

EVALUATION OF ESTROIDOGENIC EFFECT OF GIBBERRELLIC ACID IN AGING HENS

Alaa E. A. Elkomy¹, O.A. Elghalid² and Samar A. Elnagar²

1- Arid Lands Cultivation Research Institute, City of Scientific Research and Technology Applications, 2- Poultry Production Department., Faculty Of Agriculture, Alexandria University, Alexandria, Egypt

SUMMARY

To study the estroidogenic effect of Gibberrellic acid in aging hens, a total of 100 hens at the end of egg production curve (64 weeks of age) from Alexandria strain were used. Birds were assigned to 5 treatments. Groups 2 and 3 were injected subcutaneously with 200, 400 µg Gibberellic acid (GA₃)/kg body weight/week, while, groups 4 and 5 were injected with the same solution which contains 100 and 200 µg estradiol 17-β (E₂)/kg body weight/week, whereas, group 1 served as control. Overall, low and high E₂ treatments resulted in a 340 and 240% increase, respectively in LH concentrations whereas, low and high GA₃ treatments resulted in a 25 and 185% increase, respectively. Regarding FSH concentrations, it increased to reach 400% and 427% of control for low- and high-E₂, respectively, low and high GA₃ treatments resulted in a 27 and 80% increase, respectively. GA₃ treatment resulted in a 13 and 50% increase in E₂ compared to control with the low and high doses, respectively. Overall, egg production increased as a result of low- and high-E₂ treatments by 19% and 9%, respectively, over control. Whereas, it decreased by 9% with low-GA₃ and didn't differ than control with the high-GA₃. Overall, both E₂ and GA₃ treatments showed significant increases in blood calcium level by 17 and 14% over control due to the low and high-E₂ treatments and 14 and 13% over control due to the low and high-GA₃ treatments, respectively. Serum total protein increased as a result of either E₂ or GA₃ treatments.

It could be concluded that gibberrellic acid exerts effects on aged hens which are partially similar to estrogen effects mimicing its actions on egg production traits and blood profile. Moreover, estrogen hormone levels suggest that gibberrellic acid can also stimulate estrogen secretion.

Keyword: Gibberellic acid, estradiol, egg production, LH and FSH hormones

INTRODUCTION

Gibberellic acid (GA₃) is a natural hormone that can be readily extracted from common plants and acts as growth promoter (Riley, 1987). The effect of gibberellic acid on various aspects of plant growth and development has been extensively researched (Riley, 1987; Baydar, 2002; Celik *et al.*, 2007). Because of the possible use of GA₃ in spray applications for promoting plant growth in field crops and the presence of potentially high residual levels which can reach 630 µg. per lb of plant materials used in poultry feeds, subsequent studies were conducted to elucidate effects of GA₃ on birds' performance (Anderson *et al.*, 1982; Abdel-Hamid *et al.*, 1994; Azza *et al.*, 2003; and Elkomy, 2003). On the other hand, GA₃ has been reported to have number of endocrine effects (Gawienowski *et al.*, 1977 and Gawienowski and Chatterjee, 1980). Their studies have demonstrated that GA₃ is estrogenic, androgenic and acts synergistically with estradiol, in rats; GA₃ elicited an estrogen like response in uteri of ovariectomized females and kept them in continuous estrus. Moreover, results of an estrogenic bioassay on one-day

female chicks revealed that gibberellic acid mimics estradiol effect on oviduct length after 7 and 14 days injections with the dosage 100 and 200 µg of GA₃, which had the biological effects of 3 and 20 µg of Estradiol after 7 days injections, and of 10 and 33 µg of Estradiol after 14 days injections, respectively (Elkomy *et al.*, 2007). As well, gibberellic acid mimics testosterone effect on comb's relative weight of male chicks after 7 days injections, with the experimental dosage 100 and 200 µg of Gibberellic acid, which reaches the biological effect of 34 and 46 µg of Testosterone after 7 injections (Elkomy *et al.*, 2007).

The aim of the present study was to evaluate Gibberellic acid estroidogenic effects on aging hens.

