

EFFECT OF GIBBERELLIC ACID AND ROYAL JELLY INJECTION ON SOME PRODUCTIVE, REPRODUCTIVE AND PHYSIOLOGICAL TRAITS IN MATROUH CHICKENS STRAIN DURING SUMMER SEASON.

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ABSTRACT:

A total of 168 laying hens and 21 cocks of Matrouh local strain at 24 weeks of age (At 43.93 % egg production) was used in a completely randomized design among seven treatment groups with three replicates per treatment group (8 hens and 1 cock per replicate). Birds are bi-weekly injected, up to 36 weeks of age (12 weeks). The 1st treatment group of birds were injected with saline solution 0.9% only and served as control. The birds in 2nd and 3rd treatment groups, were injected subcutaneously with 100 and 200 µg Gibberellic acid (GA3)/kg body weight (BW), respectively. The 4th and 5th treatment groups were injected with 50 and 100 µg Royal Jelly (RJ)/kg BW, respectively. The 6th treatment group was injected subcutaneously with 50µg GA3 plus 25µg RJ/kg BW. The 7th treatment group was injected subcutaneously with 100 µg GA3 plus 50µg RJ/kg BW. The same 7 treatment groups were used also, on 21 cocks in individual batteries to take semen quality.

The obtained results revealed that, Injection with 100 , 200 µg GA3 or 50 , 100 µg RJ /kg BW of Matrouh chicks improved significantly ($P<0.05$ and $P<0.01$) body weight changes (BWC), fertility eggs %, hatchability/total eggs % and chicks hatch weight (g), sperm-cell concentration ($\times 10^9/ml$) and sperm motility (%), while significantly ($P<0.05$) decreased each of dead spermatozoa (%), sperm abnormalities (%), and acrosomal damage (%) as compared with the control group. Layers injected by 100 , 200 µg GA3/kg body weight showed significantly ($P\leq 0.05$ and $P\leq 0.01$) improved feed conversion ratio (FCR), egg production (EP)% and egg mass (EM) compared with those the groups

50 , 100 µg RJ /kg BW and control groups through the whole of the experimental period. Injection with 50, 100 µg of RJ /kg BW caused to increase significantly ($P\leq0.01$) in IgG and IgM and primary and secondary antibody titer against sheep red blood cells (SRBC's) in hens as compared with 100, 200 µg of GA3 and the control group. Injection 100 , 200 µg of GA3 /kg BW caused to increase significantly ($P\leq0.05$ and $P\leq0.01$) in concentration of estrogen in hens and testosterone of cocks, FSH and LH in hens and cocks when compared with those in control group.

In conclusion, injection with 100, 200 µg GA3 /kg BW improved productive, reproductive and physiological performance of males and females Matrouh. Furthermore, the RJ-treated groups had significantly improved immune responses in hens and the quality of semen parameters in cocks under summer season conditions in Egypt.

Keywords: Gibberellic acid, Royal Jelly, Heat stress, Productive, Reproductive, Matrouh chicks.

INTRODUCTION

Heat stress is one of the most general environmental challenges to poultry local. It has been widely confirmed that heat stress negatively impacts the health, safety, and productivity of broilers and laying hens (Rostagno, 2020). Also, high ambient temperatures have clear impacts on the behavior, feed and water intake, heat production, and physiological responses (body temperature, respiratory rate and heart rate) of poultry (Mutibvu *et al.*, 2017). Research has associated high environmental temperature with diminish fertility rate and sperm yield in reproducer cocks to high natural temperature (Obidi *et al.*, 2008). The cooperative effect of heat in males with oxidative breakdown of sperm film lipids because of excess production of free radicals in sperm mitochondrial plasma layer have been watched (Sabeti *et al.*, 2016). Gibberellic acid (GA3) is a characteristic hormone found in plants and parasites and goes as growth promoter (Silva *et al.*, 2013). GA3 was seen as engaged with the union of RNA and proteins (Williams and Weisburger, 1991). In addition, Abdel-Azim (2017) announced that GA3 is one of the plant development controllers that are generally utilized in Egypt, to increase the growth of fruits and vegetables. Anderson *et al.*

(1982) considered the impact injecting 72-weeks old brown egg type hens with 400 µg of GA3 for 6 substitute days. They revealed a significantly greater mean of egg production that joined by 23% less feed expended per egg and a dynamic increment in egg numbers and weight with time until the 3ed week post injection at that point, it was slightly decreased from that point. Elkomy *et al.* (2007) presumed that, GA3 copy the estrogenic impact and it is skilled to animate estrogen secretion from the ovaries of hens at the end of productive stage and copy testosteronic biological effects for male chicks. Askar and Ismaeil (2012) revealed that GA3 has a positive effect on productive performance and fertility of laying hens. Elkomy (2003) found that treated develop cockerels with GA3 directed to improve semen quality attributes (sperm concentration, sperm motility, increment live sperm and diminishing abnormal sperm). Likewise, who revealed that, the GA3 dosages had low serum testosterone concentration compare with the control group.

Royal Jelly (RJ) is item from honey bees that is utilized to take care of hatchlings in the hive just as a main nutritional source for the queen honey bee. It has different significant natural exercises including as an antioxidant, immunomodulatory, anti-inflammatory, antimicrobial, cholesterol-lowering, and growth promoter exercises and has the ability for inhibition of certain enzymatic corruption. It tends to be utilized for the improvement of growth performance, gut health and quality and safety of animal products in poultry because of its antioxidant and immune modulating properties (Saeed *et al.*, 2018 and Zhang *et al.*, 2019). The RJ is a rich source of proteins (27%–41%), essential amino acid (EAA), sugars (30%), unsaturated fats, lipids (8%–19%), phytosterols, carotenes, flavonoids, nutrients and minerals that guide the honey bee to remain alive, just as some other unknown active compounds (Maghsoudlou *et al.*, 2019). Moreover, the RJ organization can effectively decrease heat stress-induced reproductive damages through improvement of sperm attributes and MDA concentration restoration, proposing that RJ is a promising component to protect the reproduction in heat stress issue (Mahdivand *et al.*, 2019). This bit of leeway is maybe a direct result of the presence of MRJPs in the RJ (Park *et al.*, 2019). These properties supported the use of RJ as a well-known dietary enhancement and various pharmaceuticals related to fertility issue in animals (Maghsoudlou *et al.*, 2019).

