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EFFECT OF GLYCINE SUPPLEMENTATION OF MANDARAH LOCAL CHICKENS DIETARY ON PHYSIOLOGICAL AND REPRODUCTIVE PERFORMANCE

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ABSTRACT: This study was conducted to evaluate the effect of dietary supplemental of glycine (Gly) on modulating physiological and reproduction performance of Mandarah (M) local chickens. A total of 135 hens and 18 cocks, from 28 to 40 weeks of age were randomly assigned to 3 treatments, and each treatment (T) include 45 hens and 6 cocks divided in 3 replicates of 15 hens and 2 cocks each. The 1st group (T1) was fed the basal diet and served as a control group (without supplementation). The 2nd group and 3rd group were fed the basal diet supplemented with 0.1 and 0.2 %, Gly (1 and 2 gm/ kg diet), respectively.). The study showed that the cocks Gly treatments (0.1 and 0.2 %) were significantly ($P<0.05$) increased in the ejaculate volume, total sperm output, semen quality factor**,** total motile sperm, thyroxine (T4) and follicle-stimulating hormones (FSH) compared with control treatment**.** Chickens that received diet supplemented with 0.1 % Gly had significant higher sperm concentration, total antioxidant capacity (TAC), triiodothyronine (T3) than those of control T1. No significant differences among T2, T3 and control (T1) in sperm motility %, live sperm %, dead sperm %, abnormal sperm %, fertility, hatchability, catalase, glutathione enzyme and luteinizing hormone (LH). Conclusively, it could be recommended to supply layer diets with Gly for better physiologically performance during the laying period.

Key Words: Chickens, Glycine, physiological and reproduction performance, laying period.

INTRODUCTION

Amino acids can attain a variety of functions in poultry, where it preserves feathers, bone, skin, muscles, organs, and structural constituents (NRC, 1994). Moreover, amino acids play important role in metabolic functions and formation of multi substantial non-protein as a precursor for body constituents (Han and Thacker, 2011). In addition, Gly consider the simplest amino acid in nature; where it was initially isolated from amino acid hydrolysates in 1820 by a French chemist called H. Braconnot (Meister, 1965). Furthermore, the name of Gly was obtained from the Greek word "glykys", which means sweet because it is sweet such as glucose (Li and Wu, 2017).

Ohta *et al*. (1999) indicated that, amino acids administration in ovo at a late stage of incubation may boost the status of chicken embryo amino acid. Silvestroni *et al*., (1979) found that semen plasma amino acids are lower in infertile patients compared to fertile persons, so, it was proposed that amino acids can protect sperms in the hostile vaginal environment. Glycine is the second highest concentration of free amino acids in bull semen plasma (Assumpção *et al*., 2005). However, L-Gly and L-carnitine supplementation did not improve fertility and hatchability in the cryopreservation mixture Kumar *et al*., (2019). Gly supplementation can improve the anti-oxidative ability, metabolic regulation, and neurological function for chicken (Xie *et al*., 2016; Li *et al*., 2009; Wang *et al*., 2013). So, it is used as a nutrient to enhance anti-oxidative capacity.

In addition, serine/Gly has an important role in glutathione synthesis (Gheller *et al*., 2020). Sekhar *et al*., (2011) showed that there is evidence that glutathione levels with advancing age can be maintained with dietary interventions, such as correction of a persistent metabolic phenotype in red blood cells which associated with aging and increased ROS due to impaired glutathione synthesis by dietary supplementation of the glutathione precursors glycine and cysteine. In addition, Wang *et al*., (2018) indicated that gly acts as a primary antioxidant and the precursor of glutathione in the human body. Yoon and Ahn (2007) demonstrated that supplementation of 15 mM Gly betaine (GB) to Chinese hamster ovary cells culture medium increased FSH titer by 11% and 17%, respectively. .

Former researchers studied the effect of Gly supplementation to low protein diets on broiler (Salim et al. 2021), but not in wide on chickens' physiological and reproduction performance. So, the objective of this experiment was to estimate dietary Gly supplementation effects on physiological and reproduction performance of Mandarah local Egyptian strain chickens.

MATERIALS AND METHODS

This experiment was performed at Sakha's Poultry Farm, Animal Research Station, Institute of Animal Production Research, and Agricultural Research Center, Egypt.

