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EVALUATION OF ESTROGENIC RESPONSE TO SUBCUTANEOUSLY INJECTION OF GIBBERELLIC ACID (GA_3) IN AGED FEMALE FOWL

By

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ABSTRACT: *A total of 90 Gemiza hens at the end of production curve (48 wk of age) were randomly divided into 5 equal treatment groups which were injected subcutaneously (under neck skin) with 0, 100, 200, 400 or 800 µg GA_3/kg B.W./week for four weeks as a treatment period. After that, hens were allowed without treatment for four weeks as a recovery period. The effect of GA_3 on the egg production, egg weight, feed consumption, feed conversion and egg quality was studied. Also, the effect of GA_3 on blood physiological characteristics and serum 17-β estradiol were determined.*

Egg production (egg/hen/day) for the GA_3 treated groups was increased significantly than the control group, while, egg weight (g) was not affected. GA_3 administration resulted in slight increase in hen's feed consumption and also, there was a non-significant improvement in feed conversion as (kg feed/kg egg or g feed/one egg) when compared with the control group.

eggshell thickness and eggshell relative weight for GA_3 treated groups showed a non-significant increase except for the high dose of GA_3 (800 µg) which produced eggs with lowest shell thickness and eggshell relative weight compared to the control group or the other GA_3 treated groups. Also, egg albumin relative weight was insignificantly increased due to GA_3 treatment, in contrast, egg yolk relative weight showed a non-significant decrease. GA_3 treatment caused a gradual and significant increase in egg yolk total lipids and the effect was in a GA_3 dose dependent manner.

There was a significant increase due to GA_3 doses on hemoglobin concentration and packed cell volume (PCV), while, red blood cells (RBC) count was not significantly affected. GA_3 at any dose resulted in a non-

significant increase in plasma total protein and plasma total lipids concentration when compared with the control group. Serum calcium concentration at any dose was increased significantly compared with the control group. As well as, GA_3 at any doses studied resulted in a significant increase in hen's serum 17- β estradiol concentration when compared with the control group.

INTRODUCTION

Because of the possible use of Gibberellins in spray applications for promoting plant growth in field crops and the presence of potentially high residual levels on plant materials which can be used in poultry feeds, Warden and Schaible (1958) fed GA_3 for broiler chicks till four weeks of age at levels of 0.2, 2 or 20 g/ton feed. They found no significant differences on body weights and feed conversion. No morbidity or unusual symptoms developed among any of the birds during the test period. Also, Kratzer *et al.* (1958) mentioned that, when Bronze turkey poults were fed the basal ration containing 5 mg GA_3/kg feed, there was no significant effect in body weight gain. On the other hand, Alkhiat *et al.* (1981) reported positive influences of GA_3 on body weights of rats, poultry, pigs and calves. Increased feed intake was reported by Madacsi *et al.* (1988) when chicks were fed rations containing GA_3 from hatching till four weeks of age; there were no changes in their feed conversion and body weight. In addition, Abd-Elhamid *et al.* (1994) reported that feeding two-weeks-old broiler chicks on rations containing GA_3 at 0, 1, 5, 25, and 125 ppm levels respectively for 3 weeks led to no significant increase of body weight, decreased feed consumption with better feed conversion. The percentage of the carcass and other organs (liver, gizzard, heart) and glands (adrenal, thyroid and pituitary) weights were lower comparing with the control. Blood protein raised significantly, whereas, blood glucose increased but not significantly.

Anderson *et al.* (1982) studied the effect of injecting 72-weeks old brown egg type hens, with 0.4ml of solution containing 400 μg of GA_3 for 6 alternate days (a total 2.4 mg of GA_3). They found that there was no significant differences in hens body weights four weeks post injection. Also significantly greater mean of egg production, accompanied by 23% less feed consumed per egg was observed. Progressive increase in egg numbers with time reached a maximum at 3 weeks post injection, and then it was slightly decrease at the following week. As well as, egg weight was increased in the same manner, whereas, the control birds showed a progressive decline to this point. Shell thickness dropped 6% from the injection period to the end of 1st week post injection in the GA_3 birds, this dropping was not

significant, then shell thickness fluctuated with time. They suggested that differences might be related to the changes in the levels of circulating gonadal hormones, since GA_3 has been shown to have estrogenic activity in hens.

Moreover, Maillet and Bouton (1969) showed that with rats, GA_3 elicited an estrogen like response in uteri of ovariectomized females and kept them in continuous oestrus. Gawienowski *et al.* (1977) reported that, when mature female mice injected with GA_3 alone or GA_3 + estradiol, the data reflected that GA_3 treatment increased the weight of uterus for about 20-30% as compared to control. Authors indicated that GA_3 may act synergistically with exogenous or endogenous estrogen and produced an enhanced growth of uterine tissue. As well as, Alkhiat *et al.* (1981) found that feeding white rats for two weeks 20mg GA_3/rat led to a large increase in body weight, heart, liver, spleen, kidneys and ovaries. Gawienowski and Chatterjee (1980) investigated the mode of estrogenic actions of GA_3 that determined by the ovariectomized mouse uterine bioassay, the results indicated that GA_3 activity in mammalian species might act via the prostaglandin or/and estrogens. On the other hand, Ratsimamanga *et al.* (1963) observed that, in adrenalectomized rats subjected to cold chamber stress, their survival was significantly increased when treated with 0.1-3 µg GA_3. Another study revealed that, GA_3 increased adrenal cortical activity in rats (Laboratories Laroche 1967).

In this study, the main purpose was to investigate the physiological effect of Gibberellic acid GA_3 on laying hens productive and reproductive performance at the end of the production curve.