MATERIALS AND METHODS

This study was conducted at the Poultry Research center, Faculty of Agriculture, Alexandria University, during the year of 2008. A total of 100 hens at the end of egg production curve (64 weeks of age) from Alexandria strain were used in this study. Birds were fed diet that contained 2745 kcal/kg ME

and 16.2% crude protein. Feed and water were provided *ad libitum* throughout the experimental period. Birds were randomly assigned to 5 treatments of 2 replicates each (10 birds each). Groups 2 and 3 were injected subcutaneously with 0.2ml of solution containing different levels of GA₃ (200, 400 µg GA₃ /kg body weight/week) while, groups 4 and 5 were injected with the same solution which contains estradiol 17-β (100 and 200 µg E₂ /kg body weight/week), whereas, group 1 served as a control group which injected with the injection solution (ethanol-sesame oil mixture 1:11) during the first 4 weeks of the experimental period (TRT), After the 4 weeks treatment period, groups were kept without treatment for 4 weeks as a recovery period (REC)).

Egg production (egg/hen/week) and egg weight (g) were recorded for each replicate in each treatment group. Blood samples were withdrawn from the brachial vein from five females (randomly chosen) from each treatment group to obtain plasma or serum, blood samples were collected at 8 Am from hen that already laid its egg to obtain blood samples at the same stage of egg production cycle. Plasma or serum was stored at -20 °C for later analysis. Serum total protein (STP) concentration as (g/dl) was measured by the Biuret method as described by Armstrong and Carr (1964). Total cholesterol (TCh) concentration as (mg/dl) was determined according to Bogin and Keller (1987). Plasma glucose (PG) concentration as (mg/dl) was estimated according to the method of Trinder (1969). Serum calcium (SCa) concentration as (mg/dl) was measured according to the method of Tietz (1986).

Radioimmunoassay (RIA) was used for FSH, LH and E₂ determinations (Krishan *et al.*, 1993; Follett *et al.*, 1972 and Akiba *et al.*, 1982)

Statistical analysis:

Data were analyzed by analysis of variance using the general linear model procedure (Proc GLM; SAS institute, 1996). For the overall means, data was classified according to 5 treatments and the mean of each treatment was used. Differences among means were determined using Duncan test (Duncan, 1955).

RESULTS AND DISCUSSION

Estroidogenic effect of Gibberellic acid on Gonadotrophins profil (LH and FSH):

Overall, low and high E₂ treatments resulted in a 340 and 240% increase, respectively ($P=0.0001$) in LH concentrations (27.3 ± 3.4 and 21.1 ± 4.6 ng/ml) compared to

control (6.2 ± 0.12 ng/ml) whereas, low and high GA₃ treatments resulted in a 125 and 185% increase, respectively ($P=0.0001$) (Table 1). As expected, LH in control birds did not differ between TRT and REC periods. Low-E₂ boosted LH during the TRT period to 444% higher than control and it remained 236% higher during the REC period ($P=0.0001$) (Table 1). The effect of high-E₂ was less profound during the TRT period but more profound during REC period, with increases of 67% and 432% over control during the TRT and REC periods, respectively. On the other hand, low-GA₃ boosted LH during the TRT period to 33% higher than control and it remained 20% higher during the REC period ($P=0.0001$) (Table 1). The effect of high-GA₃ was more profound, with increases of 91% and 293% over control during the TRT and REC periods, respectively (Table 1).

Regarding FSH concentrations, the values shown in Table (1) represent increases ($p=0.0001$) over control of 400% and 427% for low- and high-E₂, respectively. Low and high GA₃ treatments resulted in a 27 and 80% increase, respectively ($P=0.0001$) (Table 1). Low-E₂ dose stimulated FSH secretion to be 1050% over control during TRT but this effect was reduced during REC to reach 164%, and the response to high-E₂ dose compared to control were increased FSH secretion to reach 338% and 464% during TRT and REC, respectively. On the other hand, low-GA₃ boosted FSH during the TRT period to 137% higher than control group and it didn't differ than control during the REC period ($P=0.0001$) (Table 1). The effect of high-GA₃ was more profound, with increases of 137% and 59% over control during the TRT and REC periods, respectively (Table 1).