Housing birds and management:

A number of 135 hens and 18 cocks, from 28to 40 weeks of age, are taken from the Mandarah Egyptian local strain (M), housed randomly distributed and divided into 3 treatments, each treatment has 3 replicates, and each replicate has 15 females and 2 males. Water and feed (mash form) were provided to hens and cocks *ad libitum*.

Treatments and diet composition

All birds were randomly divided into 3 equal replicates in each treatment. The basic diet was the 1st treatment (T1) and served as the control. While the 2nd (T2) and the 3rd (T3) treatments were given the basic diet with 0.1 and 0.2 % Gly, respectively Each replicate was individually weighed, housed in separated floor pens (185 x 320 cm) and submitted to the same managerial conditions in a windowed house with light cycle regimen of 16 hours light: 8 hours darkness (16L:8D). Hens were fed *ad libitum* and continuously provided with fresh water. During the experimental period, the basic experimental diet was formulated to meet the nutritional requirements of chickens (from 28 to 40 wks. of age) according to Agriculture Ministry Decree (1996). According to feed Composition Tables for Animal and Poultry Feed Stuffs Used in Egypt (2001), the composition and calculated analysis of the experimental basic diet presented in Table (1).

Measurements Fertility and hatchability%:

At 43 weeks of age, a total number of 120 eggs from each treatment were collected and incubated at standard conditions in automatic setter/hatcher incubator. Then the eggs were candled on the 18th day of incubation for embryonic development and fertile eggs were transferred into hatcher compartment. Unhatched eggs were broken open to confirm the absence of embryonic development and determine the fertile eggs. The chicks hatched on the 21st day of incubation were counted for calculating hatchability %.

$$\text{Fertility \%} = \frac{\text{Number of fertile eggs} \times 100}{\text{Total number of setting eggs}}$$

$$\text{Hatchability \%} = \frac{\text{Number of hatched chicks} \times 100}{\text{Total number of setting eggs}}$$

$$\text{Hatchability \% of fertile egg} = \frac{\text{Number of hatched chicks} \times 100}{\text{number of fertile eggs}}$$

Semen quality assays

Males were separated of females at 40 to 42 weeks to get semen samples (6/ treatment) and evaluate it. Then the males were mixed with females at the age of 43 - 45 weeks to get fertilized eggs to measure hatchability and fertility. Semen samples were individually collected from all Mandara males by the massage method described by Burrows and Quinn (1937) to determine the fresh semen characteristics (Ejaculate volume, sperm motility and concentration, dead, live and abnormal sperm, pH). The ejaculated semen was diluted with sodium citrate (2.9 gm disodium citrate + 0.04 gm citric acid anhydrous + 1.25 gm lactose).

Blood analysis:

At 40 wks. of age, 3 hens/ replicate were selected randomly and slaughtered and 3 mL of blood samples were collected in tubes kept in ice. After that, the blood samples were centrifuged at 4 C for 4000 roll per minute (rpm) for 20 min. Hemolysis-free serum samples were transferred to 1.5 mL micro centrifuge tubes and stored at -20 ^{o}C until further analysis. Serum concentrations of TAC were determined according to Koracevic *et al*. (2001), Catalase according to Aebi (1984), Glutathione according to Pagila and Valentine (1967), follicle-stimulating hormon according to Rebar *et al*. (1982), luteinizing hormone according to Uotila (1981), Triiodothyronine according to Hoffenberg (1978), Thyroxin according to Schuurs (1977), respectively.

Statistical analysis

Data from all the response variables were subjected to one way analysis of variance (SAS, 2000) $X_{ij} = \mu + Ti + e_{ij}$

Where: X_{ij} = any observation

μ = Overall mean

T_i = Treatments (i = 1, 2, and 3)

e_{ij} = Experimental error

Variables having a significant F-test ($P < 0.05$) were compared using Duncan's Multiple Range Test (Duncan, 1955).

RESULTS AND DISCUSSION

Semen quality:

As shown in Table 2, it could be observed that Gly supplementation by 0.1 and 0.2 led to increase ($P < 0.05$) significantly the ejaculate volume by about 0.087 and 0.113 ml in compared to control group, respectively. Moreover, sperm concentration was increased significantly ($P<0.05$) with supplementing diet with 0.2 % Gly (T3) compared with control (T1), whereas, the data declare that all treatments did not show any significant change in sperm motility %, live sperm %, dead sperm %, abnormality sperm % and pH of M cocks among all experiment treatments.

