

EFFECTS OF BETAININE WITH DIFFERENT LEVELS OF GUANDINO ACETIC ACID SUPPLEMENTATION ON PRODUCTIVE AND REPRODUCTIVE PERFORMANCE OF LOCAL MAMOURAH STRAIN DURING SUMMER SEASON IN EGYPT.

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ABSTRACT:

The main objective of this study was to investigate the effect of adding betaine with different levels of guandino acetic acid (GAA) on the productive and reproductive performances of the Mamourah chickens strain, under Egyptian summer conditions. A total number of 192 laying hens and 24 cocks of Mamourah chickens at 48 weeks of age (At 63.24 % egg production) was used in a completely randomized design and randomly assigned into 8 experimental similar groups in body weight and egg production (24 hens + 3 cocks in each treatment group), each group with three replications (8hens +1 cock in each replicate) and was kept in a single cage within an open system house until 60 weeks of age. The experimental 8 groups were arranged as control, and that fed a basal diet supplemented with 1600 mg betaine/kg diet, while the third up fifth groups were fed a basal diet supplemented with 600, 800 and 1000 mg guandino acetic acid/kg diet, respectively, and from the sixth to the eighth groups were fed a basal diet supplemented with 1600 mg betaine/kg diet and 600, 800 and 1000 mg guandino acetic acid/kg diet.

***The obtained results showed that,** dietary betaine and GAA had no significant ($P<0.05$) effect on body weight changes (BWC), feed intake (FI), egg weight (EW), hatchability/fertility eggs (%), chick hatch weight (gm), total antioxidant capacity (TAOC) of Mamourah layers during the whole experimental period. Feed conversion ratio (FCR), egg production (EP) and egg mass (EM) significantly*

($P < 0.01$) improved in hens fed the diet containing 1600 mg betaine + 600 mg/Kg GAA as compared to the control group. Fertility eggs (%) and hatchability/total eggs (%) were significantly ($P < 0.05$ and 0.01) higher for layers fed diets contained betaine, GAA and their mixtures., Increasing the amount of GAA significantly ($P \leq 0.05$) increased the superoxide dismutase (SOD) and glutathione peroxidase (GPX). However, Malonidialdehyde (MDA) caused decreased significantly ($P \leq 0.05$) with supplementing either dietary betaine, GAA and their mixtures as compared with the control group. Dietary supplementation of either betaine, GAA and their mixtures improved significantly ($P < 0.05$) sperm motility (%), dead spermatozoa (%), sperm abnormalities (%), sperm-cell concentration ($X 10^9/ml$) and acrosomal damage (%) as compared with the control group. Feeding Mamourah layers a diet supplemented with 1600 mg betaine + 600 mg GAA recorded the highest net revenue and the best economical efficiency.

In conclusion, dietary supplementation of betaine with various levels of GAA might be potentially used to improve each of EP, fertility eggs (%) and hatchability per total eggs (%) of Mamourah laying hens and most characters of semen quality for cocks under summer season conditions in Egypt.

INTRODUCTION

High ambient temperature in Egypt during the summer season, which joined by high relative humidity, may cause water imbalance and osmotic change in cells, bringing about lack of hydration which influence the phone movement (Sahin *et al.*, 2009). Heat stress has negative effects on feed intake, laying and reproductive performances and physiological traits in the chickens (Attia *et al.*, 2011). The adverse effect of heat stress might be ascribed to the complex interresponse of low feed intake, endocrine system, acid – base imbalance and poor physiological function of organs and mechanism connected with the entire egg production process, follicular enrollment ovulation, egg and shell arrangement, egg and yolk improvement, oviposition and oviposition interim (Oguntunji and Alabi, 2010).

Betaine is absent in most animal feedstuffs and dietary supplementations seem to be important to enhance productivity and resistance to heat stress (Wang *et al.*, 2004). Moreover, betaine is a typical term for trimethylglycine, a substrate for betaine-homocysteine methyltransferase in liver and kidney (Attia *et al.*, 2009). Betaine has two essential metabolic parts, including the main part a methyl bunch giver in the accompanying vital compound pathways of protein metabolism, creatine, adrenaline, phosphotidy choilne and DNA methylation, protein and fat metabolism (Rima, 2013) it's exceedingly prone to be identified with the union of hormones. Second part was an osmolyte that aids cell water homeostasis. The antioxidant mechanism of betaine was found to enhance nonenzymatic antioxidant defenses via the methionine–homocysteine cycle and form a protective membrane around cells (Zhang *et al.*, 2016). Supplementation of betaine in laying hen diets caused to improve of egg production (Gudev *et al.*, 2011), fertility, hatchability and chick weight at hatch (Tollba and El-Nagar, 2008), egg weight, egg mass (Park *et al.*, 2006).