The increasing effect of E₂ treatment on LH and FSH levels can be explained by the findings of Dunn *et al.* (1996) who suggested that the decrease in ovarian function in old laying hens could be mediated by a reduction in GnRH mRNA transcription and/or stability. Moreover, the discovery of a putative avian gonadotrophin inhibitory hormone GnIH, (Tsutsui *et al.*, 2000) presented a new possibility that reduced plasma LH in aging laying hens could be a consequence of increased GnIH release. This view is strengthened by the observation that increased GnIH mRNA is associated with depressed plasma LH in incubating hens (Ciccione *et al.*, 2004). On the other hand Estradiol or a combination of Estradiol and progesterone treatment caused a significant decrease in pituitary GnIHR mRNA quantity suggesting that GnIHR gene expression is possibly down regulated in response to a surge in circulating

estradiol (Maddineni *et al.*, 2008). Present findings also prove GA₃ estrogenic effect in old hens with GA₃ treatments boosting LH and FSH levels (as estradiol treatments did) in a dose dependent manner.

Estroidogenic effect of Gibberrellic acid on Estradiol hormone secretion:

As expected with estradiol treatment, low-E₂ resulted in a 50% ($p=0.0002$) increase in blood E₂ profile compared to control hens, as indicated by the values shown in Table 1. Meanwhile, The response to high-E₂ did not differ from control. On the other hand, Gibberillic acid treatment resulted in a 13 and 50% increase ($p= 0.0002$) in blood E₂ profile compared to control with the low and high doses, respectively (Table 1).

Concentrations of blood E₂ in control hens did not change over the course of the experiment. Low-E₂ treatment resulted in elevation of blood E₂ over control by 83 during TRT and it didn't differ than control during the REC period ($p=0.0009$). The response of blood E₂ to high-E₂ treatment differed considerably, whereas, blood E₂ was decreased significantly during TRT but it was did not differ during REC periods compared to control group. On the other hand, low-GA₃ resulted in a non significant (14 and 12%) increase over control during the TRT and REC periods, respectively. The response of blood E₂ to high-GA₃ did not differ than control during the TRT period but it increased by 66% during the recovery period ($p=0.0009$) (Table 1).

These findings are in harmony with previous findings, when Japanese quail hens were injected with estradiol, serum estradiol concentration was higher in the injected group than in the control group (Samar and Abd-Elhady, 2009). Present findings also prove the GA₃ estrogenic effect in old hens with GA₃ treatments and come in agreement with Elkomy *et al.* (2007) findings, who stated that GA₃ not only mimics estradiol biological effect but also have effects similar to those of estrogen with a suggestion that GA₃ can also stimulate estrogen secretion Elkomy *et al.* (2008).

Estroidogenic effect of Gibberrellic acid on Egg Production:

Overall, egg production increased ($P=0.0001$) as a result of low- and high-E₂ treatments by 19% and 9%, respectively, over control (Table 1). Whereas, it decreased by 9% with low-GA₃ and didn't differ than control with the high-GA₃.

On the other hand, egg weight responded differently to treated pulletes with E₂ as it declined ($P=0.0001$) to 98% of control egg

weight with the low-E₂ treatment, but did not differ than control with the high-E₂ treatment (Table 1). Low and high-GA₃ doses decreased egg weight to 99% and 97% of control egg weight.

Overall, low- and high-E₂ treatments resulted in a 10% and 4% increase, respectively ($P=0.0001$) in egg mass compared to control (Table 1). Whereas, it decreased by 6.5% with low-GA₃ but increased by 6% with the high-GA₃ treatment compared to control group.

These findings are consistent with those of El-Afifi and Abu Table (2002); Hamdy *et al.* (2002); Elghalid (2005); and Samar and Abd-Elhady (2009). Who reported that egg number and egg mass were significantly improved when Leghorn pullets and immature quail females were treated with estradiol. High-GA₃ apparently exerts effects on egg production traits similar to high dose of estradiol effects.

Estroidogenic effect of Gibberrellic acid on Blood profile:

Neither E₂ nor GA₃ doses affected blood cholesterol level. Low and high E₂ doses resulted in 3 and 11% in cholesterol concentrations respectively ($P=0.2858$), whereas, increasing blood cholesterol concentration was found with low GA₃ treatment only, which resulted in a 8% increase compared with control group ($P=0.2858$) (Table 2).

Regarding plasma triglycerides concentration, the values shown in Table (1) represented non significant increases ($P=0.4428$) over control of 4% for low- and high-E₂ treatments. Meanwhile, Low and high GA₃ treatments didn't differ from control ($P=0.4428$) (Table 2).

Blood calcium concentration showed significant increases ($P=0.0001$) due to low and high-E₂ treatments by 17 and 14% over control, respectively. Gibberrellic acid (GA₃) treatments showed the same trend as E₂ treatments. Blood calcium concentration increased by 14 and 13% over control group due to treated aging hens with low and high-GA₃ doses, respectively ($P=0.0001$) (Table 2).