MATERIAL AND METHODS

The present study was carried out at the Poultry Research Center, Faculty of Agriculture, Alexandria University, Egypt.

A total of 90 laying hens (Gemiza) at the end of the production curve (48 wk of age) were randomly divided into 5 equal treatment groups (18 birds each) with two replicates (9 birds each). Birds were maintained in cages (3 birds in each) with 16 hours light/day, during the 2 months as experimental period. Groups 2,3,4,5 were injected subcutaneously with 0.2 ml/kg B.W./week of ethanol-sesame oil solution (1:11 ethanol- sesame oil mixture) which contained 100, 200, 400 or 800 µg GA_3/kg B.W./week during the first 4 weeks of the experimental period only, whereas group 1 served as a control group and treated in a like manner with the ethanol-sesame oil mixture only. After the 4 weeks treatment period, groups were

kept without treatment for 4 weeks as a recovery period. Feed and water were provided *ad libitum*; the composition of the diet is shown in Table (1)

Egg production and egg weight were recorded daily, while, feed consumption was determined at two weeks intervals for each replicate within each treated group (as g/bird/day) to calculate the feed conversion expressed as kg feed/kg egg/2 weeks and also as g feed/one egg. The last ten eggs at monthly intervals from each treated group were collected and used for egg quality measurements. Shell thickness, without shell membranes, was determined using a micrometer. Albumin, yolk and shell weight were estimated as a percentage of egg weight. Yolk/albumin ratio (Y/A ratio) was determined also. Yolk total lipids content were determined using the extraction procedure of Fisher and Leveille (1957) and yolk total lipids were measured by the method of Fringes *et al.* (1972).

Blood samples were withdrawn from the brachial vein from five females (randomly chosen) from each treated group to obtain plasma or serum, which stored at –20 °C for later analysis.

Hemoglobin (Hb) concentration as (g/dl) was estimated according to Eilers (1967). Wintrobe hematocrit tubes were used for determination of the packed cell volume (PCV) as (%).Red blood cells (RBC) counts were counted on an AO Bright line hemocytometer using a light microscope at 430X magnification after diluting blood samples 200 times with a physiological saline (0.9% NaCl solution) before counting. plasma total protein (STP) concentration as (g/dl) was measured by the Biuret method as described by Armstrong and Carr (1964). Albumin (A) concentration as (g/dl) was determined by the method of Doumas *et.al.* (1971). Globulin (G) concentration as (g/dl) was calculated as the difference between total protein and albumin. Plasma total lipids (PTL) concentration as (g/dl) were estimated according to Frings *et.al.* (1972). Total cholesterol (TCh) concentration as (mg/dl) was determined according to Richmond (1973). 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 Sarkar and Chauhan (1967) using commercial kits (Stanbio kits). Estradiol 17-β concentration (pg/ml) was determined in serum with immunoassay Elisa kit.

At the end of the experimental period, three birds from each treated group were randomly chosen and slaughtered. Then carcasses were eviscerated and their livers, spleens, small intestine, pancreases, adrenal glands, and ovaries and ovary duct were removed, and then weighed

separately to the nearest 0.1g. Hens liver glycogen content (mg/g) was determined according to

Statistical analysis

Data for the present studies were statistically analyzed for ANOVA using the General linear model of MSTAT-C (1989). Duncan's multiple range test was used to detect any significant differences among the experimental means (Duncan, 1955).

RESULTS AND DISCUSSION

Egg production:

The results obtained (table 2) showed a significant improvement in egg production for the GA_3 treated groups when compared with the control during the experimental and recovery periods where the moderate dose of GA_3 (200 µg) had the highest mean of egg production. On the other hand, the high GA_3 (800 µg) treated group laid a few shell-less egg, which may be attributed to derangement in the oviduct, which occur owing to excess activity in the oviduct and this effect was continued during the recovery period. These results are in agreement with those of Anderson et al (1982) who found that GA_3 significantly increased egg production. They suggested that, this increase in egg production might be due to that GA_3 treated group had higher circulating estrogenic hormone and the metabolic activity of GA_3 enhanced ovulatory process. As well as, Khalifa *et al.* (1983) concluded that; the improvement in egg production by estradiol can be explained by the physiological effect of estrogen upon the ovary and oviduct which causing their activation and enhancing ovulatory process. Also, Hamdy et al. (2002) reported that, egg mass was significantly and positively correlated with plasma concentration of estrogen.

Egg weight:

No significant effect for GA_3 treatment on egg weight at the end of the treatment period and this effect was maintained during the recovery period. These results are similar to that of Asmundson (1931) when injected an aqueous solution of estrogen into Single pullets without apparent effect on their egg weights. Khalifa *at al.* (1983) found that, egg weight increased with estradiol treatment over control but not significantly. On the other hand, Anderson *et al.* (1982) observed a significant increase in egg weight (to 3 weeks post injection) in GA_3 treated group of 72 weeks old brown type hens.

Feed consumption

GA_3 at different doses resulted in slightly increase in hens feed consumption when compared with the non-treated group without any significant effect for the dose. In this respect, the results which reported herein are in agreement with the results of El-Afifi and Abuo Taleb (2002), who found no significant influence on hens feed consumption when egg-laying Japanese quails were treated with estradiol benzoat.

