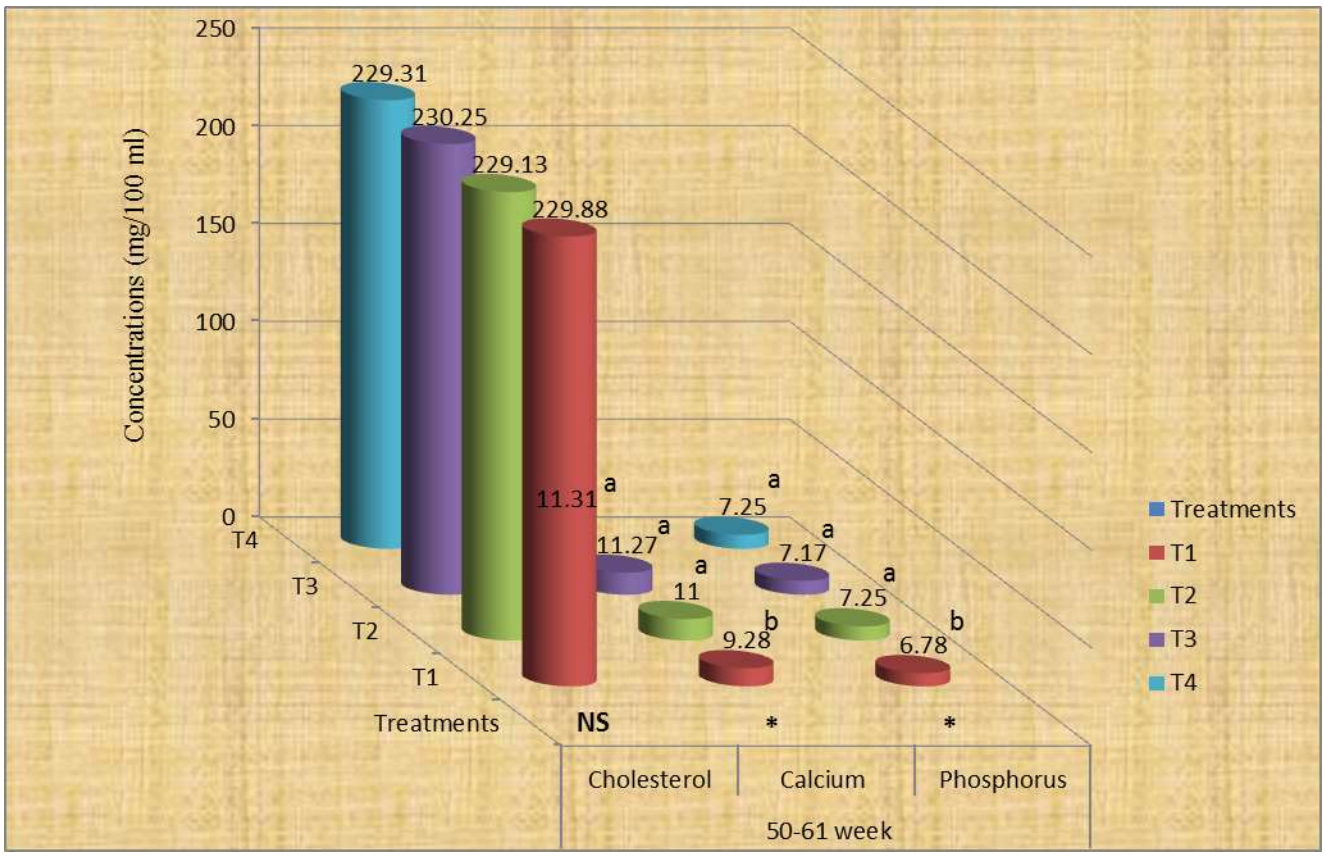

Figure 2. Effect of adding clomiphene citrate to the diet on the general average of cholesterol level and calcium and phosphorous concentrations in blood serum (mg/100 ml) for quail at 50-61 week of age