Guanidinoacetic corrosive (GAA) has been appearing to save arginine (ARG) requirements in chickens. This plays a basic part in enhancing growth and averting right ventricular hypertrophy in chickens subjected to hypobaric hypoxia (Ahmadipour *et al.*, 2018). GAA is integrated from glycine and arginine by l-arginine:glycyl amidinotransferase (AGAT) in kidney and liver of Avian (Dilger *et al.*, 2013). Decreased semen quality and fertility rate is a typical component in local cocks and hens under Egyptian hot summer condition. This decrease is related to brokenness of Sertoli cells and defective spermatogenesis. Since GAA has a guanidinium ion of conjugate base, which effectively gives an electron, higher concentrations of GAA may produce a hydroxyl radical, solid free radical and impede antioxidant capacity (Hiramatsu, 2003). GAA, as a forerunner of creatine, plays an important role in the proper functioning of Sertoli cells and energy metabolism in sperm (Tapeh *et al.*, 2016). GAA is the antecedent of creatine (Mousavi *et al.*, 2013). GAA play a key part in cellular energy metabolism since it is the single immediate precursor of creatine in animal and humans; creatine is a key energy reserve in animal tissues, and it is help to maintain a strategic distance from the exhaustion of cell adenosine triphosphate (ATP) through the immediate provision of high energy phosphates to regenerate the ATP atom from adenosine diphosphate

(Wyss and Kaddurah- Dauk, 2000) and the creatine may enhance the dependability of cellular membranes and the creatine may enhance the security of cellular membranes (Sestili *et al.*, 2011). On the other hand, GAA is a more reasonable feed additive as compared with creatine and arginine due to its lower price than them and is more chemically stable than creatine (Dilger *et al.*, 2013). Sharideh *et al.* (2016) detailed that the addition of the GAA in laying hen diets lead to improvement of fertility and motility of sperm by about 22 and 29% respectively.

Therefore, the aim of this study was to evaluate the effects of dietary betaine with different levels of guandino acetic acid on productive and reproductive performances of local Mamourah chickens strain during Egyptian summer conditions.

MATERIALS AND METHODS

Birds, diet and treatments:

The experimental work of this study was carried out at El-Serw Poultry Research Station, Domiat Governorate, Animal Production Research Institute, Agricultural Research Center, Giza, Egypt, from June to August, 2016.

A total number of 192 laying hens and 24 cocks of Mamourah chickens at 48 weeks of age (At 63.24 % egg production) was used and divided into equal 8 experimental groups (24 hens + 3 cocks), each with three replicates (8 hens +1 cock in each replicate) similar body weight and egg production in a completely randomized design to investigate the effects of dietary betaine and guandino acetic acid (GAA) supplementation on productive and reproductive performance of local Mamourah chickens strain under Egyptian summer conditions. Birds in each replicate per treatment group were kept in single cage in an open system house. The basal diet was formulated to meet the NRC (1994) recommendations as shown in Table 1 and the calculated analysis was according to Feed Composition Tables for Animal And Poultry Feedstuffs used in Egypt (2001). The experimental groups were arranged as follows: the first group fed a basal diet without any dietary supplementation (as a control), the second group was fed a basal diet supplemented with 1600 mg betaine/kg diet, where the third up fifth groups were fed a basal diet supplemented with 600, 800 and 1000 mg guandino acetic acid/kg diet, respectively, while from the sixth to the eighth groups were fed a basal diet supplemented with 1600 mg

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

Ingredients	%
Yellow corn	61.80
Soybean meal (44% CP)	15.10
Wheat bran	8.28
Corn gluten meal (60% CP)	4.75
Dicalcium phosphate	1.35
Vegetable oil	0.00
Salt	0.30
Limestone	8.10
Vit + Min. premix*	0.30
DL-Methionine	0.02
Total	100
Calculated analysis :(NRC, 1994)	
Crude protein (CP); %	16.07
ME; kcal/kg	2691
Ether extract	2.942
Crude fiber	3.434
Calcium	3.468
Av. Phosphorus	0.304
Lysine	0.653
Methionine	0.314
Methionine + cystine	0.608

*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 and carrier CaCo₃ up to 1kg.

betaine/ kg diet and 600 , 800 and 1000 mg guanidino acetic acid/kg diet, respectively. Birds were fed *ad libitum* and fresh water was continuously provided. Birds were submitted to the same managerial conditions in a window house with light cycle regimen of 16 hours light: 8 hours darkness. Birds were examined against diseases and treated with antibiotics and vaccines to keep them healthy.

The average minimum and maximum of ambient temperature during the experimental period ranged between 26.37 and 34.98°C, relative humidity from 34.28 to 76.52 % and temperature-humidity index (THI) from 23.94 to 33.47 under El-Serw, Domiat Governorate, Egypt as shown in Table 2. THI was estimated according to the formula as follows: $THI = db\ ^\circ C - \{(0.31 - 0.31 RH) (db\ ^\circ C - 14.4)\}$, where $db\ ^\circ C$ = bulb temperature in Celsius and $RH = RH\%$. 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 under environmental conditions in Egypt (Marai *et al.*, 2000).