Therefore, the present study aimed to study the effect of gibberellic acid and royal jelly injection on some productive, reproductive and physiological traits in Matrouh chickens strain under Egyptian summer conditions.

MATERIALS AND METHODS

The experimental work of this study was carried out at the Inshas Poultry Research Station, Animal Production Research Institute, Agricultural Research Center, Giza, Egypt, from May to August, 2019). One hundred sixty eight laying hens and twenty one cocks of Matrouh local strain at 24 weeks of age (At 43.93 % egg production) were used in a completely randomized design among seven treatment groups with three replicates in each treatment group (8 hens and 1 cock per replicate). Birds were housed in 21 floor pens (2 m × 2 m) furnished with wheat straw and fed *ad libitum* a basal diet up to 36 weeks of age. The basal diet was formulated to meet the NRC (1994) recommendations as shown in Table 1.

The calculated analysis was according to Feed Composition Tables for Animal and Poultry Feedstuffs used in Egypt (2001) are shown in Table 1. Birds were submitted to the same managerial condition in a window house with light cycle regimen (16 hours light: 8 hours darkness). Birds were examined against diseases and treated with antibiotics and vaccines to keep them healthy. All groups had nearly similar average body weight (1218.36 ± 5.39) to investigate the effects of gibberellic acid and royal jelly injection on some productive, reproductive and physiological traits in local Matrouh chickens strain under Egyptian summer conditions. Birds are bi-weekly injected, for a whole experimental period. The 1st treatment group of hens and cocks was injected with saline solution 0.9% only and served as control. The chicks in 2nd and 3rd treatment groups, were injected subcutaneously with 0.2 ml of saline solution 0.9%, which containing 100 and 200 µg GA3/kg body weight (BW), respectively under the skin of the neck. While, the 4th and 5th treatment groups, were injected into the mouth, using an insulin syringe directly subcutaneously by 50 and 100 µg Royal Jelly (RJ)/kg BW, respectively. The 6th treatment group was injected subcutaneously with 0.2 ml of saline solution 0.9%, which containing 100 µg GA3/kg BW and injected into the mouth subcutaneously by 25 µg RJ/kg BW. The 7th treatment was injected subcutaneously with 0.2 ml of saline solution 0.9%, which containing 200 µg GA3/kg BW and injected into the mouth subcutaneously by 50 µg RJ/kg BW. The same 7 treatment groups were used also, on 21 cocks in individual batteries to take semen quality.

Table (1): Composition and calculated analysis of the basal diet fed.

Ingredients %	%
Yellow corn	61.47
Soybean meal (44% CP)	15.20
Wheat bran	8.28
Corn gluten meal (60% CP)	4.80
Dicalcium phosphate	1.35
Salt	0.30
Limestone	8.20
Vit + Min. premix*	0.30
DL-Methionine	0.10
Total	100
Calculated analysis :	
Crude protein (CP); %	16.19
ME; kcal/kg	2687
Ether extract (EE)	2.93
Crude fiber (CF)	3.44
Calcium	3.51
Av. Phosphorus	0.30
Lysine	0.65
Methionine	0.39
Methionine + Cysteine	0.69

***Vitamin and Mineral premix:** added to the 1 kg of diet including Vit. A 10000 I.U; Vit. D3 2000 I.U; Vit. E 15 mg; Vit. K3 1 mg; Vit. B1 1mg; Vit. B2 5 mg; Vit. B12 10 µg; Vit. B6 1.5mg; Niacin 30mg; Pantothenic acid 10mg; Folic acid 1mg; Biotin 50 µg; Choline 300 mg; Zinc 50mg; Copper 4mg; Iodine 0.3 mg; Iron 30mg; Selenium 0.1mg; Manganese 60mg; Cobalt 0.1mg.

The average minimum and maximum of ambient temperature during the experimental period ranged between 25.21 and 36.45 °C, relative humidity from 26.42 to 77.51% and temperature-humidity index (THI) from 22.77 to 34.93 under Inshas, Sharkia Governorate, Egypt as shown in Table 2.

THI was estimated according to the formula as follows:

$$\text{THI} = \text{db } ^\circ\text{C} - \{(0.31 - 0.31 \text{ RH}) (\text{db } ^\circ\text{C} - 14.4)\}.$$

Table (2). Microclimatic data during the whole experimental period, under environmental conditions from Inshas, Sharkia Governorate, Egypt.

Summer months	Averages temperature (°C)		Averages RH (%)		Averages (THI)	
	Min*	Max**	Min*	Max**	Min*	Max**
May	21.99±0.52	35.61±0.85	19.55±1.26	71.39±2.05	20.10	33.73
Jun	25.45±0.23	36.34±0.38	27.97±0.94	78.67±1.26	22.98	34.89
July	26.61±0.21	36.97±0.39	29.0±1.19	78.75±1.19	23.92	35.48
August	26.79±0.19	36.91±0.32	29.17±1.32	81.23±1.46	24.07	35.60
Averages	25.21±0.28	36.45±0.48	26.42±1.17	77.51±1.48	22.77	34.93

Where db °C = Bulb temperature in Celsius and RH= RH%/100. The values obtained indicate the following: <22.2 = Absence of heat stress; 22.2 to <23.3 = Moderate heat stress; 23.3 to <25.6 = Severe heat stress and 25.6 and more = Extreme severe heat stress (Marai *et al.*, 2000).

The body weight changes of laying hens calculated by the difference between finally and initial weight, while the egg number and egg weight were recorded daily and feed intake was calculated weekly. The egg production rate(EP) was calculated during the experimental period from 24 to 36 weeks of age. Where: EP rate (%) = EN / hen/ x 100.

Egg mass (EM) was calculated by multiplying EN by average EW. Feed conversion (g feed/g egg) (FCR) was also calculated. The mortality rate (%) was recorded for daily each treatment from 24- 36 weeks of age.