Plasma glucose levels didn't change with the low-E₂ treatment while it decreased by 2.6, 7.7 and 2.2% compared to control with the high-E₂, low-GA₃ and high-GA₃, respectively and this decrease was significant with low-GA₃ only ($P=0.002$) (Table 2).

Serum total protein increased ($P=0.0001$) as a result of either E₂ or GA₃ treatments, where, it increased to reach 107 and 114% of control with the low and high-E₂ treatments, respectively and to reach 106 and 122% of

control with the low and high-GA₃ treatments, respectively (Table 2).

Results obtained due to E₂ treatment are in agreement with previous findings regarding E₂ effects on blood profile. Increases in plasma lipids can be attributed to the fact that estradiol activates lipids metabolism during vitellogenesis (Walzem, 1996) and Johnson (1986) who reported that laying hens with E₂ short-term administration had significantly higher plasma total lipids. Blood calcium increases can be attributed to estrogen increasing total blood calcium, primarily by stimulating the production of blood-calcium binding proteins (Bacon *et al.*, 1980). Glucose reduction was also reported by Samar and Abd-Elhady (2009) when immature quails were treated with estradiol.

It can be concluded that gibberellic acid exerts the same effects on aged hens which are similar to estrogen effects mimicing its actions on egg production traits and blood profile. Moreover, estrogen hormone levels suggest that gibberellic acid can also stimulate estrogen secretion.

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Table 1. Mean (±S.E.) of reproductive status parameters of old hens treated with different levels of Estradiol (E2) or Gebberellic acid (GA₃) during one month of treatment (TRT) and one month of recovery (REC)

		LH (ng/ml)	FSH (ng/ml)	E2 (pg/ml)	Egg number (hen/week)	Egg weight (g)	Egg mass (g)
Control		6.2 ± 0.12 ^E	1.5 ± 0.3 ^C	355 ± 18 ^B	4.02±0.22 ^C	56.58± 0.28 ^A	276 ± 11 ^C
E2 (100)		27.3 ± 3.4 ^A	7.5 ± 0.7 ^A	533 ± 41 ^A	4.78±0.21 ^A	55.73 ± 0.21 ^B	304± 9.8 ^A
E2 (200)		21.1 ± 4.6 ^B	7.9 ± 0.9 ^A	340 ± 32 ^B	4.38± 0.21 ^B	56.55± 0.23 ^A	287±10 ^{BC}
GA ₃ (200)		7.8 ± 0.5 ^D	1.9 ± 0.1 ^C	402 ± 26 ^B	3.67±0.22 ^D	55.76 ± 0.24 ^B	258±11 ^D
GA ₃ (400)		17.7 ± 1.5 ^C	2.7 ± 0.4 ^B	531 ± 38 ^A	4.04±0.23 ^C	54.77± 0.26 ^C	292±10 ^{AB}
P value		0.0001	0.0001	0.0002	0.0001	0.0001	0.0001
Control	TRT	6.4 ± 0.06 ^G	0.8 ± 0.05 ^F	351± 31 ^{BC}	4.28 ± 0.31	56.35 ± 0.41	277 ± 16
	REC	5.9 ± 0.05 ^G	2.2 ± 0.11 ^E	360± 26 ^{BC}	3.76 ± 0.32	56.78 ± 0.37	275 ± 16
E2 (100)	TRT	34.8±0.41 ^A	9.2 ± 0.17 ^B	643 ± 42 ^A	4.86 ± 0.29	55.28 ± 0.33	296 ± 14
	REC	19.8±0.11 ^D	5.8 ± 0.41 ^C	423 ± 21 ^B	4.70 ± 0.31	56.19 ± 0.23	313 ± 13
E2 (200)	TRT	10.7 1.01 ^E	3.5 ± 0.66 ^D	248 ± 21 ^C	4.50 ± 0.29	56.28 ± 0.34	285 ± 14
	REC	31.4±0.23 ^B	12.4±0.23 ^A	431± 4.4 ^B	4.26 ± 0.31	56.74 ± 0.29	290 ± 15
GA ₃ (200)	TRT	8.5 ± 0.49 ^F	1.9 ± 0.07 ^E	401 ± 37 ^B	3.86 ± 0.31	55.97 ± 0.36	257 ± 16
	REC	7.1±0.72 ^{FG}	1.9 ± 0.12 ^E	403 ± 18 ^B	3.48 ± 0.31	55.41 ± 0.31	259 ± 16
GA ₃ (400)	TRT	12.2±0.12 ^E	1.9 ± 0.11 ^E	466 ± 39 ^B	4.33 ± 0.31	54.37 ± 0.35	295 ± 13
	REC	23.2±0.58 ^C	3.5 ± 0.13 ^D	596 ± 27 ^A	3.75 ± 0.33	55.01 ± 0.40	288 ± 15
P value		0.0001	0.0001	0.0009	0.4716	0.2598	0.1723