These results are in agreement with Silvestroni *et al.* (1979) who found that semen plasma amino acids are lower in infertile patients compared to fertile persons, so, it was proposed that amino acids can protect sperms in the hostile vaginal environment. Glycine is the second highest concentration of free amino acids in bull semen plasma (Assumpção *et al*., 2005). In addition, He and Woods, (2003) indicated that (25 - 100 mM) L-Gly of striped bass fish sperm can increase the post-thaw sperm motility. Also, gly as a component in extender of the red jungle fowl semen (RFE) increased fertility up to 57 % (Rakha *et al*., 2016). Glycine plays important role in glutathione biosynthetic process (Wu *et al*., 2009). Moreover, it was suggested that during cryopreservation process the higher level of Gly could have supported the sperm of Indian red jungle fowl against lipid peroxidation (Rakha *et al*., 2016).

Baines *et al*., (1990) proposed that Gly as a small neutral amino acid can stabilize the protein tertiary structure of cell membrane through their physicochemical effects. Also, Anchordoguy *et al*., (1988) proposed that phospholipids amino acids might interaction with the phosphate groups in the sperm plasma membrane and form a layer on the sperm surface. In addition, gly addition in semen cryopreservation of striped bass increased ATP content and the sperm mitochondrial function (He and Woods, 2003). There are two hypotheses for this; Gly provides a positive effect on mitochondria after crossing sperm plasma membrane, or binding to its receptors on plasma membrane triggers signal transduction (Flipse, 1956).

Fertility and hatchability:

Table 3 represented the effect of dietary Gly supplementation in M laying hen diets on fertility and hatchability. It is clear that Gly supplementation had no significant effects on fertility and hatchability percentages. These observed findings were in accordance with Ohta *et al*. (1999) who indicated that, administration of 53 mg of amino acids in ovo enhanced relative weight at hatch without affecting hatchability. Similarly, Shafey *et al*. (2014) showed that hatchability was not affect with amino acid when compared to control.

Plasma constituents and biochemical:

Effect of Gly in the diets of M layer chicken at 40 weeks of age on TAC, catalase and glutathione concentration are presented in Table (4). The results of TAC revealed that there were significantly increase in constituents by 0.2% of Gly (T3) supplementation compared with the control. Moreover, no significant differences were observed between 0.1 % (T2) Gly supplementation in diets and control (T1) in this trait. However, there were no significant effects among all experiment treatments in catalase and glutathione concentration.

These results are in contrast with Hoseini *et al.*, (2022) who showed that dietary glycine supplementation at 0.25–0.5% could improve antioxidant on the fish. Moreover, Wang *et al.* (2013), Xie *et al.* (2016), Gheller *et al.* (2020), and Chen *et al.* (2018) demonstrated that the plasma levels of glutathione (GSH), catalase (CAT) and SOD increased in the STZ-induced diabetic rats by 1% glycine supplementation in the drinking water.

(Willcox *et al.*, 2004 and Valko *et al.*, 2005) showed that Endogenous compounds in cells include enzymatic antioxidants and non-enzymatic antioxidants. Where, enzymatic antioxidants which include superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx) and glutathione reductase (GRx) can neutralize ROS and RNS. While, the non-enzymatic antioxidants involved metabolic antioxidants which produced by metabolism in the body for instance glutathione, L-ariginine, lipoid acid, etc. (Droge, 2002 and Willcox *et al.*, 2004) and nutrient antioxidants which cannot be produced in the body so it must be provided through foods or supplements, for instance vitamin E, vitamin C, trace metals etc.

Since we analyzed part of the enzymatic antioxidants, also we did not analyze the non-enzymatic antioxidants; this may explain that the antioxidants that were not analyzed increased significantly as a result of adding glycine to the diet, so the TAC increased. Nevertheless, Gly supplementation had no significant effects on oxidative enzymes. Perhaps the addition of Gly at a higher-level affects catalase and glutathione and we may consider this matter in future research.

Plasma Hormones Analysis:

Table 5 represented the effect of Gly supplementation in M laying hen diets on plasma hormones.

T3 and T4 hormones:

In our study Gly supplementation in the diets had no significant effects on T3 concentration by 0.1 % Gly, but increased significantly by 0.2 % Gly at 40 (wks.) of age compared with the control. Furthermore, Gly supplementation increased significantly T4 concentration by 0.1 % and 0.2 % Gly. Our results were in agreement with the results of Namroud *et al.* (2010) who demonstrated that supplementing low CP diets with Gly did not affect significantly T3.

