Research Article

Assessing the Role of Organic Iron Supplementation in Enhancing Productive and Reproductive Performance in Chickens at Two Different Ages

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Abstract | This experiment was designed to exhibit supplementing organic iron (Ferric-glycine, Fe-Gly) comparable to ferrous sulfates ($FeSO_4$) for improving productive and reproductive performance, egg quality and some blood constituents among two ages of chickens. A total number of 224 Silver Sabahia chickens (192 hens + 32 cocks) with 2 different ages were kept in the same house. Chickens were arranged in a 2 × 4 factorial design, with two layers ages (30 and 50-wk-old) and 4 dietary iron concentrations. One hundred and twelve chickens representing each studied age (96 hens + 16 cocks) were randomly distributed to 4 treatments with 6 replicates. Chickens of the first treatment group were fed basal diet supplemented with 40 mg Fe/kg diet as $FeSO_4$, while 2nd, 3rd, and 4th groups were fed basic diet supplied with organic Fe (40, 60, and 80 mg Fe-Gly /kg diet, respectively. Results indicated that egg production percentage, egg weight, egg mass and feed conversion ratio were significantly improved ($P\leq0.05$) for birds fed diet enriched with 60 or 80 mg Fe-Gly /kg compared with other experimental groups. Regardless of iron addition, egg weight was larger ($P\leq 0.001$) for elder birds compared to younger ones. Irrespective of chicken age, albumen height, eggshell thickness, strength and iron in eggshell for chickens fed diet enriched with 80 mg Fe-Gly/ kg diet were substantially increased ($P\leq0.05$) compared with inorganic group. Irrespective of chicken age, supplementing basal diet with 60 and 80 mg Fe-Gly/kg substantially improved ($P\leq0.05$) red blood cell count, hemoglobin, total antioxidant capacity and superoxide dismutase, while significantly decreased ($P\leq0.05$) serum aspartate amino transferase, alanine amino transferase and malondialdehyde compared with those for $FeSO_4$ group. In conclusion, incorporating 60 or 80 mg Fe-Gly/kg in the layer diet could be an effective strategy for enhancing productive and reproductive performance, especially for older chickens.

Keywords | Laying hens, Organic iron, Antioxidant, Productivity, Egg production

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INDRODUCTION

Minerals are crucial for dietary and physiological utility (Khalifah *et al*., 2022). Iron (Fe) is an essential element for organisms that plays vital roles in various substantial metabolic activities, including deoxyribonucleic acid synthesis, energy metabolism, erythropoiesis, oxygen transport, immune preservation and cognitive function

(Khalid *et al*., 2023; Rehman *et al*., 2022). The main iron supplement source used in poultry feed is ferrous sulfates ($FeSO_4$) (Trivedi and Barve, 2021). Nevertheless, the inorganic iron sources have many defects, such as high oxidations, low bioavailability and excretion which pollute the environment (Zhou *et al*., 2022). These limitations have driven increasing interest in the use of organic mineral forms.

Organic trace mineral forms provide protection against the formation of indigestible compounds with some antinutritive dietary components in the intestine and against reciprocal mineral antagonisms, and these forms present substitutional methods of absorption with high availability as a source of minerals for birds (Zhou *et al*., 2022; Wang *et al*., 2021).

Chelated iron with amino acids and organic iron components are being developed now as substitute for inorganic iron and these organic trace minerals have higher bioavailability compared with inorganic elements (Song *et al*., 2023; Chen *et al*., 2021). The organic minerals relative bioavailability was detected by the chelation strength which provided a higher biological value and beneficial impact on layers (Liu *et al*., 2022). For instance, Cao *et al*. (2023) reported that iron absorption from Fe-Gly was approximately double that from $FeSO_4$. Likewise, Khalid *et al*. (2023) showed that Fe-Gly improved iron concentrations in serum, eggshells, albumen, and yolk. Furthermore, organic iron supplementation has demonstrated promising effects on reproductive traits, including fertility and hatchability (Ebbing *et al*., 2019), suggesting its broader impact on breeder performance. Given that age is a well-established factor influencing nutrient requirements and performance outcomes in laying birds (Xu *et al*., 2023; Lin *et al*., 2021), investigating the interaction between iron source and bird age is particularly relevant for optimizing both productivity and reproductive efficiency.

Therefore, this experiment was performed to exhibit the best concentration of dietary organic iron (Ferric-glycine) comparable to $FeSO_4$ for improving productive and reproductive performance, egg quality, egg iron deposition and some blood constituents among two ages of Silver Sabahia chickens. This approach is expected to enhance both nutritional efficiency and environmental sustainability in poultry production.