Monthly, about 36 eggs from each treatment group were collected and incubated. After hatching, the chicks were counted and non-hatched eggs were broken to determine the overall mean of fertility and hatchability percentages. Fertility (%) was calculated as the percentage of fertile eggs from the total number of set eggs, while the hatchability was expressed as the chicks hatched from fertile eggs and from total eggs.

At the end of the experimental period, 5 ml blood samples were withdrawn from the brachial vein from 3 hens and 3 cocks per treatment group.

Blood samples were collected in the morning from the overnight-fasted chickens. Each sample was collected in two tubs; one without anticoagulant, while in the other, heparin was used as an anticoagulant. Plasma and serum was obtained by centrifugation of blood at $1500\times g$ for 20 min and kept at -20°C until used for analysis. The plasma concentrations of estrogen (E2), FSH, LH and testosterone (T) were measured using a homologous radioimmunoassay (Krishnan *et al.*, 1993). Immunoglobulin concentrations of (IgG and IgM) were determined by enzyme-linked Immuno- sorbent assays using commercial ELISA kits according to manufacturer's Instructions (Sun Biomedical Technology Co., BeiJing, 10039).

Moreover, nine hens of each treatment group at 32 and 36 weeks of age were injected intravenously with 1 ml of 10% suspension of sheep red blood cells (SRBC's). Then blood samples were collected at 33 and 37 weeks of age and centrifuged to get serum and frozen until the measurements of anti SRBC's antibody levels for primary and secondary immune responses were determined using micro-hemagglutination procedure (Witlin, 1967). The highest dilution exhibited by hemagglutination was recorded as titer of serum sample. The titer of each sample was converted to log₂ value (Thaxton and Siegel, 1972).

After production of the primary egg, semen collected from the 7 groups of cocks twice every week. Semen was collected at three times amid the trial time frame at 28, 32 and 36 weeks of age from 5 cocks in each treatment group to determine the overall mean periods. Cocks were randomly chosen using the massage method. Immediately after semen collection, semen-ejaculate volume (ml) was measured utilizing graduate collecting tubes and hydrogen-ion concentration (pH) was measured by Universal Indicator Paper and Standard Commercial Stain. A drop of semen with the guide of a micro-pipette was set on a pre warmed microscope slide, which was then covered with a glass cover slip and inspected at a magnification of $\times 400$. Motility of semen samples was tested as the percentage of motile spermatozoa having moderate to rapid progressive movement and cells that are motile under their own power (Ommatiet *et al.*, 2013). At least 10 microscopic fields were inspected for every semen test. Eosin-Nigrosine stain was utilized to decide the percent of morphologically sperm abnormalities and dead spermatozoa. For sperm cell concentration ($\text{X } 10^9/\text{ml}$) a droplet of diluted semen (1:200 in distilled water) semen was tenderly put on both councils of a Neubauerhemocytometer and the number of spermatozoa was determined

microscopically (Ommatiet *et al.*, 2013). Acrosomal damage (%) of spermatozoa was determined according to Waston (1975). No less than 10 minuscule fields were inspected for every semen test.

The economic efficiency (EEF) of the experimental treatments was estimated depending up on feeding cost and price of egg produced as the following equation:

$$\text{EEF} (\%) = (\text{Net revenue/hen} / \text{Total cost hen}) \times 100.$$

Statistical analysis:

Data were analyzed by the least square analysis of variance according to Snedecor and Cochran (1982) using the General Linear Model Procedure (SAS, 2011) at the 5% level of significance as the following model:

$$Y_{ij} = \mu + N_i + e_{ij}$$

Where: Y_{ij} = Any observation, μ = Overall mean, N_i = Effect of treatment ($i = 1 \dots 7$), e_{ij} = Experimental random error. All percentages, data were transferred to percentage angle using arcsine equation before subject to statistical analysis. Significant differences among means were tested using Duncan's New Multiple Range Test (Duncan, 1955).

RESULTS AND DISCUSSION

Productive performance:

The effect of gibberellic acid, royal jelly and their mixtures injected on productive performance of Matrouh hens, during experimental periods under Egyptian summer conditions are presented in Table 3.

Body weight changes (BWC) of Matrouh layers injected with 100 , 200 μg GA3, or 50 , 100 RJ /kg BW and their mixtures were significantly ($P \leq 0.05$) increased as compared with the control at the end of the experimental period.

The present results are in agreement with those reported by El-Sheikh and Hanafy (2006) who found that the live body weight of females and males of Bandarah local chicken strain injected with 250 or 500 μg GA3 were significantly ($P \leq 0.01$) expanded particularly with the high dose during both treatments and recovery periods when compared with the control group. Marey (1974) showed that birds live body weight due to GA3 treatment was

Table (3). Effect of injection with gibberellic acid (GA3) royal jelly (RJ) and their mixtures on productive performance for Matrouh hens from 24-36 weeks of age.

Treatments	Body weight changes (g)	Feed intake (g)	Feed conversion (g./hen/day)	Egg production (%)	Egg (g)	Egg weight (g/hen)	Egg mass (g)	Viability (%)
Control	178.16±4.0 ^b	103.13±1.61 ^b	4.49±0.05 ^a	54.26±0.68 ^c	44.39±0.59	24.09±0.59 ^c	79.17	
100 µg GA3/kg BW	280.25±18.08 ^a	104.36±0.9 ^b	3.99±0.06 ^{bc}	60.22±0.72 ^{ab}	47.46±1.31	28.57±0.77 ^a	83.33	
200 µg GA3/kg BW	293.87±23.67 ^a	105.14±1.62 ^b	3.78±0.16 ^c	63.23±0.99 ^a	47.03±0.95	29.73±0.59 ^a	91.67	
50 mg RJ/kg BW	242.25±16.83 ^a	108.16±2.27 ^b	4.40±0.06 ^a	56.29±0.66 ^c	45.85±0.59	25.80±0.08 ^{bc}	87.5	
100 mg RJ/kg BW	249.55±23.86 ^a	112.12±1.00 ^a	4.47±0.05 ^a	55.97±0.34 ^c	46.33±0.23	25.93±0.23 ^{bc}	91.67	
50 µg GA3+25 mg RJ/kg BW	270.00±16.32 ^a	106.88±1.58 ^a	4.32±0.11 ^a	56.96±0.76 ^{bc}	45.29±0.49	25.80±0.58 ^{bc}	91.67	
100 µg GA3+50 mg RJ/kg BW	261.14±21.78 ^a	107.4±1.22 ^a	4.22±0.15 ^{ab}	57.24±2.50 ^c	46.54±0.29	26.62±1.00 ^b	91.67	
Sig.	*	*	**	**	NS	**	NS	NS

A, b, c Means having different letters in the same column, differ significantly ($P<0.05$).

