

EFFECT OF GONADOTROPHIN STIMULANT (THERIOGON) TREATMENT ON ALEXANDRIA COCKERELS REPRODUCTIVE PERFORMANCE

By

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Abstract: *Fourty, 40 weeks old Alexandria cockerels were distributed among 4 treatments to study the effect of GnRH stimulant (Theriongon) administration, on their reproductive performance. Birds of the second, third and fourth group were fed with 0.25, 0.5 and 1.00 g Theriongon/ kg feed, respectively for 2 months. Birds of the first group served as untreated control. Theriongon had significantly increased testosterone. Birds treated with the 0.25 and 0.50 and 1.00 g Theriongon/ kg feed had significantly higher ejaculated volume as it increased by 84, 84 and 87 % respectively, compared to the untreated males. Birds treated with the 0.25 and 0.5 and 1.00 g Theriongon/ kg feed had significantly higher motility as it increased to reach 119, 127 and 124 % of control respectively. Theriongon treatments from 40 to 48 weeks of age had significantly increased sperm concentration in a dose dependent manner as it increased to reach 146, 161 and 189 % of control with the three doses of Theriongon respectively. Theriongon treatments significantly increased blood hematological parameters Plasma glucose increased significantly with the GnRH stimulant treatment. Blood cholesterol was not affected by Theriongon treatment whereas; total lipids were increased significantly to reach 114, 115, and 113% of control and total protein increased significantly to reach 107, 122 and 111 % of controls level with the three doses of Theriongon, respectively. Whereas, globulin levels increased significantly with the 0.50 g dose of Theriongon only to reach 140 % of control. Seminal plasma constitutes showed a reflection of the blood status. It can be concluded that the veterinary gonadotrophin stimulant was capable of improving 40 week old Alexandria cockerels reproductive status.*

INTRODUCTION

Gonadotropin-releasing hormone (GnRH) controls the reproductive physiology and behavior of vertebrates by stimulating synthesis and release of gonadotrophin from the pituitary gland (**Ubuka *et al.*, 2008**). Testicular function both in birds and mammals is controlled by factors from the outer and inner environments, which are integrated in the brain to modulate gonadotrophin releasing hormones (GnRH) secretion which is responsible of stimulating the gonadotropes of the pituitary to secrete Luteinizing hormone (LH) and Follicle stimulating hormone (FSH) (**Sharp and Gow 1983**). Although little information is available concerning the effect of GnRH or its stimulant treatment on local chicken strains, it was well investigated in different mammalian species. **Marai *et al.*, (1998)** reported a significant improvement in semen quality and reproductive performance of male rabbits treated with GnRH, and an increase in testosterone in bulls was reported by (**Kozdera *et al.*, 1993**).

Little is known about the effect of GnRH treatment on galliform species, however, the genetic improvements of the modern strains of chickens led to impaired fertility, late sexual maturity and some reproductive problems associated with the related Neuroendocrine axis (**Weil *et al.*, 1999**). Therefore, the present study was conducted to investigate effects of different levels of GnRH stimulant treatment on 40 week old Alexandrian cockerels.

MATERIALS AND METHODS

The present study was carried out at the poultry research center, Faculty of Agriculture, Alexandria University, using 40 week old Alexandrian cockerels.

Theriogon:

Theriogon® used in this study is a veterinary gonadotropin stimulant sold as a powder, each 100 gm contain 100 gm Alpha-amino-p-hydroxyhydrocinnamic acid, Produced by Adwia, Egyptian Company for Chemicals and Pharmaceuticals.

Experimental design:

A total of forty, 40 weeks old Alexandrian cockerels were randomly and equally distributed among 4 treatment groups (10 birds each). Each group was subdivided into tow equal replicates (5 birds each). Birds of the second, third and fourth group were fed ration added with 0.25, 0.5 and 1.00 g Theriogon/ kg feed, respectively for 2 months. Birds of the first group

served as untreated control. Feed and water were provided *ad libitum* throughout the 2 months experimental period.