Table (2): Means of air temperature ($^\circ C$), relative humidity (RH %) and temperature-humidity index (THI) factors during experimental period according to the Egyptian Meteorological Authority

Factors Months	$^\circ C$		RH (%)		THI	
	Min*	Max**	Min*	Max**	Min*	Max**
June	26.17±0.54	38.7±0.57	17.87±1.28	75±3.36	23.17	36.82
July	26.52±0.29	32.94±0.1	42.59±0.99	77.13±1.21	24.36	31.62
August	26.42±0.38	33.3±0.16	42.39±1.04	77.42±1.59	24.27	31.98
Averages	26.37±0.41	34.98±0.27	34.28±1.1	76.52±2.05	23.94	33.47

Laying performance traits:

The body weight changes of laying hens calculated by the difference between final and initial weight, while, the egg number (EN) and egg weight (EW) were daily recorded. Feed consumption (FC) was weekly recorded. The egg production rate (EP) was calculated during the experimental period.

Where: Egg production rate = Egg number / hen/ x 100 and egg mass (EM) were calculated during the whole experimental period from 48-60 weeks of age. Feed conversion (g feed/g egg) (FCR) was also calculated. The mortality rate was daily recorded for each treatment from 48 weeks of age until the end of the experiment (60 weeks of age).

Hatching traits:

After production of the primary egg, all females were artificially inseminated twice times every week with a blend of semen collected from the same group of cocks. At 56 weeks of age, about 60 eggs from each treatment group were collected and incubated. After hatching, the chicks were counted and non-hatched eggs were broken to determine the percentages of fertility and hatchability. 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.

Blood samples:

At the end of the experimental period, 3 hens arbitrarily browsed every treatment and blood tests were acquired from the brachial vein. Blood serum was separated by centrifugation of the blood at 3000 rpm for 15 min and was then frozen at -20°C for analysis. Antioxidant components and antioxidant enzymes {superoxide dismutase (SOD), glutathione peroxidase (GPX), total antioxidant capacity (TAOC) and Malonidialdehyde (MDA)} were determined using commercial Kits produced by Bio-diagnostic, Egypt.

Semen quality:

Semen was collected at three times amid the trial time frame in 48, 52 and 60 weeks of age from 5 cocks in each treatment which 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 (Ommati *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 ($\times 10^9/\text{ml}$) a droplet of diluted semen (1:200 in distilled water) semen was tenderly put on both councils of a Neubauer hemocytometer and the number

of spermatozoa was determined microscopically (Ommati *et al.*, 2013). Acrosomal damage of spermatozoa (%) was determined according to Waston (1975). Not less than 10 minuscule fields were inspected for every semen test.

The economical efficiency (EEF):

The economical efficiency (EEF) of the experimental treatments was estimated depending up on feeding cost and price of egg produced as the following equation: $EEF = (\text{Net revenue/hen} / \text{Total cost hen}) \times 100$.

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, 2004) 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 ($i = 1 \dots 7$), R_j = Replicates ($j=1,2,3$), e_{ij} = Experimental random error.

All percentages data were transferred to percentage angle using arcsine equation before subjected to statistical analysis. Significant differences among means were tested using Duncan Multiple New Range Test (Duncan, 1955).

RESULTS AND DISCUSSION

Productive performance:

Dietary betaine and guanidinoacetic acid (GAA) supplementation had no significant ($P < 0.05$) effect on body weight changes (BWC), feed intake (FI) and Egg weight (EW) of Mamourah layers during the whole experimental period (Table 3). The present results were in agreement with the reports of Khakran *et al.* (2018) who in indicated that the GAA had no significant effect on the BWC and FI of laying hens. Omer *et al.* (2018) noted that 0.5% betaine supplementation did not affect the body weight (BW) changes and EW of laying hens. Feed conversion ratio (FCR), egg production (EP) rate and egg mass (EM) were significantly ($P < 0.01$) improved in hens fed the diet

Table (3). Effect of dietary supplementation of betaine, guanidinoacetic acid (GAA) and their mixtures on productive performance of Mamorah hens during experimental period under Egyptian summer conditions.