Feed conversion:

The present results (table 2) revealed that, GA_3 treatment did not significantly affect feed consumption whereas egg production was significantly increased and this was reflected on feed conversion (as kg feed/kg egg or g feed/ one egg), which was non-significantly improved. From the data, it can be seen that, the group treated with 200 µg GA_3 had the highest improving effect on feed conversion compared with the control or the other treated group. The present results concerning the improving hens feed conversion when the hens treated by different doses of GA_3 during the two treatment periods are in a good agreement with those of Anderson *et al.* (1982) who found that, GA_3 treatment induced significant greater mean of egg production accompanied by 23% less feed conversion per egg. Also, Abd-Elhamid *et al.* (1994) reported that, when chicks were fed on rations containing GA_3, it was found to decrease feed consumption with better-feed conversion.

Blood hematological parameters:

Overall means at the end of the treatment and recovery periods (table 3) indicated a significant effect ($P<0.05$) of GA_3 doses on hens Hb concentration at the end of the treatment period, but these differences were not statistically significant at the end of the recovery period. there was a significant increase effect ($P<0.01$) of the GA_3 doses on PCV at the end of the treatment period, while, at the end of the recovery period this effect was not statistically significant. At the end of the treatment period the results indicated that hens red blood cell count was not significantly affected by the GA_3 treatment, while, during the recovery period the results revealed a significant decrease effect ($P<0.01$) in red blood count of the hens treated with GA_3 when compared with the control group.

Some clinical chemistry parameters:

At the end of the treatment period (table 4), plasma glucose concentration was significantly higher ($P<0.01$) with the lowest GA_3 dose (100 µg GA_3) when compared with the control group and the other treated

groups. Within the GA_3 treated groups, it can be seen that plasma glucose concentration was gradually decreased and the decreasing effect was in a GA_3 dose dependent manner. At the end of the recovery period no significant differences were found except that with 200 µg GA_3 that had significantly higher plasma glucose concentration than the control group and the other GA_3 groups. The changes in carbohydrate metabolism induced by GA_3 treatment increase of the hen's blood glucose concentration, this may be correlated with the effect of GA_3 on the activities of hepatic enzymes system, which are intimately involved in glucose production, storage and metabolism (Bell and Freeman 1971), since, the data of liver glycogen revealed a significant increase in liver glycogen content (table 5) in the GA_3 treated groups compared with the control at the end of the treatment period, and/or correlated with the endocrine activity of the pancreas, whereas, pancreatic weight was increased significantly. This increase in plasma glucose found with GA_3 in our study is in agreement with the results of Abd-Elhamid *et al.* (1994) who found that, when the broiler chicks fed on rations containing GA_3, at different levels, blood glucose increased but not significant.

There was an increase in hen's serum total protein (table 4) due to GA_3 treatments and this effect was significant ($P<0.05$) during the recovery period only. The increasing of hens serum total protein in the GA_3 treated groups observed in the present study is in agreement with the results of Abd-Elhamid *et al.* (1994) who found that, GA_3 raised blood protein significantly when the broiler chicks were fed rations containing different levels from GA_3.

Serum albumin concentration was not significantly affected by GA_3 at any dose during the treatment and recovery periods when compared with the control group.

Plasma total lipids (table 4) showed that no significant differences were found between the GA_3 treated groups and the control group during the two experimental periods, But, the means of plasma total lipids for hens treated with GA_3 doses were higher than that of the control group. Furthermore, within the GA_3 treated groups it can be seen that there was a gradual increase in hens plasma total lipids which was in a GA_3 dose-dependent manner. Increasing plasma total lipids concentration in the GA_3 treated groups compared to the control, may be due to that GA_3 activates the fat metabolism to provide the yolk with lipids required for yolk lipids formation. Whereas, the yolk lipids content was increased for the hens treated with GA_3 during the two treatment periods (table 6). This effect of GA_3 is similar with that of estrogen hormone. The results obtained in the

present study are in accordance with the results of Pearce and Johnson (1986) who found that, blood total lipids of fowls were increased during the laying period. Also, estrogen administration caused a similar rise in the immature fowl blood total lipids.

During the treatment period, hens treated with GA_3 doses had significantly higher plasma cholesterol means (table 4) when compared with the control ($P<0.05$) by the high(400 and 800 µg). Within, the GA_3 treated groups it can be seen that there was a gradually and significantly increase ($P<0.05$) in plasma cholesterol concentration, which was in a GA_3 dose-dependant manner. The results, at the end of the recovery period, indicated that hen's plasma cholesterol concentration was slightly decrease without any significant when treated with the low GA_3 doses (100, 200 and 400 µg) when compared with the control group or 800 µg GA_3 treated group.

serum Ca concentration at any GA_3 doses (table 4) had a significant higher means when compared with the control group during the two experimental periods. Increasing serum calcium obtained by GA_3 doses during the two experimental periods was reflected on eggshell quality, whereas, the eggshell thickness and relative eggshell weight (table 6) were improved. On the other hand, this increase in hen's serum calcium concentration during and after GA_3 treatment may be due to increase the intestinal calcium absorption. And this increase in serum calcium concentration was corresponding by the increasing of hens egg production (table 3). While, the reduction of hens serum calcium concentration with the high GA_3 dose (800 µg) may be refer to increase calcium excreted in excreta which concomitant with lied shell-less eggs. Clagett *et al.* 1977, found that, more calcium was excreted in the thin shell egg than in the thick shell egg line during egg production), in spite of, serum calcium levels of the hens treated with 800 µg GA_3 was higher than the control during the two experimental periods. Also, the results obtained herein are in a good agreement with the effect of estrogen upon blood calcium. Jackson *et al.* (1976) reported that, increasing of total plasma calcium after estrogen injection is due to increase calcium binding protein in blood. Bar *et al.* (1978) reported that, estrogen hormones increased intestinal absorption of calcium that has been described in laying birds. Baski and Kenny (1977 and 1981) reported that, estrogen treatment increased calcium absorption and blood calcium concentration in young and old Japanese quail hens. Grunder *et al.* (1983) and Tsang & Grunder (1985) reported that, estradiol treatment had a great effect in increasing blood calcium levels. Sommerville *et al.* (1989) found that, calcium absorption from the duodenum responded poly nominally to the dose of estradiol.