Data collected:

Semen specimens were artificially collected biweekly free of transparent fluid by abdominal massaging the back of male and collecting semen by suction in a clean collecting tube. The collecting tube was held in an insulated block to maintain a suitable temperature during the period of semen collection according to the method of **Burrows and Quinn (1937) and Khalifa (1977)**. technique. Ejaculated volume was measured to the nearest 0.01 ml. A weak eosin-formalin (10 % formalin) solution was used for evaluation of sperm concentration by the improved Newbuer hemocytometer slide as described by **Smith and Mayer, (1955)**. Sperm motility was estimated at 400 X magnification on a warm stage adjusted at 38 °C (**Kamar, 1960**). Seminal plasma was obtained by centrifugation of semen samples at 3500 rpm for 20 min at 4 °C, and then stored at -20 °C until later analysis. Seminal plasma samples were analyzed for total protein, albumin, total lipids, and cholesterol levels, calorimetrically using available commercial kits (Diagnostic kits in 29 Tahreer St., Dokki, Giza, Egypt).

Blood samples were collected biweekly from the wing vein from five cockerels (randomly chosen) from each treated group at 9.0 a.m. before access to feed and water . Heparin was used as anticoagulant but a part of samples was withheld to obtain serum. Plasma or serum were obtained by centrifugation the blood at 3000 rpm for 20 minutes, and were stored at -20°C for biochemical analysis. Serum glucose, total protein, albumin, total lipids, and cholesterol, levels were determined calorimetrically using available commercial kits. Serum testosterone (ng/ ml) was determined by enzyme immunoassay using commercial kits purchased from Biosource.

Statistical analysis:

Means and standard errors were estimated for each studied trait. Data were analyzed using **SAS, (1996)** program, using general linear model. Significant differences among treatments and age were separated using Duncan's multiple range procedure (**Duncan, 1955**).

RESULTS AND DISCUSSION

Testosterone:

Data concerning testosterone concentration as influenced by Theriogon administration are presented in Table (1). Overall, birds treated with different doses of Theriogon from 40 to 48 weeks of age had

significantly higher testosterone level compared to the untreated males ($p \leq 0.0001$). Testosterone levels were increased in a dose dependent manner, as it increased by 112, 113 and 127 % compared to control with 0.25, 0.50 and 1.00 g/ kg feed of Theriogon, respectively. Regarding interaction of treatments with months ($p \leq 0.0215$), testosterone concentrations reached 98, 101 and 127 % by the end of the first month, and 125, 125 and 128 % by the end of the second month compared to control with 0.25, 0.50 and 1.00 g/ kg feed of Theriogon, respectively. This indicates that the first two doses (0.25 and 0.50 g/ kg feed) of Theriogon needed two months of treatment to show their effect on testosterone concentration.

The increase in testosterone concentration as a result of GnRH stimulant treatments comes in good agreement with the finding of **Barna and Mezes, (1994)** reported that treating cockerels with a GnRH analogue increased testosterone concentration compared to the untreated cockerels. **Hirschenhauser et al. (2000)** who reported that treatment of white geese with GnRH analogue (Ovurelin) resulted in an increased plasma testosterone concentration compared to the untreated birds. Also, **Bollwein et al. (2008)** reported elevation in testosterone of stallion after administration of human chorionic gonadotropin. Moreover, **Samar Elnagar, (2009)** reported that treating cockerels with a GnRH analogue (Receptal) increased testosterone concentration compared to the untreated birds.

Ejaculated volume:

Effect of GnRH stimulant treatments on semen ejaculated volume is illustrated in Table (1). Overall, birds treated with the 0.25 and 0.50 and 1.00 g Theriogon/ kg feed from 40 to 48 weeks of age had significantly higher ejaculated volume as it increased by 84, 84 and 87 % respectively, compared to the untreated males ($p \leq 0.0001$). Regarding interaction of treatments with months ($p \leq 0.0510$), Regarding interaction of treatments with months ($p \leq 0.0500$), ejaculated volume reached 177, 172 and 142 % by the end of the first month, and 194, 200 and 240 % by the end of the second month compared to control with 0.25, 0.50 and 1.00 g Theriogon/ kg feed of Theriogon, respectively. Treating cocks with Theriogon at any dose caused a significant increase in their semen volume that correlated with increased sperm concentration, this increase in semen volume may be used as indicator for increased nutrition elements that is needful for the sperm life

(**Elkomy, 2003**).