Traits Treatments	Body weight changes (g.)	Feed intake (g. / hen/ day)	Feed conversion (g. feed/ g. egg mass)	Egg production %	Egg weight (g.)	Egg mass (g./hen)	Viability %
Control	197.50 ±6.96	123.36 ±3.00	4.80 ±0.29 ^a	51.64 ±2.16 ^d	50.41 ±0.29	25.91 ±0.95 ^c	94.96
1600 mg betaine	197.50 ±6.96	123.88 ±2.78	4.34 ±0.05 ^{bc}	56.89 ±1.00 ^b	50.65 ±0.3	28.56 ±0.37 ^b	96.64
600 mg GAA	253.34 ±22.76	123.74 ±2.55	4.38 ±0.06 ^{bc}	56.94 ±0.85 ^{bc}	50.45 ±0.45	28.42 ±0.39 ^b	95.8
800 mg GAA	205.42 ±47.37	123.01 ±2.35	4.35 ±0.06 ^{bc}	57.44 ±0.69 ^{bc}	49.92 ±0.54	28.44 ±0.15 ^b	96.64
1000 mg GAA	221.25 ±19.38	124.20 ±2.48	4.49 ±0.02 ^{ab}	56.45 ±1.17 ^c	49.61 ±0.5	27.81 ±0.72 ^b	95.8
1600 mg betaine + 600 mg GAA	235.42 ±27.29	123.82 ±2.51	4.04 ±0.01 ^c	62.85 ±1.23 ^a	49.27 ±0.43	30.76 ±0.68 ^a	96.64
1600 mg betaine + 800 mg GAA	220.42 ±17.45	124.04 ±2.33	4.20 ±0.03 ^{bc}	60.71 ±0.88 ^{ab}	49.44 ±0.91	29.71 ±0.51 ^{ab}	97.48
1600 mg betaine + 1000 mg GAA	200.63 ±33.23	119.20 ±2.31	4.10 ±0.05 ^c	58.83 ±1.04 ^{bc}	50.19 ±0.34	29.22 ±0.48 ^{ab}	98.32
Sig.	NS	NS	**	**	NS	**	NS

Means having different letters in the same row, differ significantly. * = (P<0.05); ** = (P<0.01); NS= Not significant.

supplemented with 1600 mg betaine + 600 mg/kg GAA diet as compared to control groups (Table 3). The improvement of feed conversion ratio may be due to that betaine had effect as a methyl donor for methionine and its diverse physiological properties that could improve the gut environment and thus enhance the ability of absorption (Remus *et al.*, 2004). They added, it may be enhanced utilization of dietary amino acids for protein synthesis and release a few amino acids available for domination and eventual synthesis of adipose tissue or to support intestinal growth, function, increased cell proliferation and improves microbial fermentation activity, which in turn, may enhance nutrient

digestibility (Ratriyanto *et al.*, 2009). Betaine accumulation in the cell may protect it from osmotic stress and allows regular metabolic activities in conditions that would normally inactivate the cell (Saunderson and MacKinlay, 1990). These results were similar to those obtained by Ryu *et al.* (2002) observed that addition of betaine to laying hen diets at a level of 5.00 and 2.00 ppm significantly improved the numerically egg production, egg mass and feed conversion ratio compared with the control group. Also, Park *et al.* (2006) suggested that dietary betaine supplementation could improve feed conversion of laying hens. Ezzat *et al.* (2011) indicated that betaine supplementation in the diets of laying hens increased EP and improved FCR when compared to the control under summer conditions in Egypt. Gudev *et al.* (2011) reported that egg production and egg mass were significantly improved ($P < 0.05$) when betaine supplemented to laying diet up to 1.5 g/kg for hens during heat stress. Laying performance improvement may be due to the fact that betaine supplementation increased the secretion of blood insulin-like growth factor binding protein-3 (IGFBP-3), consequently extended the half-life of blood IGF-I and increased preservation, enhancing the productivity and liver tissue differentiation (Park *et al.*, 2006). Also, it could reduce the requirement for other methyl group donors such as methionine and choline and its osmotic properties as well as the potential to improve the digestibility of specific nutrients (Remus *et al.*, 2004). Moreover, it improved protein and fatty acids synthesis in the liver (Rima, 2013). These results were in agreement with those obtained by Park *et al.* (2006) who reported that egg production was insignificantly improved by betaine supplementation up to 1.2 g /kg to laying hen diets as compared to the control group.

On the other hand, the viability percentage (%) of laying hens fed diet supplemented with different betaine levels only or with GAA was insignificantly better than those fed the control diet (Table 3). These results may be due to that betaine consumption resulted in suppressing insulin-like growth factor binding protein-1 (IGFBP-1) secretion in the livers of laying hens as the result of the reduction in catabolic functions which resulting from stress, infection and immune deficiency (Jones and Clemmons, 1995). These results were in agreement with those obtained by Zhan (2001) who reported that betafin (betaine) addition significantly improved ($P < 0.05$) the immune response and alleviated the response of body temperature. Awad *et al.* (2014)

reported that betaine supplementation at different levels (0, 0.5, 1.0 and 1.5 g/kg diet) to Domyati duck's diet resulted in the highest viability values.