Serum 17-β estradiol concentration (E_2):

During the two treatment periods, treated hens by GA_3 at any dose (table 4)resulted in a significant increase ($P<0.01$) of hen's serum estradiol concentration when compared with the control group. However, within the GA_3 treated groups it can be noted no significant differences were found between them, but serum estradiol was increased with increase the GA_3 dose. Results obtained here revealed that, GA_3 can mimic estrogen effects only but also can stimulate its secretion in old hens. This observation from our results is in a good agreement with that of Maillet and Bouton (1969) they found that, with rats, GA_3 elicited an estrogen like response in uteri of ovariectomized females and kept them in continuous estrus. Gawienowski *et al.* (1977) found that, GA_3 increase the weight of uterus mature female mice as compared with control and this increase was did not due to the increased water retention only whereas the dry uterus weight was increased. Also, Gawienowski and Chatterjee (1980) found that GA_3 stimulated an increase of rat's uteri net weight as compared with the control and they suggested that GA_3 activity, in mammalian might act via the estrogens.

Liver glycogen content:

Hens liver glycogen content (mg/g) (table 5) at the end of the treatment period was significantly affected ($P<0.05$) by GA_3 treatment, which increased in the GA_3 treated groups and the increase was in a GA_3 dose-dependent manner. The present results showed a gradually reduction in hens blood glucose levels for GA3 treated groups (table 4) that was in GA_3 dose-dependent, however, the liver glycogen content was significantly increased, which was in GA_3 dose-dependent manner, also. This increase in liver glycogen content induced after treated the hens by GA_3, may be correlated with the effect of GA_3 on the activities of hepatic enzyme systems that are intimately involved in glycogen synthesis (glycogensis) and storage (Bell and Freeman 1971); or/and due to stimulate pancreatic activity or insulin secretion which decreased blood glucose level (table 4) and increased liver glycogen content. Since, treated hens pancreatic relative weight was significantly increased (table 5). and/or also may be due to increase effect of adrenal gland activity which has an important role in carbohydrate metabolism, since adrenal weight was significantly increased in the GA_3 treated hens (table 5).

Some organs relative weight:

Relative weights of liver, spleen, pancreas, intestine, ovary, oviduct, adrenal gland and abdominal fat (as a percentage of live body weight) for the experimental groups at the end of the treatment period are presented in

Table (5). There was a non-significant increase effect of GA_3 treatment on the relative liver weight. This increase in liver weight in the GA_3 treated groups may be referred to the increase in liver glycogen content (Table) or may be attributed to the physiological stimulation of GA_3 on increase liver cell division (Hifny, 1974 and Marey, 1974). Also, the present results are in accordance with those of Alkhiat *et al.* (1981) who found that, feeding white rats for two weeks on 20 mg GA_3/ rat led to a large increase in liver weight. On the other hand, this effect of GA_3 on liver weight was similar to the effect of estrogen. Pearce and Johnson (1986) found that, in laying hens, short-term estradiol administration (up to 6 day) had significantly increased in their liver size. Qin and Klandorf (1995) reported that, estradiol administration in molted hens, increased liver weight but the differences were not significant.

It can be noted that there was a gradual increase in the spleen weight in a GA_3 dose-dependent manner, but the difference was not statistically significant when compared with the control group. The present results are in a good agreement with those of Alkhiat *et al.* (1981) who found that, feeding white rats with GA_3 led to a large increase in spleen weight.

Pancreatic relative weight was higher in the GA_3 groups than the control and this increase was statistically significant with the GA_3 doses 100, 200 and 400 µg. Within the GA_3 treated groups there was a gradual and significant decrease in the pancreatic weight that was in a GA_3 dose-dependent manner. The results of pancreatic weight were in parallel with the trend of plasma glucose concentration and liver glycogen content and these results may be attribute to the increase in activity and secretion of insulin from the pancreas, which responsible for the level of plasma glucose and liver glycogen content.

Adrenal gland relative weight was significantly affected ($P<0.05$) by the GA_3 treatment, which significantly increased in the GA_3 treated groups when compared with the control group. The increase in adrenal gland relative weight by the GA_3 treatment may be attributed to the increase of physiological activity of the gland. Our results is in a good agreement with those of Ratsimamanga *et al.* (1963) who found that, survival of adrenalectomized rats subjected to cold chamber stress was significantly increased when treated with GA_3. Laboratories Laroche (1967) it is also found that GA3 increased adrenal corticol activity in rats. This increase in adrenal weight and activity may be responsible, in part, upon the changes in hen's blood glucose level and their liver glycogen content that found in the present study.

Hen's abdominal fat relative weight was significantly affected ($P<0.05$) by the GA_3 treatment, which significantly increased in the GA_3 treated groups when compared with the control group. Within the GA_3 treated groups, it can be noted that there was no statistically difference due to the GA_3 treatment on abdominal fat relative weight. Increasing relative abdominal fat weight in the GA_3 treated groups refer to an increase in fat metabolism, which increase the blood total lipids concentration. The results obtained here are in accordance with those of Abd-Elhamid *et al.* (1994) who concluded that, the percentage of muscular fat deposition was increased in broiler chicks fed rations containing GA_3. It can be observed that this effect of GA3 is similar to the effect of estrogen. According to Detwiler *et al.* (1950), implanting chicks with stilbesterol increased their fat deposition.