MATERIALS AND METHODS

This experiment was performed at El-Sabahia Poultry Research Station, Animal Production Research Institute, Egypt. The experimental protocol (Protocol No. 01-05-03-429) was approved by the Scientific Council of the Animal Production Research Institute, Agricultural Research Center, Egypt. The authors declare that all procedures involving the birds were conducted in accordance with Directive 2010/63/EU of the European Parliament and of the Council of 22 September 2010, concerning the protection of animals used for scientific purposes.

Animals, management and experimental design

A total of 224 Silver Sabahia chickens, an Egyptian-developed breed (192 hens and 32 cocks), were used in the study. Birds of two different ages were included and housed under identical conditions while being fed the same basal diet. The experimental design followed a 2 × 4 factorial arrangement, consisting of two age groups: a younger flock aged 30 weeks (Y) and an older flock aged 50 weeks (O), along with four dietary iron treatments.

For each age group, 112 birds (96 hens and 16 cocks) were randomly assigned to four dietary treatments with six replicates per treatment. The experiment lasted for 12 weeks. The first group (T1) received the basal diet supplemented with 40 mg Fe/kg as inorganic iron ($FeSO_4$). The second, third, and fourth groups (T2, T3, and T4) received the basal diet supplemented with 40, 60, and 80 mg Fe/kg, respectively, in the form of organic iron (Fe-Gly), i.e., iron glycinate.

The basic diet was prepared depending on the energy, protein, and mineral consideration of NRC (1994), without adding exogenous iron. The composition and nutritional level of basal diet are shown in Table 1. The experiment adopted a 3-layer ladder cage (45×45×43 cm), with 2 chickens in each cage and 2 neighboring cages considered as one replicate. For each age group, the males were divided into four groups (4 males per group) and fed the same experimental diets as their corresponding treatment groups and replicates. Hens were artificially inseminated using semen from the males within their respective groups. Water and feed were provided *ad libitum* during the experimental period. The housing conditions were carefully controlled throughout the study. Environmental temperature was maintained between 20°C and 22°C, with relative humidity at 65 ± 5%. A lighting regimen of 16 hours light and 8 hours dark was applied, with a light intensity of 15 lux. Regular daily ventilation was ensured, and strict disinfection weekly protocols were implemented. Standard veterinary care and vaccinations were provided also provided.

Data collection

Productive and egg quality parameters

Daily egg production percentage (EP, %), egg weight (EW, g), and egg mass (EM, g egg/hen/day) were recorded for each replicate and treatment group. Additionally, daily feed consumption (FC, g feed/hen/day) and feed conversion ratio (FCR, g feed/g egg mass) were monitored. At the end of the trial, three eggs per replicate were randomly selected for egg quality assessment.

Table 1: Ingredient composition and nutrient levels of the basal chicken diet (air-dry basis).

Feedstuffs	**Kg/ton**
yellow Corn	603
Wheat Bran	14
Soybean Meal	260
NaCl	3.7
Vit.-mineral mixture[1]	3
Di-Calcium phosphate	17
Limestone	86
Vegetable oil	12
DL.Methionine	1.3
Total	1000
Calculated analysis	
Crude protein%	16.55
ME kcal/kg diet	2752
Calcium %	3.33
Available phosphorus %	0.45
Arginine %	1.06
Lysine %	0.84
Arginine/Lysine	1.26
Methionine %	0.38
Cysteine %	0.29
Na^+%	0.016
K^+%	0.91
Cu, mg/kg diet	20.44
Zn, mg/kg diet	107.02
Cu, %	0.002
Zn, %	0.0107

[1]Premix provided per kilogram of diet: vitamin A, 12,500 IU; vitamin D3, 4,125 IU; vitamin E, 30 IU; vitamin K3, 2 mg; thiamine, 1 mg; riboflavin, 8.5 mg; calcium pantothenate, 50 mg; nicotinic acid, 32.5 mg; vitamin B6, 8 mg; folic acid, 5 mg; vitamin B12, 5 mg; biotin, 1 mg; choline chloride, 600 mg; Iodine, 0.35 mg; Copper, 10 mg; Manganese, 80 mg; Zinc, 80 mg; Selenium, 0.3 mg.