Reproductive performance:

Percentages of fertile eggs, hatchability per total eggs and hatchability /fertility eggs of Mamourah hens as affected by dietary supplementation of betaine, GAA levels and their mixtures during summer conditions are presented in Table (4). Decreasing of fertility percentage under heat stress may be due to that the heat stress could decrease number of spermatozoa stored in the sperm host gland in the hen's reproductive tract (Brillard, 2003). Also, hatchability percentage was significantly lower ($P<0.05$) under heat stress, which may be due to increase embryonic death during incubation period as a result of increase endogenous (metabolic) heat production (Awad *et al.*, 2013). In this study, the results showed that fertility eggs (%) and hatchability per total eggs (%) were significantly ($P<0.05$ and 0.01) higher for layers fed diets supplemented with either betaine, GAA or their mixtures. These results may be due to the good semen quality traits of the cocks treated with betaine, GAA levels and their mixtures. However, hatchability per fertility eggs (%) and chick hatch weight (g) were not affected with either betaine, GAA levels or their mixtures at the end of the experimental period (60 weeks of age). These results are in the range of those obtained by Tollba and El-Nagar (2008) who reported that increasing betaine supplementation significantly ($P<0.05$) altered the percentages of fertility, hatchability, and average values of relative hatched chick weight and absolute chick weight. Awad *et al.* (2014) found that fertility percentage was insignificantly improved ($P<0.05$) by supplementing of 0.5 and 1.0 g betaine /kg diet, while, hatchability percentage of both total set and fertile eggs was significantly improved ($P<0.05$) by supplementing of 0.5 g betaine /kg diet as compared to the control. On the other hand, Tapeh *et al.* (2016) stated that fertility rate of broiler breeder roosters (Ross 308) was increased by feeding of all the levels of GAA (0, 600, 1,200 and 1,800 mg GAA/kg diet) from 29–54 wks of age. Also, Sharideh *et al.* (2016) found that adding 1200 mg GAA/kg diet caused the fertility increase by approximately 22% and the number of sperm holes in Ross 308 line broiler breeder hens.

Table (4). Effect of dietary supplementation of betaine, guanidinoacetic acid and their mixtures on Fertility and hatchability traits of Mamorah hens during experimental period under Egyptian summer conditions.

Traits Treatments	Fertile Eggs (%)	Hatchability /Total eggs (%)	Hatchability / Fertile eggs (%)	Chick weight (g)
Control	67.74 ±2.06 ^b	58.54 ±1.05 ^c	86.61 ±3.39	36.57 ±0.39
1600 mg betaine	76.67 ±1.67 ^a	65.98 ±1.84 ^b	86.12 ±2.78	36.52 ±0.34
600 mg GAA	76.8 ±2.70 ^a	66.78 ±2.48 ^{ab}	86.95 ±0.28	35.13 ±0.58
800 mg GAA	76.69 ±1.04 ^a	69.01 ±2.02 ^{ab}	89.97 ±1.70	34.49 ±0.49
1000 mg GAA	76.39 ±2.93 ^a	69.83 ±2.62 ^{ab}	91.42 ±0.26	36.20 ±1.63
1600 mg betaine + 600 mg GAA	81.28 ±3.14 ^a	71.79 ±2.62 ^{ab}	88.5 ±3.70	37.30 ±0.91
1600 mg betaine + 800 mg GAA	77.73 ±2.77 ^a	70.75 ±2.07 ^{ab}	91.34 ±5.10	37.26 ±0.46
1600 mg betaine + 1000 mg GAA	81.61 ±2.62 ^a	73.53 ±1.48 ^a	90.19 ±1.18	37.48 ±0.53
Sig.	*	**	NS	NS

Means having different letters in the same row, differ significantly (P<0.05). * = (P<0.05); ** = (P<0.01); NS= Not significant.

Thus, in the current study, it seems that increased fertility rate in GAA groups may be due to increased sperm motility and sperm cell concentration in cock's semen. This positive effect of GAA on egg fertility may not be directly related to the number of spermatozoa presented in the vitelline membrane of eggs but rather to a possible improvement in the viability and

motility of spermatozoa. GAA supplementation may be important to provide additional levels of creatine in the female reproductive tract, enabling the maintenance of the viability of a greater number of spermatozoa until to perform fertilization, considering that birds may store spermatozoa after mating for long periods of time in the tubular gland of the uterovaginal junction (Santos *et al.*, 2013) and that creatine may improve the stability of cellular membranes (Sestili *et al.*, 2011). Murakami *et al.* (2014) demonstrated that the GAA (0.00, 0.06, 0.12, 0.18, and 0.24%) caused to improved hatchability and total fertility of eggs. GAA may be beneficial in poultry diets because it may be able to spare Arginine (Arg); this is an important point considering Arg is the fifth limiting AA in corn-soybean diets for poultry (Dilger *et al.*, 2013).