In addition, T3 and T4 hormones are important growth promoters and related to metabolism regulation in chickens (Yahav, 2000), associated with feed intake (Yahav *et al.,* 1996; 1998).

Moreover, the addition of Gly significantly improved FCR of broilers (Yuan *et al*. 2012), the gain: feed ratio in broiler chicks increased (0.9–2.1%) with digestible Glycineequ (Gly + serine) intake (Hofmann *et al.,* 2020).

Also, Hanaa *et al*. (2020) showed that Gly addition to laying hens diets led to improve egg production through improve feed efficiency which may be due to better nutrient digestibility and small intestine health.

LH and FSH hormones:

Gly supplementation increased FSH concentration significantly by T2 and T3 compared to control. Our results were in agreement with the results of Yoon and Ahn (2007) demonstrated that supplementation of 15 mM Gly betaine (GB) to Chinese hamster ovary cells culture medium increased FSH titer by 11% and 17%, respectively.

However, Gly supplementation had no significant effects on LH concentration by T2 and T3. This is agreement with Morishita *et al.,* (1981) who showed that 50 and 100 mg Gly had no significant effect on rat’s serum LH levels, While, 200 mg Gly increased serum LH levels significantly. So, it was suggested that Gly has important role in LH secretion neural regulation at a higher level.

Moreover, 200 mg Gly increased serum LH levels significantly. So, it was suggested that Gly has important role in LH secretion neural regulation (Morishita *et al*., 1981). Our treatments and chickens are the same of (Hanaa *et al.,* 2020) were found that the increase of anti-oxidant and hormones (LH and FSH) led to an increase in egg production.

CONCLUSION

the present study indicated that Gly supplementation to the diets during laying period from 29 to 40 weeks of age could be modulate and optimize semen quality characteristics of cocks and antioxidant traits, Likewise, the dietary supplementation of Gly can improve some hormones of local laying hens' strains.

Table (1): Composition and calculated analysis of the experimental diets

Ingredients (%)	**Control diet**	**Gly. 0.1%**	**Gly. 0.2%**
Yellow corn	63.37	63.44	63.50
Soybean meal (44%CP)	24.60	24.35	24.20
Wheat bran	2.07	2.15	2.14
Limestone	7. 80	7. 80	7. 80
Di calcium phosphate	1.50	1.50	1.50
Premix[1]	0.30	0.30	0.30
Salt	0.30	0.30	0.30
DL– Methionine	0.06	0.06	0.06
Glycine	0.00	0.10	0.20
Total	100	100	100
Calculated analysis[2]:			
CP %	16.00	16.00	16.00
ME (kcal/kg)	2700	2700	2700
Calcium %	3.30	3.30	3.30
Available phos. %	0.42	0.42	0.42
Dl-Methionine %	0.35	0.35	0.35
Meth. +cyc.	0.62	0.62	0.62
L- lysine-Hcl	0.89	0.88	0.88
Glycine	0.67	0.77	0.86
Glycine+Serine	1.47	1.55	1.64

[1]Vitamin and mineral premix provides per 3kg: Vitamin A 12000 IU; Vitamin D3 2000 IU; Vitamin E. 10mg; Vitamin k3 2mg; VitaminB1 1mg; Vitamin B24mg; Vitamin B6 1.5 mg; Pantothenic acid 10mg; VitaminB12 0.01mg; Folic acid 1mg; Niacin 20mg; Biotin 0.05mg; Choline chloride (50% choline) 500 mg; Zn 55mg; Fe 30mg; I 1mg; Se 0.1mg; Mn 55mg; ethoxyquin 3000 mg. [2]According to Feed Composition Tables for Animal and Poultry Feedstuffs Used in Egypt (2001).

Table (2): Effect of glycine supplementation of Mandarah cocks' diets on some semen quality.

Traits	Dietary treatments			SEM
	Control	0.1 % Glysine	0.2% Glysine	
Ejaculate volum (ml)	0.213[b]	0.300[a]	0.326[a]	0.018
Sperm concentration ($x10^6$)	743.33[b]	770.00[ab]	786.66[a]	7.63
Total sperm output ($x10^6$)	158.70[b]	230.93[a]	257.00[a]	15.45
Sperm motility %	76.66	81.66	83.33	1.30
Total motile sperm ($x10^6$)	121.42[b]	188.80[a]	214.05[a]	14.43
Live sperm %	83.66	84.33	87.00	1.14
Dead sperm %	16.33	15.66	13.00	1.14
Abnormality sperm %	5.33	4.00	3.66	0.50
Semen quality factor	132.81[b]	194.91[a]	223.63[a]	14.33
pH	7.86	7.76	7.70	0.037

[a, b] Means bearing different superscripts within the same row are significantly different ($P<0.05$).