* = ($P<0.05$); ** = ($P<0.01$) and NS= Not significant.

increased, this might be credited to GA3 physiological impact on guideline of the cell division; incitement of digestive enzymes production and creation of RNA and protein. Impacts of administrating RJ in trial animals were accounted for after its injection or ingestion, where the most apparent outcome was proposed to be the expanded BW turkey (Bonomiet *et al.*, 2001). On the other hand, Seven *et al.* (2016) detailed that the last weight of female quails in the RJ enhanced group (500 mg royal jelly/kg BW) was higher than in the control groups ($P < 0.05$).

Seven *et al.* (2014) revealed that RJ improve growth performance of quails under stress resultant in increased BW and FI and lower FCR. The impact of these dynamic fixings, particularly flavonoids, on quails performance was accounted for beforehand (Bonomiet *et al.*, 2001; Seveniet *et al.*, 2012 and Seven *et al.*, 2014). Saeed *et al.* (2018) found that supplementation with RJ at the level of 10 and 15 mg/kg in layer diets have showed a significant increase in body weight. The expanded last body weight of the RJ group might be linked to the expanding impact on oxygen digestion of tissues on account of expanded concentration and utilization of the blood glucose (Gonnard and N'Guyen, 1957). Notwithstanding this the expanding impact of RJ on tissue oxygen utilization cause last to improve final body weight and continuance because of the advancing impact of RJ on breath and oxidative phosphorylation (Krylov and Sokolskii, 2000).

Results uncovered that, viability percentage and egg weight (EW) of Matrouh layers injected with 100 , 200 μg of GA3 or 50 , 100 RJ /kg BW and their mixtures was not significantly affected and being higher than those control group. Layers injected by GA3/BW showed significantly ($P \leq 0.05$ and $P \leq 0.01$) improved feed conversion ratio (FCR), egg production (EP)% and egg mass (EM)when compared with those the RJ /kg BW and control group through the whole of the experimental period. The present results are in agreement with those reported by Elkomy *et al.* (2007) announced that with 100,200, 400 and 800 μg GA3/kg BW to laying hens at 48 weeks old enough came about in marginally increment in hens FI when contrasted and the non-treated group without any significant impact of the amount. Anderson *et al.*(1982)found that GA3 treated group of 72 weeks old brown type hens significantly expanded egg production % and egg weight. El-Sheikh and Hanafy (2006) demonstrated that the egg production percentage was

significantly ($P \leq 0.01$) expanded, particularly with the low amount of GA3 (250 µg GA3/kg body weight) during both treatment and recuperation periods contrasted and the control group and Elkomy *et al.*(2007)indicated a significant improvement in egg production of Gemiza hens that treated by GA3 where the moderate amount of GA3 (200 µg) had the most elevated mean of egg production. This expansion in egg production may be due to the GA3 treated group had higher coursing estrogen hormone and the metabolic movement of GA3 improved ovulatory process (Anderson *et al.*, 1982). As well as, Khalifa *et al.*(1983)inferred that; the improvement in egg production by estradiol can be clarified by the physiological impact of estrogen upon the ovary and oviduct which causing their activation and upgrading ovulatory process. Also, Saeed *et al.* (2018)found that the unsaturated fats, natural mixes and flavonoids in RJ have been appeared to upgrade the growth rate of quail resulting in improved body weight, feed intake and feed conversion ratio of layer hens. Hamdy *et al.*(2002)announced that, egg mass was significantly and decidedly related with plasma concentration of estrogen. Saeed *et al.* (2018)found that supplementation of RJ at the degree of 10 and 15 mg/kg in layer diets positively influenced egg weight (by 5.0% and 4.8%,individually), egg production (10.5% and 11.0%,separately) and weight gain of hens (7.0% and 6.5%,separately).Additionally, Seven *et al.*(2016)who detailed that the feed consumption of RJ-supplemented groups was higher significantly than those of the control group. Seven *et al.* (2014)announced that these compound segments can improve growth performance of quail under pressure resulting in increased FI. Royal jelly has bunches of flavonoids, organic compounds and unsaturated fats.

Reproductive performance:

Percentages of fertile eggs, hatchability /total eggs and hatchability /fertility eggs of Matrouh hens injected with 100 , 200 µg of GA3 or 50 , 100 RJ /kg BW and their mixtures, under Egyptian summer conditions are presented in Table 4.

Diminishing of fertility percentage under heat stress might be because of that the heat stress could diminish numbered of spermatozoa put away in the sperm have an organ in the hen's regenerative tract (Brillard, 2003). Likewise, hatchability percentage was significantly lower ($P < 0.05$) under heat stress, which might be because of the increment early stage demise during the brooding period because of increment endogenous (metabolic) heat creation

Table (4). Effect of injection with gibberellic acid (GA3), royal jelly (RJ) and their mixtures on productive performance for Matrouh hens during experimental period.

Traits Treatments	Fertile eggs (%)	Hatchability Total eggs (%)	Hatchability/ fertile eggs (%)	Chick weight at one day (g)
Control	75.93±2.40 ^c	64.82±3.21 ^d	85.27±2.08	31.83±0.33 ^c
100 µg GA3/kg BW	83.34±1.86 ^{ab}	75.00±0.93 ^{abc}	90.16±2.52	33.26±0.29 ^{ab}
200 µg GA3 /kg BW	85.19±1.52 ^a	77.78±2.62 ^a	91.31±2.51	33.97±0.20 ^a
50 mg RJ/kg BW	83.59±2.47	72.74±2.32 ^{abc}	87.14±2.95	33.30±0.46 ^{ab}
100 mg RJ/kg BW	84.26±1.78 ^{ab}	73.15±1.78 ^{abc}	86.85±1.72	32.03±0.45 ^{bc}
50 µg GA3+ 25 mg RJ/kg BW	77.78±2.62 ^{abc}	67.60±1.78 ^{cd}	87.03±1.93	32.68±0.32 ^{abc}
100 µg GA3+ 50 mg RJ/kg BW	81.49±1.52 ^{abc}	68.52±2.40 ^{bcd}	84.08±2.29	32.14±0.70 ^{bc}
Sig.	*	**	NS	*

A, b, c Means having different letters in the same column, differ significantly ($P<0.05$).