Antioxidant capacity and enzyme activity:

It is evident from Table 5 shows that total antioxidant capacity (TAOC) was not significantly affected due to supplementing dietary either betaine, GAA and their mixtures. While, increasing the level of GAA significantly ($P \leq 0.05$) increased the superoxide dismutase (SOD) and glutathione peroxidase (GPX). However, Malondialdehyde (MDA) caused significantly ($P \leq 0.05$) decreased with dietary supplementation of betaine, GAA levels or their mixtures as compared with the control group. Generally, antioxidant enzymes are the first defence level in the antioxidant system of animal cells. A significant increase ($P \leq 0.05$) in free radical production, which was reported along with an increase in the expression of antioxidant enzymes during a period of non-damaging exercise (McArdle and Jackson 2000). The increments in antioxidant enzyme activities have been considered as protective responses to oxidative stress (Altan *et al.*, 2003). Also, the condition of oxidative stress results in enhanced production of reactive oxygen species (ROS) that induces lipid peroxidation reactions, which are in turn manifested by an increased level of MDA in plasma and tissues (Sahin *et al.*, 2002). These results were in agreement with those of Nasiroleslami *et al.* (2018) who stated that lower activity of the liver SOD and MDA were shown in broilers fed diet supplemented with the betaine (600 mg/kg). Zhang *et al.* (2016) reported that betaine exerts its antioxidant activity via two mechanisms. One mechanism

Table (5). Effect of dietary supplementation of betaine, guanidinoacetic acid (GAA) and their mixtures on antioxidant components and antioxidant enzymes of Mamorah hens at the end of the experimental period.

Traits Treatments	Superoxide dismutase (SOD) (U/ml)	Glutathione peroxidase (GPX) (mU/ml)	Total antioxidant capacity (TAOC) (mM/l)	Malonidialdehyde (MDA) (nmol/ml)
Control	2.18 ±0.17 ^{bc}	6.34 ±0.50 ^c	0.95 ±0.05	3.50 ±0.21 ^a
1600 mg betaine	1.99 ±0.15 ^c	6.39 ±0.34 ^{bc}	0.83 ±0.06	2.28 ±0.18 ^b
600 mg GAA	2.69 ±0.13 ^{ab}	7.99 ±0.40 ^{abc}	0.92 ±0.05	2.57 ±0.16 ^b
800 mg GAA	2.77 ±0.16 ^a	8.28 ±0.51 ^{ab}	0.89 ±0.05	2.52 ±0.21 ^b
1000 mg GAA	2.80 ±0.09 ^a	9.20 ±0.63 ^a	0.88 ±0.02	2.34 ±0.21 ^b
1600 mg betaine + 600 mg GAA	2.47 ±0.15 ^{abc}	7.95 ±0.74 ^{abc}	0.91 ±0.05	2.62 ±0.22 ^b
1600 mg betaine + 800 mg GAA	2.42 ±0.17 ^{abc}	7.66 ±0.70 ^{abc}	0.89 ±0.04	2.45 ±0.19 ^b
1600 mg betaine + 1000 mg GAA	2.22 ±0.23 ^{bc}	7.37 ±0.64 ^{abc}	0.89 ±0.03	2.60 ±0.24 ^b
Sig.	*	*	NS	*

Means having different letters in the same row, differ significantly. * = (P<0.05); ** = (P<0.01); NS= Not significant.

involves scavenging ROS in cells via up-regulation of endogenous nonenzymatic antioxidant defences. Betaine was able to increase the levels of S-adenosylmethionine and methionine via the methionine–homocysteine cycle and improve the ROS-scavenging ability of the methionine sulfoxide reductase system. Another mechanism inhibits ROS generation by isolating cells from the oxidative stress inducer. Betaine may be an amphiphilic molecule with a hydrophilic group, carboxyl terminal, and the three hydrophobic methyl groups

at the N terminal, and may form a protective membrane around cells to prevent the contact of free radicals with the cytomembrane. The structure of the protective membrane is such that the three hydrophobic methyl groups at the N terminus are close to the lipid bilayer, and the carboxyl terminus is close to the water. Betaine is a zwitterion, and its carboxyl terminus is electronegative, which means that the outside surface of the protective membrane is also electronegative. Free radicals were molecules with one or more unpaired electrons. Therefore, free radicals were electronegative. The electronegative outside surface of the protective membrane may repel free radicals, preventing them from damaging the cytomembrane (Zhang *et al.*, 2016). On the other hand, Wang *et al.* (2012) demonstrated that dietary GAA improved antioxidant status by elevating total antioxidant capacity and the activities of several antioxidant enzymes. The same authors reported that GAA-related metabolites (creatine and arginine) might be able to quench free radicals after GAA ingestion, suggesting indirect antioxidant effect of GAA utilization.

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 Mamourah cocks as affected by dietary supplementation of betaine, guanidinoacetic acid (GAA) and their mixtures during hot summer condition are presented in Table 6.

Semen ejaculate volume and hydrogen-ion concentration (pH) of cocks were not significantly affected by dietary supplementation of betaine, GAA and their mixtures. However, dietary supplementation of either betaine, GAA 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. These results may be due to antioxidant properties of betaine which plays a major role in stimulating host defense and superoxide anion scavenging (Messadek, 2010). Also, betaine is a major osmolyte in the cell, it regulates the cell volume and stabilizes proteins, and it acts as a modulator of nitric oxide synthesis (Lever and Slow, 2010).