Non-significant increase effect was found on ovary and oviduct relative weight due to the treatment when compared with the control. As well as, the ovary and oviduct relative weight were increased in a similar manner. Increase ovary and oviduct weights of the GA_3 treated groups compared to the control refer to the estrogenic effect of GA_3, whereas, the egg production was increased significantly in the hens treated by GA_3. The estrogenic effect of GA_3 that found in the present study is in accordance with those of Anderson *et al.* (1982) who concluded that, a part of the metabolic activity of GA_3 in the birds is to enhance or induce an increase in estrogen levels and/or produce a direct estrogen-like action. And also, with the results of Gawienowski *et al.* (1977a) with mouse uterine tissue. Moreover, there is identity between the effect of GA_3 and estrogen on ovary and oviduct. Khalifa *et al.* (1983) reported that, weight of the hens ovary and oviduct increased rapidly when the hens changes its reproductive phase from rest to laying condition. El- Afifi and Abu Taleb (2002) results showed, a slight increase in oviduct and ovary weight of old egg-laying Japanese quail group fed estradiol-supplementation diets was observed.

Egg quality:

eggshell thickness (table 6) during the treatment period was a gradually increase by treated hens with GA3doses. Furthermore, the birds treated with 400 µg GA_3 had the highest ($P<0.05$) eggshell thickness mean when compared with the control group or the 800 µg GA_3 treated group that did not differ than the control group. On the other hand, during the recovery period, the results of egg shell thickness revealed that the effect of GA_3 treatment was still continue, since, there was a gradually increase but not significant in eggshell thickness with increase the GA_3 dose except in the 800 µg GA_3 treated group which was slightly decreased. However, relative eggshell weight (table 6) during the treatment and recovery periods

indicated that there were a non-significant increase dut to treatments hens by GA_3 dose during the two experimental periods except the high dose of GA_3 (800 µg) which produced eggs with lower shell thickness and have also the lowest eggshell relative weight. The present results concerning the increase of eggshell thickness and relative eggshell weight during the two experimental periods revealed that, this increase in eggshell quality followed that increase in serum calcium levels (Table 4) and may be enhancing calcium transport in eggshell gland. Also, the slightly decrease in eggshell quality with the high GA_3 dose (800 µg) during the recovery period was corresponded with the reduction of serum calcium concentration with this dose. Our results are in agreement with the influence of estrogen on hens eggshell quality. According to Grunder *et al.* (1983) observed a good correlation between estradiol administration and eggshell quality. El-Afifi and Abou Taleb (2002) investigated the effect of dietary supplementation with estradiol benzoat on old egg-laying Japanese quail (by a level of 9 mg/kg diet) and the results showed that, eggshell weight was significantly improved compared to the control and the enhancing in eggshell weight was reflected on eggshell thickness which improved also.

During the two experimental periods, treated hens with different doses of GA_3 resulted in a non-significant increase in egg albumin relative weight (table 6) when compared with those eggs of the control group and this increase was in a GA_3 dose-dependent manner, while, the egg yolk relative weight revealed a non-significant decrease in the GA_3 treated groups than the control. This increase in egg albumin relative weight may be referring to excitation of magnum to albumin secretion. Whether estrogen administrated or supplied by the maturing ovary, results in an increase in oviduct size (Sturkie 1965) followed by enhanced capacities for protein synthesis (Campbell *et al.* 1971).

From table (6) it can be seen that egg yolk/egg albumin ratio was decreased in the GA_3 treated groups when compared with the control group, but these differences were not statistically significant. The decreases in egg yolk/egg albumin ratio were due to the increase in egg albumin weight and decrease the egg yolk weight.

Changes in the egg total yolk lipids (mg/g yolk) for GA_3 treated groups during the treatment and recovery periods are presented in table (table 6). Hens treated by GA_3 doses revealed a gradual and significant increase in their total egg yolk lipids when compared with the control eggs and the effect was in a GA_3 dose dependent manner. Moreover, within GA_3 treated groups, no significant differences between the groups were found. Treatment of hens with GA_3 may be stimulating vitellogensis and increased

the level of total lipids transport from plasma to yolk, and that level was correlated with the GA_3 dose. The present results are in accordance in part with the results about estrogen effect obtained by Common *et al.* (1947) who reported that, in laying hens the metabolism of fat is increased to provide the formation of the egg yolk lipids. Turner (1948) when fed various levels of synthetic estrogen to hens, he found that the treatment was influenced markedly the metabolism of fat and the stimulus of fat production which utilized in eggs.

Conclusion

From the present study it could be concluded that, GA_3 mimic the estrogenic effect. And it is capable to stimulate estrogen secretion from the ovaries of hens at the end of productive stage.

Table (1) : The composition and calculated analysis of diets used throughout the first and the second experiment.

Ingredients		Percentage of diet
Yellow corn		66
Soybean meal		15
Protein concentration[1]		10
Bone meal		1
Limestone		7.97
Premix[2]		0.03
Calculated analysis:	100	
Crude protein	%	16.2
ME	kcal/kg	2745
C/P ratio		169
Crude fat	%	3.2
Crude fiber	%	2.8
Calcium	%	4.1
Phosphorus available	%	0.39
Methionine	%	0.33
Cystine	%	0.25
Lysin	%	0.78
Arginine	%	1.06
Linoleic acid		1.31

1= Layer concentrate contained 40% CP.