***: - mean significant differences among the treatments at the level (P <0.05).**

N.S: - No significant differences between treatments

T1; Control , T2,T3 and T4 supplemented with 0.25, 0.50 and 0.75 mg clomiphene citrate /female /day respectively

The reason for the improvement in the productive traits may be due to the role of clomiphene in stimulating the gonadotropin-releasing hormone (GnRH) (28), and thus increasing the secretion of FSH and LH hormones that are important for the development of the ovaries and oviducts, the growth of ovarian follicles and ovulation (32). These hormones lead to the maturation of the small ovarian follicles (33) and an increase in their number as well as the preparation of the ovarian follicles by the effect of luteinizing hormone, which the follicle needs to reach full size, and the secretion of estrogen as its effect on granulosa cells and theca cells (32), theca cells do not respond to LH when the ovarian follicle is less than 12 mm in diameter (34) while granulosa cells respond to FSH when the ovarian follicle is 6-8 mm in diameter (32). The increase in the secretion of FSH and LH occurs through the effect of clomiphene on the pituitary gland (19), which has a major role in the increase in the concentration of estrogen in the blood plasma through the role of these hormones in stimulating the secretion of estrogen from the theca cells of young follicles (17, 22). Estrogen affects the process of inducing ovulation as a result of its effect on the effectiveness of follicle stimulating hormone and luteinizing hormone (1,12), and plays a major role in promoting the growth of the oviduct (10, 14, 21) with increasing the secretion of tubular glands responsible for the production of ovalbumin, conalbumin, lysozyme , antimicrobial and ovotranferrin (18,29). Also, it helps in the manufacture of special proteins in the oviduct and the precursor to the yolk proteins vitellogenin. It also stimulates in general, the manufacture of yolk vitellogenesis by acting directly on the liver in addition to its role in modifying the progesterone receptors found in the cytoplasm of the genital tract (32), on the other hand, estrogen plays a major role in the deposition of calcium within the pulpal part of the long bones, which in turn is a reserve source of

calcium during the period of high egg production. It is also important for regulating proteins involved in calcium metabolism necessary for eggshell calcification, which include epithelial calcium channels (TRPVS) and Calbindin (2). On the other hand, the secretion of FSH and LH hormones increases the effectiveness and activity of the ovary and increases egg production by increasing the number and size of mature follicles which leads to an increase in the secretion of estrogen that increases the deposition of yolk proteins (31). Artificial estrogen treatments with diethylstilbestrol to chicken increases the growth, development, and differentiation of the oviduct and changes expression of genes related to tubular gland formation, epithelial cell differentiation, hormone interactions, nerve development, and tissue remodeling (30).The high concentration of estrogen is accompanied by an increase in calcium and phosphorous levels in the blood plasma to meet the body's production needs of the necessary elements (26), Estrogen receptors were detected in the duodenal tissue (3). On the other hand, the complex interaction between calcium and estrogen also includes estrogen activation of vitamin D, which in turn enhances the transport of calcium from the gastrointestinal tract and increases its concentration in the blood (9). The increasing in estrogen concentration in blood plasma plays a major role in increasing the production of the active form 1,25 (OH) 2D 3 as a result to the decrease in the concentration of calcium, as the active form of vitamin D3 (1,25 (OH) 2D 3) acts to control on the process of transferring calcium from the small intestine or bones to the uterus to be deposited in the calcareous shell there, as the increase in egg production is accompanied by an increase in the absorption of calcium from the small intestine (32). The increase in the concentration of both calcium and phosphorous directly affects the increase in thyroid activity as a result of its secretion of hormones responsible for regulating their level in the blood and thus increasing metabolic processes within the body, which positively affects the productive performance, especially egg production (8).

CONCLUSION

The results of the experiment indicated that the supplementation of clomiphene citrate to the laying Japanese quails diet improved productive traits and physiological performance, especially when adding Clomiphene citrate at a concentration of 0.75 mg / bird / day.

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