* = ($P<0.05$); ** = ($P<0.01$) and NS= Not significant.

(Awad *et al.*, 2013). In this investigation, the results indicated that hatchability/fertility eggs % of Matrouh layers injected with 100 , 200 µg of GA3, 50 , 100 RJ /kg BW and their mixtures were not significantly affected. However, fertility eggs % , hatchability/total eggs % and chicks hatch weight (g) were significantly ($P<0.05$ and $P<0.01$) higher for layers injected with 100 , 200 µg of GA3 or 50 , 100 RJ /kg BW as compared with those in the control group. These results agreed with those of Askar and Ismaeil (2012) revealed that fertility percentage of laying hens was affected significantly ($P<0.05$) by GA3 treatment, where hens that injected by 400µg of GA3 showed the highest value (86.67 %) with 29 % more than that of control group (66.67%). El-Sebai *et al.* (2003), demonstrated that GA3 treatment had a little and insignificant effect on fertility percentage of female Japanese quails, while hatchability one was significantly ($P<0.05$) improved due to GA3 treatment. These results might be because of the great semen quality characteristics of the cocks treated with injected with 100 , 200 µg of GA3 kg BW. The improvement in fertility; hatchability and chick characteristics might be ascribed to the helpful impacts of GA3 on egg quality, since part of the metabolic activity in birds is prompting an expansion in estrogen levels and additionally produce direct estrogen-like activity (Gawienowski and Chatterjee, 1980).

On the other hand, the improvement of fertility rates and hatchability/total eggs in heat-stressed animals that treated with RJ could be attributable to the significant compounds of RJ for example proteins, micronutrients, antioxidants and phenolic that enhance liver and kidneys work to decrease in lipid peroxidation and oxidative stress synthesis (Abdelnour *et al.*, 2020). RJ can have the option to restrain the creation of Reactive Oxygen Species (ROS) and protein oxidation (El-Hanoun *et al.*, 2014 and Elnagar, 2010). EL-Tarabany *et al.* (2017) revealed that RJ may improve the negative effects of senescence in laying hens at the late phase of production.

The immunoglobulin, IgG, IgM and sheep red blood cells (SRBC's) :

It's known, heat stress significantly decreases the plasma levels of IgA, IgG and IgM in chickens (Chen, 2015). This decrease of antibody titer with heat stress be credited to the arrival of a continued elevated level of corticosterone bringing about diminished IgM and IgG combination (Habibian *et al.*, 2013). Figures (1 and 2) exhibited that immunoglobulin G (IgG), immunoglobulin M (IgM) and primary and secondary antibody titer against sheep red blood cells (SRBC's), in hens Matrouh injected with 100 , 200 µg of

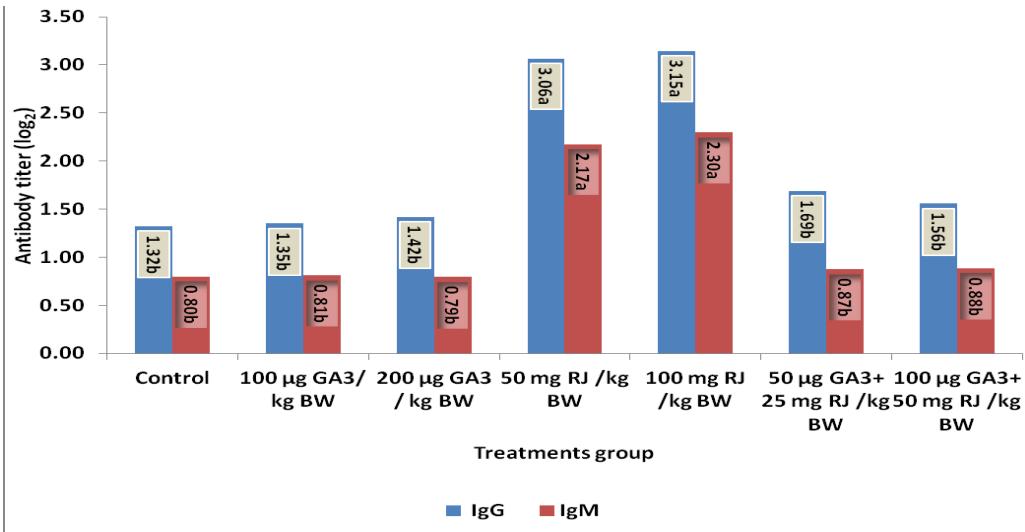


Figure (1): IgG and IgM antibody titre (\log_2) as affected by gibberellic acid and royal jelly and their mixtures injection in Matrouh hens strain under Egyptian summer conditions.