Table (6). Effect of dietary supplementation of betaine, guanidinoacetic acid (GAA) and their mixtures on semen quality of Mamorah cocks at the end of the experimental period.

Traits Treatments	Ejaculate volume (ml)	Hydrogen-ion concentration (pH)	Sperm motility (%)	Dead spermatoz oa (%)	Sperm abnormali ties (%)	Sperm cell concentration (X 10 ⁹ /ml)	Acrosomal damage (%)
Control	0.29 ±0.01	7.18 ±0.04	63.40 ±2.93 ^c	18.20 ±0.8 ^{ab}	13.60 ±1.6 ^a	3.18 ±0.14 ^c	7.80 ±0.97 ^a
1600 mg betaine	0.27 ±0.02	7.10 ±0.05	72.60 ±2.51 ^b	19.60 ±1.0 ^a	11.60 ±1.3 ^{ab}	3.85 ±0.17 ^b	7.60 ±0.98 ^a
600 mg GAA	0.26 ±0.02	7.20 ±0.04	75.20 ±1.78 ^{ab}	16.60 ±1.0 ^{abc}	9.00 ±0.71 ^b	4.12 ±0.11 ^{ab}	6.40 ±0.60 ^{ab}
800 mg GAA	0.28 ±0.02	7.20 ±0.05	75.60 ±1.70 ^{ab}	17.00 ±1.0 ^{abc}	10.40 ±0.5 ^b	4.07 ±0.17 ^{ab}	6.60 ±1.03 ^{ab}
1000 mg GAA	0.27 ±0.02	7.24 ±0.05	73.60 ±1.87 ^{ab}	19.00 ±1.0 ^a	11.60 ±0.6 ^{ab}	3.92 ±0.20 ^b	7.00 ±0.71 ^{ab}
1600 mg betaine + 600 mg GAA	0.29 ±0.02	7.18 ±0.03	78.00 ±1.23 ^{ab}	15.40 ±1.0 ^{bc}	10.80 ±0.3 ^{ab}	4.27 ±0.19 ^{ab}	4.60 ±0.51 ^{bc}
1600 mg betaine + 800 mg GAA	0.27 ±0.03	7.12 ±0.06	76.00 ±1.88 ^{ab}	15.80 ±0.9 ^{bc}	10.40 ±0.5 ^b	4.12 ±0.13 ^{abc}	3.80 ±0.74 ^c
1600 mg betaine + 1000 mg GAA	0.27 ±0.02	7.22 ±0.04	79.60 ±1.64 ^a	14.20 ±0.7 ^c	9.60 ±0.82 ^b	4.46 ±0.16 ^a	3.60 ±0.68 ^c
Sig.	NS	NS	**	**	*	**	**

Means having different letters in the same row, differ significantly (P<0.05). * = (P<0.05); ** = (P<0.01); NS= Not significant.

These results were in agreement with Hood (1999) who reported that heat exposure caused to increase in the percentage of dead sperm (29.1%) and a decrease (10.2%) in the sperm quality index (SQI). Awad *et al.* (2014) reported that feeding betaine diets (0, 0.5, 1.0 and 1.5 g/kg diet) resulted in a significant (P≤0.05) improvement of live sperms percentage, whereas, it had a significant (P≤0.05) decrease in dead and abnormal sperms percentages as compared to the control. Ezzat *at al.* (2011) in chickens who found that supplementation of dietary betaine to chickens diet under summer conditions, significantly (P<0.05) increased sperm motility and decreased (P<0.05) dead spermatozoa compared with the control group.

On the other hand, Tapeh *et al.* (2016) found that supplementation of 1200 mg GAA/kg diet increased the semen concentration and sperm forward motility, this higher value for semen concentration in GAA-(1200) may be due to optimal creatine concentration and its role on spermatogenesis. It has been suggested that the function of Sertoli cells is impaired in low-fertile roosters, and they entrap mature spermatozoa during aging and do not allow them to release (Muncher *et al.*, 1995). However, human spermatozoa have higher creatine kinase activity when compared to that in rooster spermatozoa (Wallimann *et al.*, 1986). Therefore, it seems that creatine kinase play a key role in energy homeostasis in sperm (Yesilli *et al.*, 2005). According to previous reports, it seems that GAA supplemented diet may increase sperm energy content and consequently increases sperm forward motility. Guanidinoacetic acid, a precursor of creatine, plays a central role in the male reproduction and support the energy metabolism and storage in the Sertoli cells and spermatozoa (Wallimann *et al.*, 2011).

Economical efficiency (EEf):

Data shown in Table (7) clear that Mamourah layers as affected by 1600 mg betaine + 600 mg GAA fed diet were recorded the highest net revenue and the best economical efficiency followed by those fed level 1600 mg betaine + 800 mg GAA diet, while the control group had the lowest net revenue and economical efficiency (%).