2= Premix each kg contain vit. A (12M.I.U.), vit. D_3 (2U.I.U.), vit E (10g), vit. K_2 (1g), vit. B_1 (1g), vit. B_2 (4g), vit. B_6 (1. 5g), vit. B_{12} (10g), Pantathenic acid (10g), Nicotinic acid (20g), Folic acid (1000 mg), Bidin (50g), Choline

chloride (500g), Copper (10g), Iodine (1g), Iron (30g), Manganese (55g), Zinc (55g), Selenium (0.1g).

Table (2): Effect of different GA_3 doses on hens Egg production (Egg/hen/day), Egg weight (g), feed consumption (g/hen/day) and feed conversion (kg feed/kg egg or g feed/egg)

Parameters	Treatments	GA3 doses (µg/kg B.W. /wk) 0	100	200	400	800
Egg production (Egg/hen/day)	Treated period	56.94±2.4 ^{C}	62.28±1.9 ^{BC}	69.04±2.3 ^{A}	63.29±4.6 ^{AB}	64.87±0.6 ^{AB}
	Recovery period	52.82±2.8 ^{B}	53.85±5.3 ^{B}	63.42±3.7 ^{A}	62.95±1.9 ^{A}	57.81±2.3 ^{AB}
Egg weight (g)	Treated period	53.19±0.9 ^{NS}	53.81±0.7 ^{NS}	53.07±0.5 ^{NS}	55.29±0.6 ^{NS}	53.24±0.5 ^{NS}
	Recovery period	55.75±1.0 ^{NS}	54.50±0.6 ^{NS}	53.59±0.6 ^{NS}	56.30±0.5 ^{NS}	53.81±0.3 ^{NS}
feed consumption (g/hen/day)	Treated period	101.92±2.2 ^{NS}	103.75±2.4 ^{NS}	106.23±3.0 ^{NS}	105.86±3.6 ^{NS}	104.78±1.4 ^{NS}
	Recovery period	109.18±1.9 ^{NS}	109.08±2.8 ^{NS}	116.69±5.8 ^{NS}	114.24±3.2 ^{NS}	114.76±2.2 ^{NS}
feed conversion (kg feed/kg egg)	Treated period	3.386±0.17 ^{NS}	3.140±0.17 ^{NS}	2.903±0.05 ^{NS}	3.062±0.18 ^{NS}	3.035±0.06 ^{NS}
	Recovery period	3.678±0.19 ^{NS}	3.783±0.33 ^{NS}	3.429±0.14 ^{NS}	3.210±0.10 ^{NS}	3.625±0.09 ^{NS}
feed conversion (g feed/egg)	Treated period	180.08±9.3 ^{A}	169.13±5.1 ^{AB}	153.99±2.1 ^{B}	169.04±8.7 ^{AB}	161.57±3.0 ^{B}
	Recovery period	204.68±9.0 ^{NS}	206.33±8.7 ^{NS}	183.42±7.7 ^{NS}	183.29±5.1 ^{NS}	195.16±5.9 ^{NS}

Means within a row with different subscript are significantly different at $p<0.05$.

Table (3): Effect of different GA_3 doses on hens Hb concentration (g/dl), P.C.V. (%) and Red blood cell count ($n*10^6$/ml).

Parameter	Treatment	GA3 doses (µg/kg B.W. /wk)				
		0	100	200	400	800
Hb concentration (g/dl)	Treated period	15.85 ± 0.47^{AB}	15.26 ± 0.46^{B}	15.98 ± 0.28^{AB}	16.69 ± 0.24^{A}	15.11 ± 0.50^{B}
	Recovery period	17.25 ± 0.46^{NS}	17.40 ± 0.34^{NS}	17.17 ± 0.54^{NS}	17.76 ± 0.31^{NS}	17.88 ± 0.32^{NS}
P.C.V. (%)	Treated period	29.83 ± 0.77^{BC}	31.19 ± 1.15^{AB}	31.83 ± 0.50^{AB}	33.94 ± 0.78^{A}	28.74 ± 0.53^{C}
	Recovery period	33.83 ± 0.99^{NS}	33.50 ± 0.67^{NS}	33.49 ± 0.87^{NS}	34.23 ± 0.61^{NS}	34.18 ± 0.67^{NS}
Red blood cell count ($n*10^6$/ml)	Treated period	1.604 ± 0.07^{NS}	1.591 ± 0.05^{NS}	1.624 ± 0.04^{NS}	1.624 ± 0.05^{NS}	1.584 ± 0.06^{NS}
	Recovery period	2.064 ± 0.10^{A}	1.740 ± 0.05^{B}	1.696 ± 0.05^{B}	1.705 ± 0.05^{B}	1.828 ± 0.06^{B}

Means within a row with different subscript are significantly different at $p<0.05$.

Table (4): Effect of different GA_3 doses on hens Plasma glucose concentration (mg/ dl), Serum total protein (g/dl), Serum albumin (g/dl), Plasma total lipids (g/dl), Serum calcium concentration (mg/dl) and serum 17-β estradiol (pg/ml).