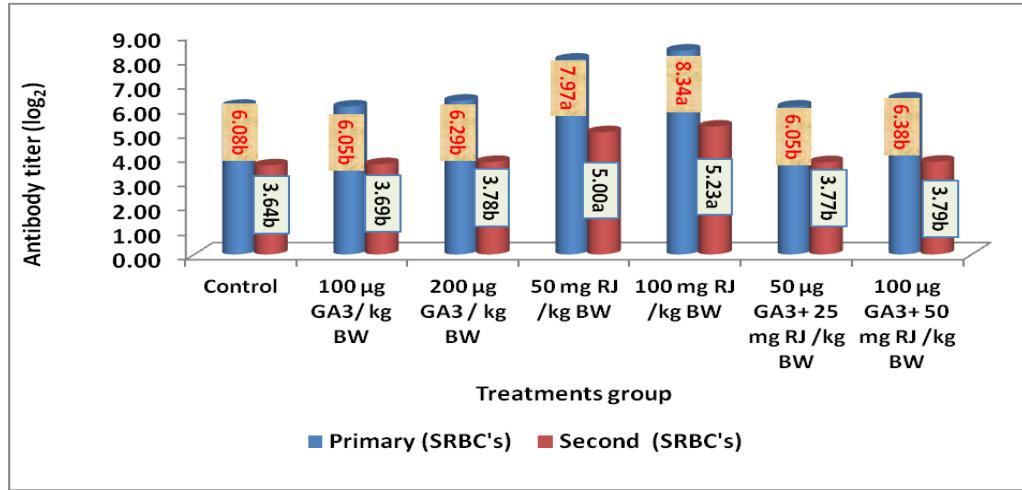


Figure (2): Primary and secondary antibody titer against sheep red blood cells (SRBC's) as affected by gibberellic acid and royal jelly and their mixtures injection in Matrouh hens strain under Egyptian summer conditions.

GA3 /kg BW only or mixtures with the levels of 50 , 100 RJ /kg BWRJ were not significantly affected. However, injection 50 , 100 µg of RJ /kg BW caused to significantly ($P\leq 0.01$) increased in IgG, IgM and primary and secondary antibody titer against sheep red blood cells (SRBC's) in hens. The improvement of the immune system might be RJ contains amino and gamma globulin, unsaturated fats, hormones, catalysts, proteins, nutrient E and A that help the invulnerable framework battle disease (Crenguta *et al.*, 2011). These results agreed with those of Mufarrej and Ei- Sarag (1997)saw that directed RJ (200 mg) significant increased antibody production against SRBC's in chickens when contrasted with the control group. Taha *et al.* (2019)announced that RJ injection in ovo at levels 0.25 and 0.5 ml/egg upgraded antibody titres against SRBC's of chicken strains El-Salam and Dokki-4 when contrasted with partner control chicks. Similarly, Elnagaret *et al.* (2010)found that RJ treatments expanded IgG and IgM levels in heat-stressed growing rabbits. El-Hanoun *et al.* (2014) announced that treated heat-stressed male rabbits with 50, 100 and 150 mg RJ/kg BW significantly ($P<0.05$) increased sheep red blood cells (SRBC's), IgG, IgM and IgA levels, and this expansion was dose-dependent as compared with the control group.

Some blood serum hormones concentration:

Blood serum estrogen, FSH, LH in females and testosterone, FSH and LH in males Matrouh injected with 100 , 200 µg of GA3 or50 , 100 RJ /kg BW and their mixtures during hot summer condition are presented in Table 5. In this investigation, the outcomes indicated that blood serum estrogen, FSH, LH in hens and FSH and LH in the cocks of Matrouh layers injected with 50 , 100 RJ /kg BW only or mixtures with the levels of GA3were not significantly affected. In any case, injection 100, 200 µg of GA3 /kg BW caused to significantly ($P\leq 0.05$ and $P\leq 0.01$) increased in concentration of estrogen in hens and testosterone of cocks, FSH and LH in hens and cocks compared with those in control groups. These results agreed with those of Elkomy (2003) who revealed that GA3 can stimulate estrogen emission in hens. Elkomy *et al.* (2007)presumed that gibberellic acid can have both estrogenic organic impacts on female chicks and testosterone natural consequences for male chicks. The reason for the high levels of estrogen in the blood serum of the two female injections might be ascribed to the job of gibberellic acid in stimulating the ovaries to discharge estrogen (Elkomy *et al.*,

Table (5). Effect of injection with gibberellic acid (GA₃), royal jelly (RJ) and their mixtures on some hormones concentration for Matrouh chicks at the end of experimental period.

Traits	Hens treated			Cocks treated		
	Estrogen ($\mu\text{g}/\text{ml}$)	FSH ($\mu\text{g}/\text{ml}$)	LH ($\mu\text{g}/\text{ml}$)	Testosterone ($\mu\text{g}/\text{ml}$)	FSH ($\mu\text{g}/\text{ml}$)	LH ($\mu\text{g}/\text{ml}$)
Treatments						
Control	182.17 \pm 14.87 ^a	2.06 \pm 0.09 ^b	1.80 \pm 0.08 ^c	2.51 \pm 0.18 ^c	0.60 \pm 0.07 ^b	0.85 \pm 0.09 ^c
100 μg GA ₃ /kg BW	253.34 \pm 15.68 ^a	2.80 \pm 0.18 ^a	2.50 \pm 0.19 ^a	3.32 \pm 0.11 ^{ab}	1.00 \pm 0.12 ^a	1.36 \pm 0.11 ^a
200 μg GA ₃ /kg BW	259.17 \pm 13.61 ^a	2.73 \pm 0.21 ^a	2.43 \pm 0.22 ^{ab}	3.51 \pm 0.06 ^a	1.08 \pm 0.12 ^a	1.31 \pm 0.13 ^{ab}
50 mg RJ/kg BW	200.17 \pm 11.00 ^b	2.07 \pm 0.18 ^b	1.83 \pm 0.17 ^c	3.03 \pm 0.20 ^{ab}	0.63 \pm 0.08 ^b	0.85 \pm 0.14 ^c
100 mg RJ/kg BW	195.67 \pm 15.62 ^b	2.12 \pm 0.14 ^b	1.87 \pm 0.15 ^c	3.20 \pm 0.18 ^{ab}	0.64 \pm 0.09 ^b	0.93 \pm 0.15 ^c
50 μg GA ₃ + 25 mg RJ/kg BW	189.17 \pm 9.33 ^b	2.25 \pm 0.20 ^b	1.92 \pm 0.21 ^{bc}	2.99 \pm 0.16 ^b	0.64 \pm 0.10 ^b	0.89 \pm 0.15 ^c
100 μg GA ₃ + 50 mg RJ/kg BW	192.84 \pm 14.96 ^b	2.33 \pm 0.24 ^b	1.82 \pm 0.19 ^c	3.00 \pm 0.13 ^b	0.65 \pm 0.14 ^b	0.95 \pm 0.12 ^c
Sig.	**	*	*	**	*	*

A, b, c Means having different letters in the same column, differ significantly ($P<0.05$).