The increase in semen volume has been also shown in Holstein Friesian and Jersey bulls treated with GnRH (**Bhaskaran and Dubey, 2004 and 2005**). Similar results were also obtained when cockerels were treated with GnRH analogue (Receptal), (**Samar Elnagar, 2009**).

Sperm motility:

Effect of GnRH analogue treatments on sperm motility is illustrated in Table (1). Overall, birds treated with the 0.25 and 0.5 and 1.00 g Theriogon/ kg feed from 40 to 48 weeks of age had significantly higher motility as it increased to reach 119, 127 and 124% of control respectively ($p \leq 0.0001$). Interaction of treatments with months showed significant effect ($p \leq 0.0209$), where motility percent reached 123, 136 and 128 % by the end of the first month and 116, 119 and 120 % by the end of the second month compared to control with 0.25, 0.50 and 1.00 doses of Theriogon, respectively. Increased sperm motility in consequence of treating cocks with Theriogon may reflect on increased semen fructose (**Darre and Harrison, 1977**) concentration and increased live sperm percentage (**Ronayne et al., 1993**) in the Theriogon treated groups compared to the control.

This effect was also reported by **Bhaskaran and Dubey (2004 and 2005)** when Holstein Friesian and Jersey bulls were treated with GnRH. Also **Fathi et al., (2000)** observed increase in motility of naked neck cocks sperms when they were treated with GnRH, and **Samar Elnagar (2009)** reported improved sperm motility as a result of GnRH analogue treatment in cockerels.

Sperm concentration:

Data concerning sperm concentration as influenced by Theriogon administration are presented in Table (1). Overall, Theriogon treatments from 40 to 48 weeks of age had significantly increased sperm concentration in a dose dependent manner as it increased to reach 146, 161 and 189 % of control with the three doses of Theriogon respectively, ($p \leq 0.0001$). Regarding interaction of treatments with months ($p \leq 0.0001$), the increase in sperm concentrations reached 152, 163 and 217 % by the end of the first month and 139, 159 and 159 % by the end of the second month compared to control with 0.25, 0.50 and 1.00 doses of Theriogon, respectively. Theriogon treatment may be responsible for stimulating and supporting spermatogenesis (**Ronayne et al., 1993**) as the sperm concentration was significantly increased in the Theriogon treated groups compared to the control in a dose-dependent manner.

The increase in sperm concentration was also shown in Holstein Friesian and Jersey bulls treated with GnRH (**Bhaskaran and Dubey, 2004**). Also, **Fathi *et al.*, (2000)** reported that GnRH treatment improved sperm cell concentration of the naked neck cockerels especially after long-term treatment.

Seminal plasma:

Seminal plasma total protein increased significantly to reach 102, 125 and 114%, where albumin reached 116, 145 and 148 % and globulin increased significantly to reach 112, 131, 106 % with the low, medium and high dose of Theriogon, respectively, which comes in agreement with the findings of **Muske *et al.*, (1994)**, who reported an increase in the globulin level of chicken treated with GnRH. Seminal plasma constitutes showed a reflection of the blood status (Table 2). Theriogon treatment resulted in an increase in seminal plasma total lipids as it increased by 8, 18 and 9 %, cholesterol also increased by 3, 24 and 18 % with the low, medium and high dose of Theriogon, respectively.

It can be concluded that the veterinary gonadotropin stimulant was capable of improving 40 weeks old cockerels reproductive status as it increased circulating testosterone, ejaculated volume, sperm concentration, and sperm motility.

Blood Analysis:

Table (2) elucidates effects of GnRH stimulant treatment on some blood hematological and biochemical constitutes. Overall, Theriogon treatments significantly increased blood hematological parameters (Red blood cell count, Hemoglobin and Packed cell volume), which comes in agreement with the findings of **Fathi *et al.*, (2000)**, who reported an increase in the hematocrit value of naked neck cocks treated with GnRH. This increase in blood hematological parameters may be responsible for enhancing the oxygen delivery to the testes (**Fathi *et al.* 2000**). Since, this increase in cocks' blood hematological parameters was associated with improved semen quality. The increase in testosterone concentration observed in this study can be also explained by the findings of **Atwal *et al.* (1964)** and **Bierer *et al.* (1964)** who reported that, the increased number of cocks red blood cell and percentage of hematocrit, corresponds with the time of androgen production. **Helene and Murray (1990)** suggested that, the increase in hematocrit could be used to predict spermatozoa maturation in testes, sexual maturity and the start of semen production.