Parameters	Treatments	GA3 doses (µg/kg B.W. /wk)				
		0	100	200	400	800
Plasma glucose concentration (mg/ dl)	Treated period	172.56 ± 7.2^{CD}	205.73 ± 3.2^{A}	187.33 ± 6.0^{B}	182.50 ± 3.5^{BC}	167.55 ± 3.9^{D}
	Recovery period	197.54 ± 7.3^{B}	199.48 ± 2.4^{B}	212.60 ± 1.6^{A}	203.48 ± 5.2^{B}	201.56 ± 3.5^{B}
Serum total protein (g/dl)	Treated period	5.15 ± 0.06^{NS}	5.33 ± 0.17^{NS}	5.38 ± 0.12^{NS}	5.27 ± 0.14^{NS}	5.20 ± 0.10^{NS}
	Recovery period	4.72 ± 0.16^{B}	5.34 ± 0.18^{A}	4.94 ± 0.18^{AB}	5.33 ± 0.16^{A}	5.01 ± 0.23^{AB}
Serum albumin (g/dl)	Treated period	$2..91\pm0.08^{NS}$	2.91 ± 0.13^{NS}	$2..99\pm0.04^{NS}$	2.94 ± 0.08^{NS}	2.88 ± 0.12^{NS}
	Recovery period	2.57 ± 0.10^{NS}	3.03 ± 0.14^{NS}	2.88 ± 0.10^{NS}	3.02 ± 0.12^{NS}	2.83 ± 0.10^{NS}
Plasma total lipids (g/dl)	Treated period	3.26 ± 0.13^{NS}	3.29 ± 0.15^{NS}	3.44 ± 0.31^{NS}	3.62 ± 0.16^{NS}	3.79 ± 0.22^{NS}
	Recovery period	3.68 ± 0.11^{NS}	3.84 ± 0.11^{NS}	3.87 ± 0.19^{NS}	3.84 ± 0.15^{NS}	3.80 ± 0.23^{NS}
Plasma cholesterol (g/dl)	Treated period	186.62 ± 3.6^{B}	190.79 ± 4.4^{B}	196.10 ± 4.5^{AB}	204.51 ± 3.5^{A}	202.42 ± 6.1^{A}
	Recovery period	198.25 ± 4.1^{NS}	196.19 ± 4.2^{NS}	193.99 ± 5.1^{NS}	195.34 ± 4.1^{NS}	200.23 ± 2.5^{NS}
Serum calcium concentration (mg/dl)	Treated period	16.21 ± 0.51^{C}	19.86 ± 1.1^{AB}	19.86 ± 1.0^{AB}	21.11 ± 0.85^{A}	18.83 ± 0.87^{B}
	Recovery period	17.65 ± 0.42^{C}	22.28 ± 0.56^{A}	21.07 ± 0.40^{AB}	20.46 ± 0.82^{B}	18.65 ± 0.69^{C}
serum 17-β estradiol (pg/ml)	Treated period	469.43 ± 11.0^{B}	585.68 ± 7.9^{A}	612.57 ± 16.5^{A}	625.64 ± 7.0^{A}	605.58 ± 17.9^{A}
	Recovery period	470.18 ± 15.3^{A}	587.79 ± 16.2^{A}	595.87 ± 9.0^{A}	599.77 ± 10.7^{A}	623.51 ± 15.1^{A}

Means within a row with different subscript are significantly different at $p<0.05$.

Table (5): Effect of different GA_3 doses on hens some organs relative weight (% to live body weight) and liver glycogen content (mg/g).

Treatments / Parameters	GA3 doses (µg/kg B.W. /wk) 0	100	200	400	800
Liver	1.864±0.16^{NS}	2.167±0.07^{NS}	2.106±0.11^{NS}	2.131±0.08^{NS}	2.046±0.07^{NS}
Liver glycogen content	0.96±0.02^{B}	1.61±0.24^{AB}	1.86±0.44^{A}	2.01±0.13^{A}	2.16±0.04^{A}
Spleen	0.078±0.022^{NS}	0.099±0.001^{NS}	0.099±0.015^{NS}	0.090±0.002^{NS}	0.116±0.011^{NS}
Pancreas	0.153±0.003^{C}	0.213±0.015^{A}	0.199±0.013^{AB}	0.190±0.011^{AB}	0.169±0.004^{BC}
Intestine	3.65±0.14^{NS}	3.92±0.12^{NS}	4.22±0.32^{NS}	4.22±0.23^{NS}	4.42±0.18^{NS}
Adrenal gland	0.006±0.0006^{C}	0.009±0.0001^{AB}	0.008±0.0004^{B}	0.008±0.0006^{B}	0.010±0.0006^{A}
Abdominal fat	1.57±0.14^{B}	2.81±0.27^{A}	2.74±0.31^{A}	2.14±0.37^{AB}	2.53±0.16^{A}
Ovary	0.338±0.01^{NS}	0.418±0.04^{NS}	0.421±0.01^{NS}	0.460±0.04^{NS}	0.446±0.04^{NS}
Oviduct	2.31±0.17^{NS}	2.40±0.231^{NS}	2.61±0.16^{NS}	2.88±0.18^{NS}	2.51±0.10^{NS}

Means within a row with different subscript are significantly different at $p<0.05$.

Table (6): Effect of different GA_3 doses on hens Eggshell thickness (mm), relative eggshell weight (% to egg weight), relative egg albumin weight (% to egg weight), relative egg yolk weight (% to egg weight), egg yolk/ albumin ratio (Y/A) and Total yolk lipids (mg/g yolk).