In conclusion

From these results it could be concluded that the addition of 1600 mg betaine + 600 mg GAA to the diet of Mamourah laying hens showed better productive performance, especially feed conversion, egg production, as well as, it recorded the highest net revenue and the best economical efficiency. While, the group of 1600 mg betaine + 600 mg GAA of the diet elevated the reproductive performance of laying hens and semen quality of cocks under summer season conditions, in Egypt .

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تأثير إضافة البيتين مع مستويات مختلفة من الجوانيديين حمض خليك على الأداء الإنتاجي والتناسلي لسلالة المعمورة المستنبطة أثناء موسم الصيف في مصر .

وحيد عزت، عرفات عبد الهادي حبيب، ياسر صديق رزق، ملاك منصور بشارة
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كان الهدف الرئيسي من هذه الدراسة هو دراسة تأثير إضافة البيتين مع مستويات مختلفة من الجوانيديين حمض خليك على الأداء الإنتاجي والتناسلي لدجاج المعمورة المر باه تحت ظروف الصيف المصري. وقد تم استخدام ١٩٢ دجاجة بياضه و ٢٤ ديك من دجاج المعمورة عند عمر ٤٨ أسبوعاً (عند معدل إنتاج بيض ٦٣.٢٤%) في تصميم عشوائي تام وتم تقسيم الطيور عشوائياً إلى ٨ مجموعات تجريبية متساوية (٢٤ دجاجة + ٣ ديوك / كل معاملة)، كل منها بها ثلاث مكررات في أقفاص مفردة في عنبر من النظام المفتوح حني ٦٠ أسبوع من العمر. وقد تم ترتيب المجموعات الثمانية التجريبية علي النحو كنترول ، وعلي عليه أساسيه مضاف إليها ١٦٠٠ ملجم بيتين / كجم عليه ، تم تغذية المجموعات من الثالثة حتى الخامسة بإضافة ٦٠٠ و ٨٠٠ و ١٠٠٠ ملجم من الجوانيديين حمض خليك / كجم عليه ، على التوالي ، وغذيت المجموعات من السادسة إلى الثامنة علي عليه أساسيه مضاف إليها ١٦٠٠ ملجم من البيتين و ٦٠٠ و ٨٠٠ و ١٠٠٠ ملجم من الجوانيديين حمض خليك/ كجم عليه.

وأظهرت النتائج أن التغذية علي البيتين و الجوانيديين حمض خليك لم يكن لهما تأثير معنوي (عند مستوي ٠.٠٥) على تغيرات وزن الجسم ، والغذاء المأكول ، ووزن البيض ، والنسبة المئوية للفقس بالنسبة للبيض المخصب، ووزن الكتاكيت الفاقسة، مضاد الأكسدة TAOC لدجاج المعمورة خلال الفترة التجريبية الكلية. تحسنت نسبة التحويل الغذائي، وإنتاج البيض، وكتلة البيض معنويا (عند مستوي ٠.٠٥) في عليه الدجاج الذي تم تغذيته علي عليه مضاف إليها ١٦٠٠ ملجم بيتين + ٦٠٠ ملجم من الجوانيديين حمض خليك/ كجم عليه عند المقارنة بمجموعه الكنترول. وكانت النسبة المئوية للبيض المخصب والنسبة المئوية للفقس بالنسبة للبيض الكلي اعلي معنويا (عند مستوي ٠.٠٥ ، ٠.٠١) للدجاج المغذي علي عليه

تحتوي علي كل من البيتين والجوانيديين حمض خليك أوهما معا. كلما زاد مستوي الجوانيديين حمض خليك زاد معنويا (عند مستوي ٠.٠٥) مضادات الأوكسدة SOD، GPX ومع ذلك انخفض معنويا(عند مستوي ٠.٠٥) MDA مع التغذية علي كل من البيتين والجوانيديين حمض خليك أو هما معا عند المقارنة بمجموعة الكنترول. تحسنت معنويا (عند مستوي ٠.٠٥) النسبة المئوية لحركة الحيوانات المنوية و نسبة الحيوانات المنوية الميتة والشاذة وتركيز الحيوانات المنوية الطبيعية والحيوانات المنوية الحية والأكروسوم الشاذ علي التغذية علي البيتين والجوانيديين حمض خليك أو معا عند المقارنة بمجموعة الكنترول. تغذية دجاج المعمورة علي عليقه مضاف إليها ١٦٠٠ ملجم بيتين + ٦٠٠ ملجم من الجوانيديين حمض خليك/ كجم عليقه أو هما معا أعطي أعلى إيراد صافي وأفضل كفاءة اقتصادية.

التوصية: عليه نوصي باستخدام إضافة البيتين مع مستويات مختلفة من الجوانيديين حمض خليك لتحسين إنتاج البيض ، والنسبة المئوية للفقس بالنسبة للبيض الكلي لدجاج المعمورة البياض ومعظم صفات جودة السائل المنوي للديوك تحت ظروف موسم الصيف في مصر.