Plasma glucose increased significantly ($p \leq 0.0008$) with the GnRH stimulant treatment. This effect of GnRH on glucose was also reported by **Singh *et al.*, (2006)** and **Khasatiya *et al.*, (2005)**. Changes in the treated cocks plasma glucose concentration may be attributed to the effect of Theriogon on the activities of the hepatic enzyme system, which are intimately involved in glucose production, storage and metabolism and/or attributed to the endocrine activity of the pancreas. Total protein increased significantly ($p \leq 0.0075$) with Theriogon treatments as it increased to reach 107, 122 and 111 % of controls level with the three doses of Theriogon, respectively. The significant increases in serum total protein, which found in the Theriogon treated groups, may be refer to amino acid balance, which initiates protein metabolism. Whereas, globulin levels increased significantly with the 0.50 g dose of Theriogon only to reach 140 % of control. Blood cholesterol was not affected by Theriogon treatment whereas, total lipids were increased significantly to reach 114, 115, and 113 % of control with the three Theriogon levels, respectively. The data of plasma total lipids concentration during the experimental period revealed that, Theriogon doses enhanced fat metabolism. Similar results were reported by **Singh *et al.*, (2006)** in buffaloes treated with GnRH analogue (Receptal) as total serum protein increased significantly. Also **Khasatiya *et al.*, (2005)** reported an increased total protein in buffaloes treated with 5 ml Receptal. On the other hand, **Patel and Dharni (2005)** reported significant increases in both cholesterol and triglycerides after Holstein-Friesian cows were treated with Receptal.

Table (1): Effect of different doses of GnRH stimulant (Therigon) treatments on cockerels' Reproductive performance (Mean ± SE).

Items	Testosterone (ng/ml)	Ejaculated volume (ml)	Motility (%)	Sperm concentration (x10 ⁷ /ml semen)	Seminal Plasma					
					Total protein (g/dl)	Albumin (g/dl)	Globulin (g/dl)	Total lipids (mg/dl)	Cholesterol (mg/dl)	
Control	1.79±0.07 C	0.141±0.01 B	71.67±1.49 C	165.33±6.01 D	1.30±0.03 C	0.554±0.03 C	0.674±0.01 D	290.85±6.99 C	26.20±0.62 B	
0.25 g/kg feed Therigon	2.00±0.10 B	0.260±0.02 A	85.60±1.16 B	240.75±7.19 C	1.33±0.04 C	0.586±0.04 B	0.754±0.03 B	314.90±6.64 B	26.88±0.70 B	
0.50 g/kg feed Therigon	2.03±0.08 B	0.260±0.01 A	90.83±1.19 A	266.33±10.03 B	1.63±0.07 A	0.747±0.02 A	0.883±0.04 A	343.77±10.3 A	32.40±1.18 A	
1.00 g/kg feed Therigon	2.28±0.05 A	0.263±0.02 A	88.75±1.21 AB	311.75±12.98 A	1.48±0.03 B	0.760±0.03 A	0.717±0.04 C	317.32±6.04 B	31.03±2.21 A	
<i>P value</i>	<i>0.0001</i>	<i>0.0001</i>	<i>0.0001</i>	<i>0.0001</i>	<i>0.0001</i>	<i>0.0001</i>	<i>0.0001</i>	<i>0.0001</i>	<i>0.0001</i>	
Control	Month1	1.80±0.13 B	0.158±0.02 C	67.50±1.90 D	169.00±6.61 D	1.31±0.04 C	0.425±0.02 E	0.882±0.04 B	312.00±10.66 CD	28.97±0.33 B
	Month2	1.78±0.05 B	0.125±0.01 C	75.83±1.61 C	161.67±10.26 D	1.47±0.02 B	0.767±0.04 C	0.706±0.02 C	269.20±2.36 F	23.43±0.332 D
0.25 g/kg Therigo	Month1	1.76±0.17 B	0.279±0.03 AB	82.88±1.21 B	256.50±10.19 BC	0.90±0.02 D	0.370±0.01 F	0.529±0.01 F	288.47±4.74 EF	28.27±1.1 BC
	Month2	2.23±0.05 A	0.242±0.03 AB	88.33±1.67 A	225.00±8.21 C	1.28±0.01 C	0.658±0.01 D	0.618±0.01 DE	341.33±5.92 B	25.50±0.52 BCD
0.50 g/kg Therigo	Month1	1.82±0.12 B	0.271±0.02 AB	91.67±1.67 A	276.17±11.33 B	1.34±0.06 C	0.658±0.03 D	0.684±0.03 CD	298.10±0.43 DE	26.90±0.52 BCD
	Month2	2.23±0.09 A	0.250±0.02 AB	90.00±1.74 A	256.50±16.57 BC	1.92±0.02 A	0.836±0.02 B	1.08±0.01 A	389.43±8.28 A	37.90±0.17 A
1.0 g/kg feed Therigon	Month1	2.28±0.09 A	0.225±0.03 B	86.67±1.67 AB	367.00±9.96 A	1.51±0.01 B	0.630±0.03 D	0.882±0.01 B	302.90±1.18 DE	24.13±1.86 CD
	Month2	2.28±0.03 A	0.300±0.02 A	90.83±1.61 A	256.50±7.09 BC	1.44±0.05 B	0.890±0.02 A	0.551±0.03 EF	331.73±10.65 BC	37.93±2.89 A
<i>P value</i>	<i>0.0215</i>	<i>0.0500</i>	<i>0.0209</i>	<i>0.0001</i>	<i>0.0001</i>	<i>0.0001</i>	<i>0.0001</i>	<i>0.0001</i>	<i>0.0001</i>	