Parameters	Treatments	GA3 doses (µg/kg B.W. /wk)				
		0	100	200	400	800
Eggshell thickness (mm)	Treated period	0.295 ± 0.006^{B}	0.303 ± 0.009^{AB}	0.323 ± 0.007^{AB}	0.327 ± 0.007^{A}	0.296 ± 0.012^{B}
	Recovery period	0.318 ± 0.008^{NS}	0.326 ± 0.008^{NS}	0.328 ± 0.011^{NS}	0.329 ± 0.007^{NS}	0.312 ± 0.009^{NS}
Relative eggshell weight (% to egg weight)	Treated period	10.53 ± 0.30^{NS}	10.70 ± 0.30^{NS}	10.79 ± 0.19^{NS}	10.93 ± 0.21^{NS}	10.24 ± 0.43^{NS}
	Recovery period	10.12 ± 0.29^{NS}	10.27 ± 0.22^{NS}	10.32 ± 0.39^{NS}	10.15 ± 0.27^{NS}	9.74 ± 0.21^{NS}
Relative egg albumin weight (% to egg weight)	Treated period	57.96 ± 0.99^{NS}	58.37 ± 0.66^{NS}	59.73 ± 0.73^{NS}	59.93 ± 0.79^{NS}	60.33 ± 0.89^{NS}
	Recovery period	58.93 ± 0.57^{NS}	60.44 ± 0.40^{NS}	60.08 ± 0.77^{NS}	61.37 ± 0.99^{NS}	61.32 ± 0.92^{NS}
Relative egg yolk weight (% to egg weight)	Treated period	31.49 ± 0.91^{NS}	30.82 ± 0.67^{NS}	30.01 ± 0.62^{NS}	29.11 ± 0.69^{NS}	28.94 ± 0.88^{NS}
	Recovery period	30.78 ± 0.47^{NS}	29.79 ± 0.51^{NS}	29.58 ± 0.48^{NS}	28.54 ± 0.80^{NS}	28.55 ± 0.78^{NS}
Hens egg yolk/ albumin ratio (Y/A)	Treated period	0.554 ± 0.027^{NS}	0.529 ± 0.017^{NS}	0.504 ± 0.016^{NS}	0.487 ± 0.017^{NS}	0.483 ± 0.020^{NS}
	Recovery period	0.520 ± 0.011^{NS}	0.493 ± 0.011^{NS}	0.498 ± 0.015^{NS}	0.466 ± 0.020^{NS}	0.468 ± 0.018^{NS}
Total yolk lipids (mg/g yolk)	Treated period	4.342 ± 0.06^{C}	4.817 ± 0.15^{B}	5.127 ± 0.04^{AB}	5.244 ± 0.14^{A}	5.277 ± 0.06^{A}
	Recovery period	4.856 ± 0.11^{B}	5.271 ± 0.04^{A}	5.284 ± 0.08^{A}	5.364 ± 0.11^{A}	5.538 ± 0.08^{A}

Means within a row with different subscript are significantly different at $p<0.05$.

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الملخص العربي

تقييم الإستجابة الأستروجينية لحقن هرمون الجبريلليك (GA_3) أسيد في الدجاجات البياضة في نهاية مرحلة الأنتاج

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٩٠ دجاجة بياضة من سلالة الجميزة (عمر ٤٨ أسبوع) قسمت عشوائيا إلي خمس مجموعات عوملت عن طريق الحقن تحت جلد الرقبة بمستويات من حمض الجبريلليك (GA_3) ٠، ١٠٠، ٢٠٠، ٤٠٠ و ٨٠٠ ميكروجرام GA_3 / كجم وزن جسم حي/ أسبوع لمدة أربعة أسابيع متتالية كفترة معاملة . تركت الدجاجات بعد ذلك بدون معاملة لمدة أربعة أسابيع أخرى كفترة أسترجاع. تمت دراسة تأثير المعاملة بـ GA_3 علي معدل أنتاج البيض اليومي، وزن البيضة، معدل أستهلاك العلف اليومي، الكفاءة الغذائية و جودة البيضة. كذلك تمت دراسة التغيرات الكيموحيوية للدم و مستوى إفراز هرمون الأستروجين.

حدثت زيادة معنوية في أنتاج البيض اليومي (بيضة/دجاجة/يوم) في المجاميع المعاملة بهرمون GA_3 مقارنة بالكنترول بينما لم يتأثر وزن البيضة. كانت هناك زيادة طفيفة في أستهلاك العلف في المجاميع المعاملة بالهرمون و لكنها غير معنوية و هذه الزيادة كان يقابلها تحسن غير معنوي في الكفاءة الغذائية(كجم علف /كجم بيض أو جرام علف/بيضة).

المعاملة بهرمون GA_3 لم تؤدي إلي حدوث تغيرات معنوية في وزن قشرة البيضة أو سمك القشرة فيما عدا المجموعة المعاملة بمستوى ٨٠٠ ميكروجرام GA_3 و التي أظهرت إنخفاض غير معنوي في هاتين الصفتين مقارنة بالمجموعة الكنترول أو باقي المجاميع المعاملة بالهرمون. أيضا حدثت زيادة غير معنوية في وزن البياض في كل المجاميع المعاملة بالهرمون مقارنة بالكنترول بينما وزن الصفار أظهر إنخفاض غير معنوي. مستوي دهون صفار البيضة أزداد معنويا في المجموعات المعاملة بالهرمون مقارنة بالكنترول و هذا التأثير كان يزداد بزيادة مستوي المعاملة.

حدثت زيادة معنوية نتيجة المعاملة بهرمون GA_3 في مستوى هيموجلوبين الدم و حجم كرات الدم المحبوسة (PCV) مقارنة بالكنترول بينما عدد كرات الدم الحمراء لم يتأثر معنويا بالمعاملة. معاملة الدجاجات بهرمون GA_3 عند أي من مستويات المعاملة أدي إلي زيادة غير معنوية في مستوى بروتينات و دهون الدم مقارنة بالكنترول. معاملة الدجاجات بهرمون GA_3 مع أي من المستويات المستخدمة أدت إلي زيادة معنوية في مستوي كالسيوم الدم و كذلك مستوي هرمون الأستروجين مقارنة بالمجموعة الكنترول.