* = ($P<0.05$) and ** = ($P<0.01$).

2007). The purpose behind the high concentration of hormone testosterone in the blood serum of male might be because of the high rates of weights, sizes of testicles for these birds and testosterone fixation (Garamszegi *et al.*, 2005). Elkomy *et al.* (2007) showed a significant level of FSH, LH hormone in the serum of old female chickens who were injected with gibberellic acid at a concentration of 200, 400 µg / kg of body weight compared to the control group. The explanation behind the significant levels of FSH and LH hormones in female and male blood serum injections might be due to the expansion in testosterone in the male blood serum and estrogen in the female blood serum, which was appeared by Tsuisui and Ishii, (1980) who found that FSH and testosterone of Japanese quail increase FSH official by Sertoli cells, and together apply a synergistic impact on FSH binding. The effect of RJ on testosterone is in agreement with the findings of Elnagar (2010) demonstrating a significant increment in testosterone level when heat stressed male rabbits were orally managed 200, 400 and 800 mg RJ/kg BW. El-Hanoun *et al.* (2014) revealed that treated heat-stressed buck rabbits with 50, 100 and 150 mg RJ/kg BW supported blood testosterone concentration to arrive at 10, 20 and 28%, separately, over the untreated group values.

Semen physical characteristics:

Semen ejaculate volume, hydrogen-ion concentration (pH), sperm motility, dead spermatozoa, sperm abnormalities (%), sperm cell concentration ($\times 10^9/\text{ml}$) and acrosomal damage (%) of Matrouh cocks injected with 100, 200 µg of GA3, 50 , 100 RJ /kg BW and their mixtures, under Egyptian summer conditions are presented in Table 6.

Semen ejaculate volume and hydrogen-ion concentration (pH) of cocks were not significantly affected by the injection of GA3, RJ /kg BW and their mixtures. However, injection of 100 , 200 µgGA3, 50 , 100 RJ /kg BW and their mixtures caused to improve significantly ($P<0.05$) sperm-cell concentration ($\times 10^9/\text{ml}$) and sperm motility (%), while significantly ($P<0.05$) decreased each of dead spermatozoa (%), sperm abnormalities (%), and acrosomal damage (%) as compared with the control group. The improvement of semen physical properties might be GA3 animating spermatogenesis prose or /and may be have direct testosterone-like action on testicles, thus initiate enactment of testicular germinal epithelium in the seminiferous tubules (El- Sheikh and Hanafy, 2006). These results agreed with those of Elkomy

Table (6). Effect of dietary supplementation of gibberellic acid, royal jelly and their mixtures on semen quality of Matrouh cocks at the end of the experimental period.

Traits	Ejaculate volume (ml)	Hydrogen-ion concentration (pH)	Sperm motility (%)	Dead spermatozoa (%)	Sperm abnormalities (%)	Sperm cell concentration ($\times 10^6$ /ml)	Acrosomal damage (%)
Treatments							
Control	0.25±0.03	7.19±0.12	80.00±2.89 ^b	13.75±1.25 ^a	10.25±0.48 ^a	3.29±0.30 ^b	9.00±1.09 ^a
100 μg GA3/kg BW	0.43±0.08	7.19±0.12	87.50±3.23 ^{ab}	7.50±1.05 ^{bc}	6.25±0.63 ^d	4.53±0.23 ^a	5.00±0.92 ^{bc}
200 μg GA3/kg BW	0.45±0.07	7.18±0.12	92.50±1.45 ^a	6.25±1.04 ^c	5.75±0.48 ^d	4.68±0.26 ^a	3.75±0.86 ^c
50 mg RJ/kg BW	0.38±0.05	7.30±0.13	85.00±3.54 ^{ab}	10.50±1.56 ^{bc}	9.50±0.65 ^{ab}	4.00±0.33 ^{ab}	5.50±0.90 ^{bc}
100 mg RJ/kg BW	0.38±0.05	7.50±0.21	86.25±1.25 ^{ab}	11.75±1.44 ^{abc}	8.50±0.29 ^{bc}	3.85±0.26 ^{ab}	7.50±1.05 ^{ab}
50 μg GA3+ 25 mg RJ/kg BW	0.30±0.05	7.50±0.21	91.25±2.40 ^a	8.25±1.66 ^{bc}	6.50±0.29 ^d	4.38±0.36 ^a	5.00±0.82 ^{bc}
100 μg GA3+ 50 mg RJ/kg BW	0.45±0.07	7.30±0.13	90.00±2.05 ^a	9.75±1.32 ^{abc}	7.00±0.71 ^{cd}	4.48±0.36 ^a	5.50±1.05 ^{bc}
Sig.	NS	NS	*	*	**	*	*

A, b, c Means having different letters in the same column, differ significantly ($P<0.05$).
 * = ($P<0.05$); ** = ($P<0.01$) and NS= Not significant.

(2003) found that treated cockerels with GA3 conducted to improve semen quality attributes (sperm concentration, sperm motility increase live sperm and decrease abnormal sperm). GA3 improved semen attributes and has a constructive effect on semen quality and amount. These perceptions allude to that GA3 might be caused animating and supporting spermatogenesis process in seminiferous tubules to create spermatozoa and furthermore, sex embellishment organs to discharge original plasma (Kamel *et al.*, 2009).

On the other, hand, Elnagar (2010), who detailed that the oral organization of RJ can check summer infertility (significant improvement of a series of spermography parameters) and improve the physiological status in male rabbits. The improvement in motility of sperm might be because of the increase in the level of testosterone that is responsible for sperm motility (Table 6).

Economical efficiency(%):

Data shown in Table (7) clear that Matrouh layers injected with 200 µg of GA3 /kg BW were recorded the highest net revenue and the best economical efficiency followed by those 100 µg of GA3/kg BW as compared with the control groups, while 50 or 100 µg of RJ/kg BW had the lowest net revenue and economical efficiency (%).

Conclusively, data revealed that injection with 100, 200 µg GA3 /kg BW of caused to improving productive, reproductive and physiological performance of males and females Matrouh. Furthermore, the RJ-treated groups had significantly improved immune responses in hens and the quality of semen parameters in cocks under summer season conditions in Egypt.