A, B, C, D Different letters within a column denote significant differences between treatments.

Cockerels, GnRH, Testosterone, Semen volume, and motility, Seminal plasma

Table (2): Effect of different doses of GnRH stimulant (Therionog) treatments on cockerels' blood analysis (Mean ± SE)

Items	RBC (x 10 ⁶ /mm ³)	HB (g/100ml)	PCV (%)	Glucose (mg/dl)	Total Protein (g/dl)	Albumin (g/dl)	Globulin (g/dl)	Total lipids (mg/dl)	Cholesterol (mg/dl)
Control	2.74±0.06 B	14.72±0.26 B	40.52±0.82 B	216.35±3.57 B	3.76±0.09 B	1.56±0.04	2.15±0.09 B	418.60±11.53 B	106.66±3.04
0.25 g/kg feed Therionog	3.11±0.07 A	16.08±0.18 A	44.08±0.66 A	232.95±3.41 A	4.04±0.12 B	1.64±0.05	2.40±0.09 B	478.24±20.12 A	104.76±4.26
0.50 g/kg feed Therionog	3.09±0.10 A	16.29±0.25 A	43.95±0.57 A	235.21±3.71 A	4.57±0.16 A	1.61±0.04	3.01±0.14 A	482.99±20.64 A	97.51±1.71
1.00 g/kg feed Therionog	3.05±0.11 A	15.92±0.26 A	43.79±0.60 A	234.82±5.49 A	4.16±0.24 AB	1.65±0.05	2.51±0.21 B	475.01±18.77 A	99.75±3.49
<i>P value</i>	0.0083	0.0001	0.0001	0.0008	0.0075	0.4686	0.0003	0.0430	0.1549
Control	Month1	2.54±0.07	14.62±0.24	37.33±0.68 E	209.28±4.91 D	3.70±0.16	1.56±0.07 AB	2.22±0.15	415.53±16.17 ABC
	Month2	2.94±0.09	14.83±0.47	43.71±0.71 BCD	223.43±4.50 BCD	3.82±0.09	1.56±0.04 AB	2.08±0.09	421.68±17.11 AB
0.25 g/kg Therionog	Month1	3.04±0.09	16.25±0.30	42.70±0.99 D	227.86±4.68 BC	4.18±0.19	1.67±0.08 AB	2.51±0.15	494.65±34.53 A
	Month2	3.18±0.11	15.92±0.23	45.46±0.69 AB	238.03±4.70 AB	3.89±0.15	1.61±0.07 AB	2.28±0.10	461.83±21.25 ABC
0.50 g/kg Therionog	Month1	3.03±0.17	16.82±0.33	43.10±0.78 CD	232.78±6.13 BC	4.72±0.31	1.48±0.03 B	3.16±0.25	482.05±31.22 BC
	Month2	3.15±0.10	15.76±0.31	44.79±0.79 ABC	237.64±4.34 AB	4.42±0.10	1.74±0.04 A	2.87±0.11	483.93±28.41 ABC
1.0 g/kg feed Therionog	Month1	2.78±0.16	15.54±0.38	41.71±0.77 D	217.62±7.96 CD	4.49±0.46	1.70±0.07 A	2.79±0.39	466.07±25.65 AB
	Month2	3.32±0.12	16.30±0.33	45.87±0.34 A	252.02±2.97 A	3.83±0.12	1.59±0.05 AB	2.24±0.14	483.95±28.29 C
<i>P value</i>	0.2332	0.0571	0.0064	0.0292	0.4172	0.0127	0.7472	0.7750	0.0270