Table: (3): Effect of glycine supplementation of Mandarah laying hens diets on fertility and hatchability treatments.

Items	Level of glysine %			SEM
	Control (0)	0.1	0.2	
Fertility %	89.50	90.33	90.66	1.1395
Hatchability% from total eggs	76.66	78.83	79.50	1.6029
Hatchability% from fertile eggs	85.60	87.21	87.64	0.8699

Table (4): Effect of glycine supplementation of Mandarah laying hens diets on some constituents and biochemical analysis of chicken plasma.
a, b Means bearing different superscripts within the same row are significantly different ($P<0.05$).

Items	Level of glysine %			SEM
	Control (0)	0.1	0.2	
Total Antioxidant Capacity (mM/l)	1.14[b]	1.31[ab]	1.43[a]	0.05
Catalase (u/l)	27.13	34.90	38.78	2.80
Glutathione (mu/ml)	0.321	0.403	0.455	0.09

[a, b] Means bearing different superscripts within the same row are significantly different ($P<0.05$).

Table (5): Effect of glycine supplementation of Mandarah laying hens diets on some hormones.

Items[1]	Level of glysine %			SEM
	Control (0)	0.1	0.2	
T3 (ng/ml)	1.116[b]	1.016[b]	1.916[a]	0.227
T4 (µg/dl)	1.583[b]	3.316[a]	2.833[a]	0.347
LH (mlu/ml)	2.50	3.00	2.45	0.194
FSH (mlu/mlu)	2.450 [b]	3.416 [a]	3.666 [a]	0.166

a, b Means bearing different superscripts within the same row are significantly different ($P<0.05$).
1-T3=triiodothyronine; T4=thyroxin

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الملخص العربي

تأثير إضافة الجليسين الى عليقة دجاج المندرة أثناء فترة إنتاج البيض على الأداء الفسيولوجي والتناسلي

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أجريت هذه الدراسة لتقييم تأثير إضافة الحمض الاميني الجليسين على الأداء الفسيولوجي والتناسلي لسلالة دجاج المندرة. تم تقسيم إجمالي ١٣٥ دجاجة و١٨ ديك (عمر ٢٨ إلى ٤٠ أسبوع) بشكل عشوائي إلى ٣ معاملات، وكل معاملة تشمل ٤٥ دجاجة و٦ ديوك مقسمة إلى ٣ مكررات (١٥ دجاجة و٢ ديك / مكرر). تم تغذية المجموعة الأولى (الكنترول) بالعليقة الأساسية بدون أي إضافات. المجموعة الثانية والثالثة غذيت على العليقة الأساسية مع إضافة ٠.١ و٠.٢٪، جليسين (١ و٢ جم / كجم علف) على التوالي. أوضحت الدراسة أن معاملات الجليسين (٠.١ و٠.٢٪) للديوك أدت الى زيادة معنوية (P <0.05) في حجم السائل المنوي، إجمالي إنتاج الحيوانات المنوية، جودة السائل المنوي، إجمالي الحيوانات المنوية المتحركة، هرمون الثيروكسين (T4) والهرمون المنشط للحوصلة المبيضية (FSH) بالمقارنة بمعاملة الكنترول. الدجاج الذي تناول عليقة مضاف لها الجليسين بنسبة ٠.١٪ كان لديه تركيز أعلى معنويا للحيوانات المنوية، تركيز مضادات الأكسدة الكلية (TAC) للسائل المنوي، ثلاثي أيودوثيرونين (T3) عن تلك الموجودة في مجموعة الكنترول. لا توجد فروق معنوية بين المعاملات في النسبة المئوية لحركة الحيوانات المنوية، نسبة الحيوانات المنوية الحية، الحيوانات المنوية الميتة، الحيوانات المنوية الشاذة، نسبتي الخصوبة والفقس، إنزيم الكتاليز والجلوتاثيون وهرمون التبويض (LH). لذلك يمكن التوصية بزيادة نسبة الجليسين في علائق الدجاج البياض لتحسين الأداء الفسيولوجي خلال فترة وضع البيض.