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Table (7). Inputs- output analysis and economical efficiency (%) of laying hens reared during summer season at the end of the experimental period.

Traits Treatments	Control	100 μg GA3/kg BW	200 μg GA3 /kg BW	50 mg RJ/kg BW	100 mg RJ/kg BW	50 μg GA3+ 25 mg RJ /kg BW	100 μg GA3+ 50 mg RJ/kg BW	Sig. test
Egg number	45.57	50.58	53.11	47.28	47.01	47.84	48.08	
Price/egg (LE)	1.30	1.30	1.30	1.30	1.30	1.30	1.30	
Total revenue hen (LE)	59.24	65.75	69.05	61.45	61.11	62.19	62.51	
Total feed intake/hen(kg)	8.66	8.77	8.83	9.09	9.42	8.98	9.02	
Price/Kg feed	4.75	4.75	4.75	4.75	4.75	4.75	4.75	
Total feed cost/hen (LE)	41.15	41.64	41.95	43.15	44.73	42.64	42.85	
Cost of hen treated (LE)	3.00	4.87	6.75	5.02	7.04	4.95	6.89	
Total cost hen (LE)	44.15	46.51	48.70	48.17	51.77	47.59	49.74	
Net revenue/hen(LE)	15.09 ^b	19.24 ^a	20.35 ^a	13.28 ^{bc}	9.34 ^c	14.60 ^b	12.76 ^{bc}	**
Economical efficiency (E.Ef.)%	34.19 ^b	41.38 ^a	41.78 ^a	27.58 ^{bc}	18.03 ^c	30.69 ^b	25.66 ^{bc}	**
Relative E.Ef.	100.00	121.02	122.19	80.65	52.74	89.75	75.04	

A, b, c Means having different letters in the same row differ significantly($P<0.05$).

** = ($P<0.01$).

The price of (ml) Gibberellic acid (GA3) = 2.50 LE/Kg . The price of (gm) Royal Jelly (RJ) = 12.0 LE/Kg
Net revenue/hen (LE) = Total revenue - Total cost/hen E.Ef= Net revenue/hen(LE)/ Total cost/hen (LE)

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تأثير حقن حمض الجبريليك والرويال جيلي على بعض الصفات الإنتاجية والتسلية والفيسيولوجية في سلالة دجاج مطروح خلال موسم الصيف

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تم استخدام 168 دجاجة بياضه و21 ديك من سلالة مطروح المحلية عند عمر 24 أسبوعاً (عند 43.93% إنتاج بيض) في تصميم عشوائي تام وتم تقسيم الطيور عشوائياً في 7 مجموعات تجريبية لكل منها ثلاثة مكررات (8 دجاجة + 1 ديك لكل مكررة). تم حقن الطيور كل أسبوعين حتى 36 أسبوع من العمر. تم حقن طيور المجموعة الأولى

بمحلول ملحي 9%. كنترول، وتم حقن الطيور في المعاملتين الثانية والثالثة تحت الجلد ب 100 و 200 ميكروجرام من حمض الجبريليك (GA3) / كجم من وزن الجسم ، على التوالي. في حين تم حقن المعاملتين الرابعة والخامسة ب 50 و 100 ميكروجرام روبيال جيلي (RJ) / كجم وزن الجسم، على التوالي. وتم حقن المعاملة السادسة تحت الجلد مع 50 ميكروجرام GA3 بالإضافة إلى 25 ميكروجرام RJ / كجم وزن الجسم. تم حقن المعاملة السابعة تحت الجلد مع 100 ميكروجرام GA3 بالإضافة إلى 50 ميكروجرام RJ / كجم وزن الجسم. واستخدمت نفس المعاملات السبع على 21 ديك في بطاريات فردية لأخذ عينات جوده السائل المنوي.

وأظهرت النتائج: سبب الحقن ب 100 ، 200 ميكروجرام GA3 او 50 ، 100 ميكروجرام RJ / كجم من وزن جسم طيور مطروح في تحسن كبير (على مستوى احتمال 0.5 و 0.01) في التغير في وزن الجسم و % النسبة المئوية للبيض المخصب والنسبة المئوية للفقس بالنسبة للبيض الكلي وزوزن الكتاكيت الفاقسة (بالGram) وتركيز الحيوانات المنوية الطبيعية وقدرة الحيوانات المنوية الحية على الحركة بينما انخفض معنويا كلًا من نسبة المئوية للحيوانات المنوية الميتة والشاذة والاكروسوم الشاذ عند المقارنة بمجموعه الكنترول 0 وتحسن معنوي(على مستوى احتمال 0.5 و 0.01) معامل التحويل الغذائي، إنتاج وكتله البيض لدجاج المحققون ب 100 ، 200 ميكروجرام /GA3/ كجم من وزن الجسم مقارنه ب 50 ، 100 ميكروجرام RJ ومجموعه الكنترول خلال فترات التجربة. وزاد معنويًا (على مستوى احتمال 0.01) (تركيز الجلوبيلين المناعي G و الجلوبيلين المناعي M والاستجابة المناعية لخلايا الدم الحمراء للأغذام لدجاج المحققون ب 50 ، 100 ميكروجرام RJ / كجم من وزن جسم طيور مقارنه 100 ، 200 ميكروجرام /GA3/ كجم من وزن جسم طيور ومجموعه الكنترول. وزاد معنويًا (على مستوى احتمال 0.5 و 0.01) تركيز هرمون الاستروجين في الدجاج والتستوستيرون في الديوك ، FSH و LH في الدجاج والديوك مقارنه مع مجموعه الكنترول.

التوصية: نستخلص من هذه النتائج أن الحقن ب 100 ، 200 ميكروجرام /GA3 كجم من وزن الجسم أدي لتحسين الأداء الإنتاجي والتسلسي والفيسيولوجي لذكور وإناث مطروح. وعلاوة على ذلك، تحسنت معنويًا الاستجابة المناعية في الدجاج وجودة السائل المنوي في الديوك للمجموعات المعاملة ب RJ في ظل ظروف موسم الصيف في مصر.