A, B, C, D Different letters within a column denote significant differences between treatments

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الملخص العربي

تأثير المعاملة بالثيريوجون كمنشط لهرمونات الجونادوتروبين على الأداء التناسلي لذكور دجاج الاسكندراني

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أجرى هذا البحث لدراسة تأثير المستويات المختلفة من الثيريوجون (كمنشط لهرمونات الجونادوتروبين) بالعليقة على الأداء الإنتاجي لذكور سلالة الاسكندراني. استخدمت الدراسة عدد 40 ذكر من سلالة دجاج الاسكندراني عمر 40 أسبوع تم تقسيمهم إلى 4 مجاميع غذيت على مستويات مختلفة من الثيريوجون (صفر ، 0.25 ، 0.50 ، 1.00 جم ثيريوجون / كجم علف) على الترتيب لمدة شهرين من عمر 40 أسبوع حتى عمر 48 أسبوع.

أشارت النتائج الى ان المعاملة بالثيريوجون أدى إلى زيادة نسبة هرمون التيستسترون في سيرم الدم زيادة معنوية وكانت الطيور المعاملة بـ 0.25 ، 0.50 ، 1.00 جم ثيريوجون / كجم علف أكثر معنوية في حجم القذف وكانت نسبة الزيادة بمعدل 84 ، 84 ، 87% على الترتيب بالمقارنة بالمعاملة الكنترول.

تركيز الحيوانات المنوية زاد زيادة معنوية بالمقارنة بالمعاملة الكنترول بنسبة تصل إلى 146 ، 161 ، 189% على الترتيب. الذكور المعاملة بمادة الثيريوجون بالجرعات الثلاثة كانت أعلى معنوية في حركة الحيوانات المنوية بنسب 119 ، 127 ، 124% بالمقارنة بالكنترول على الترتيب.

تقديرات الدم الهيموتولوجية (PCV – HB – RBC) زادت معنوياً بإضافة الثيريوجون. إما بالنسبة لتقديرات الدم البيوكيماوية فكان محتوى بلازما الدم من الجلوكوز أعلى معنوياً في المعاملات بالثيريوجون عن المعاملة الكنترول. بينما لم تتأثر نسبة كوليسترول الدم بالمعاملة بالثيريوجون. كما لوحظ ان تركيز الدهون الكلية ببلازما الدم زاد زيادة معنوية بنسبة تصل إلى 114 ، 115 ، 113% عن المعاملة الكنترول وأيضاً نسبة البروتينات الكلية زادت بنسبة 107 ، 122 ، 111% على الترتيب.

بينما وجد ان مستوى جلوبيولين الدم زاد زيادة معنوية بالمعاملة بـ 0.50 جم ثيريوجون / كجم علف وكانت نسبة الزيادة 140% عن المعاملة الكنترول. كما لوحظ ان التحسن في صفات بلازما السائل المنوي

(البروتينات الكلية و الالبومين والدهون الكلية) يعود إلى التحسن الحادث في بلازما الدم .