^{A,B,C} Different letters within a column denote significant differences between treatments (p<0.05).

Table 2. Mean (\pm S.E.) of reproductive status parameters of old hens treated with different levels of Estradiol (E2) or Gebberellic acid (GA₃) during one month of treatment (TRT) and one month of recovery (REC)

		Cholesterol (mg/dl)	Triglycerides (mg/dl)	Calcium (mg/dl)	Glucose (mg/dl)	T. protein (g/dl)
Control		180 \pm 10	389 \pm 6	14.39 \pm 0.52 ^B	271 \pm 5 ^A	4.97 \pm 0.17 ^C
E2 (100)		185 \pm 9	404 \pm 4	16.77 \pm 0.53 ^A	276 \pm 6 ^A	5.32 \pm 0.12 ^{BC}
E2 (200)		200 \pm 8	403 \pm 2	16.40 \pm 0.56 ^A	264 \pm 4 ^{AB}	5.67 \pm 0.17 ^B
GA ₃ (200)		195 \pm 13	377 \pm 9	16.38 \pm 0.52 ^A	250 \pm 7 ^B	5.28 \pm 0.14 ^{BC}
GA ₃ (400)		179 \pm 12	391 \pm 7	16.19 \pm 0.79 ^A	265 \pm 7 ^{AB}	6.08 \pm 0.10 ^A
P value		0.2858	0.4428	0.0001	0.0002	0.0001
Control	TRT	166 \pm 16	390 \pm 9	12.7 \pm 0.65	283 \pm 9	4.70 \pm 0.26 ^D
	REC	194 \pm 12	388 \pm 9	16.1 \pm 0.44	258 \pm 4	5.24 \pm 0.19 ^{CD}
E2 (100)	TRT	182 \pm 16	397 \pm 8	15.5 \pm 0.66	288 \pm 7	5.49 \pm 0.13 ^{BC}
	REC	187 \pm 11	410 \pm 1	18.1 \pm 0.65	264 \pm 7	5.14 \pm 0.20 ^{CD}
E2 (200)	TRT	185 \pm 10	405 \pm 1	15.3 \pm 0.62	267 \pm 4	6.22 \pm 0.19 ^A
	REC	215 \pm 11	402 \pm 5	17.5 \pm 0.84	261 \pm 6	5.13 \pm 0.16 ^{CD}
GA ₃ (200)	TRT	168 \pm 18	381 \pm 12	15.2 \pm 0.61	271 \pm 4	5.37 \pm 0.15 ^C
	REC	222 \pm 9	374 \pm 13	17.5 \pm 0.71	229 \pm 10	5.20 \pm 0.23 ^{CD}
GA ₃ (400)	TRT	170 \pm 18	382 \pm 11	13.3 \pm 0.83	284 \pm 5	5.96 \pm 0.18 ^{AB}
	REC	188 \pm 17	399 \pm 7	19.1 \pm 0.68	247 \pm 10	6.21 \pm 0.08 ^A
P value		0.5599	0.6193	0.0594	0.1350	0.0004

^{A,B,C} Different letters within a column denote significant differences between treatments (p<0.05).

قياس التأثير الأستروجيني لحمض الجبريليك (هرمون نباتي) علي الدجاجات البيضاء في نهاية مرحلة إنتاج البيض

علاء السيد الكومي^١، أسامة أحمد حسن^٢، سمر علي النجار^٢

١ - معهد بحوث زراعة الأراضي القاحلة، مدينة الأبحاث العلمية والتطبيقات التكنولوجية، ٢ - قسم إنتاج الدواجن، كلية الزراعة، جامعة الإسكندرية

لقياس تأثير الفعل الأستروجيني لحمض الجبريليك (هرمون نباتي) علي الدجاجات البيضاء في نهاية مرحلة إنتاج البيض تم استخدام ١٠٠ دجاجة بيضاء في نهاية مرحلة إنتاج البيض (عمر ٦٤ أسبوع) من سلالة الإسكندرية المحلية. قسمت الدجاجات إلي خمس مجموعات تجريبية (كل مجموعة تحتوي علي ٢٠ دجاجة). المجموعة الثانية و الثالثة تم حقنها تحت جلد الرقبه بمحلول حقن يحتوي حمض الجبريليك بمستويات ٢٠٠ و ٤٠٠ ميكروجرام حمض الجبريليك/كجم وزن جسم/ أسبوع بينما المجموعة الرابعة و الخامسة تم حقنها بنفس محلول الحقن و الذي يحتوي علي هرمون الأسترايول بمستويات ١٠٠ و ٢٠٠ ميكروجرام أسترايول/كجم وزن جسم/ أسبوع. في حين المجموعة الأولى استخدمت كمجموعه مقارنه (كنترول).

أظهرت النتائج أن حقن الدجاجات في نهاية مرحلة إنتاج البيض بهرمون الأسترايول (بمستوي ١٠٠ و ٢٠٠ ميكروجرام) أدت إلي زيادة مستوي هرمون LH في الدم بمعدل ٣٤٠ و ٢٤٠ % علي الترتيب زياده عن المجموعه الكنترول بينما المعامله بحامض الجبريليك (بمستوي ٢٠٠ و ٤٠٠ ميكروجرام) أدت إلي زياده في مستوي هرمون LH في الدم بمعدل ٢٥ و ١٨٥ % علي الترتيب زياده عن مجموعه الكنترول. أيضا فيما يتعلق بمستوي هرمون FSH في الدم أظهرت النتائج حدوث زياده في افراز الهرمون بمعدل ٤٠٠ و ٤٢٧ % زياده عن المجموعه الكنترول نتيجة الحقن بمستويات ١٠٠ و ٢٠٠ ميكروجرام من هرمون الأسترايول علي الترتيب بينما كانت الزياده بمعدل ٢٧ و ٨٠ % في المجموعات المعامله بحامض الجبريليك. المعامله بحامض الجبريليك بمستوي ٢٠٠ أو ٤٠٠ ميكروجرام نتج عنها زياده في مستوي هرمون الأسترايول في الدم بمعدل ١٣ و ٥٠ % علي الترتيب مقارنه بمجموعه الكنترول.

تلاحظ حدثت زياده في معدل إنتاج البيض بنسبة ١٩ و ٩ % في المجموعات المعامله بهرمون الأسترايول بمستوي ١٠٠ و ٢٠٠ ميكروجرام مقارنه بمجموعه الكنترول بينما حقن الدجاجات بحامض الجبريليك بمستوي ٢٠٠ ميكروجرام تسبب في انخفاض معدل إنتاج البيض بنسبة ٩ % في حين أن مستوي ٤٠٠ ميكروجرام لم يكن له تأثير علي معدل إنتاج البيض مقارنه بمجموعه الكنترول. كل من هرمون الأسترايول و حامض الجبريليك أدت إلي زياده معنوية في مستوي كالسيوم الدم مقارنه بمجموعه الكنترول و هذه الزياده كانت بمعدل ١٧ و ١٤ % لكل من مستوي ١٠٠ و ٢٠٠ ميكروجرام من الأسترايول و أيضا ١٤ و ١٣ % لكل من مستوي ١٠٠ و ٢٠٠ ميكروجرام من حامض الجبريليك علي الترتيب. أيضا حدثت زياده معنويه في مستوي بروتينات الدم في كل من المجموع المعامله بهرمون الأسترايول و كذلك المجموع المعامله بحامض الجبريليك مقارنه بمجموعه الكنترول.

من النتائج السابقة يتضح أن حامض الجبريليك اظهر تأثير مشابه لهرمون الأسترايول في الدجاجات البيضاء في نهاية مرحلة إنتاج البيض وذلك عند دراسة تأثيره علي صفات إنتاج البيض و بعض صفات الدم الفسيولوجية. كذلك أظهر مستوي هرمون الأسترايول في دم الدجاجات المعامله أن حامض الجبريليك له تأثير علي زياده تنبيه افراز الهرمون من المبيض.