Albumen height (mm), yolk percentage, and albumen percentage were measured. Eggshell thickness (excluding membranes) was evaluated using a micrometer, and eggshell strength (N) was determined using a Digital Force Gauge (FGC-50) following the method of Bennett *et al.* (1988). Yolk color was assessed using the Roche Yolk Color Fan (Vuilletjmier, 1969), while yolk iron content (mg/100 g) was determined according to Revy *et al.* (2004). Eggshell crude ash was obtained by incineration overnight in a muffle furnace, and iron concentration (mg/100 g) in the ash was measured using atomic absorption spectrophotometry.

Hatching traits

One thousand hatching Silver Sabahia eggs representing the treatment groups and replicates were settled in an Egyptian-made incubator at 99 °F and 55% Relative humidity (RH) during the setting phase. At 18th day of incubation, the eggs were candled and those with live embryos were transferred to the hatcher and incubated at 98.7 °F and 70% RH. Percentages of fertility and hatchability of fertile eggs were determined. Eggs that failed to hatch were broken out and examined macroscopically to determine embryonic mortality as percentage of fertile eggs. Chick weight at pull out is the weight (g) of the chicks at the time of removal from the hatcher. All percentages data were subjected to arcsine square root percentages transformation prior to analysis.

Blood analysis

At the end of the experiment, blood samples (4 mL) were collected from the brachial vein of three birds per replicate. Each sample was divided into two portions. The first portion (fresh blood) was immediately used to measure hemoglobin (Hb, g/dL), packed cell volume (PCV, %), red blood cell count (RBCs, $\times10^6/mm^3$), and white blood cell count (WBCs, $\times10^6/mm^3$), along with differential WBC counts, including percentages of lymphocytes and heterophils.

The second portion was centrifuged, and the resulting serum was stored at −20°C until further analysis. Serum parameters included iron concentration (Fe, mg/L), total protein (g/dL), globulin (g/dL), uric acid (mg/dL), and creatinine (mg/dL). In addition, the activities of aspartate aminotransferase (AST, U/L), alanine aminotransferase (ALT, U/L), total antioxidant capacity (TAC, μmol/L), glutathione peroxidase (GSH-Px, μmol/L), superoxide dismutase (SOD, U/mg), and malondialdehyde (MDA, μmol/L) were evaluated using commercial diagnostic kits. The kits were obtained from Sentinel CH (Milano, Italy) and CAL-TECH Diagnostics Inc. (Chino, CA, USA), and analyses were performed according to the manufacturers' instructions.

Statistical analysis

Data were analyzed using two-way analysis of variance (ANOVA) through the General Linear Model (GLM) procedure in SAS software version 9.2 (SAS Institute Inc., 2018, Cary, NC, USA). The statistical model included the main effects of chicken age, dietary iron concentration, and their interaction. Results are presented as means ± standard error of the mean (SEM). Differences between means were assessed using Tukey's post hoc test, and statistical significance was considered at $P \leq 0.05$. The following statistical model used is:

$$Y_{ijk} = \mu + S_i + P_j + (SP)_{ij} + e_{ijk}$$

Where, Y_{ijk} is each dependent observation under study, μ = overall mean, S_i is the iron addition effect, P_j is an age effect, $(SP)_{ij}$ is interaction between iron and age and e_{ijk} is the experimental random residue error.

RUSULTS

Productive performance

Data of Table 2 represent the effect of dietary iron supplementation, chicken age and their interactions on productive performance of Silver Sabahia chickens. Irrespective of chicken age supplementing the diet with 80 mg organic iron (FeGly)/kg diet (T_4) significantly increased (P≤0.001) EW followed by 60 mg (T_3) compared with those for other supplemented groups. Egg production percentage, EM and FCR were improved (P≤0.001) for chickens of T_3 and T_4 groups compared to those for T_1 and T_2 groups. In addition, birds supplemented with FeGly for T_3 and T_4 groups consumed lower amounts (P≤0.001) of feed compared with those for $T_1$and T_2 groups. Regardless of iron addition, EW was larger (P≤0.001) for elder chickens compared with those for younger ones. Moreover, young chickens represented significant improvement of EP% compared with older chickens. While, EM, FC and FCR traits did not show any statistical change between studied ages.

Interaction between dietary iron concentration and flock age revealed that the best significant result of egg weight was detected for older chickens supplemented with 80 mg Fe-Gly/kg diet (T_4 x O) compared with the other values of interaction and the least value was detected for (T_1 x Y) interaction. Highest significant interactions of EP were recorded for (T_3 x Y) and (T_4 x Y) interactions followed by those for (T_4 x O) compared with the others. The interaction between chicken age and dietary iron concentration represented that young chickens fed 40 mg $FeSO_4$ substantially consumed highest amount of feed (T_1 x Y) compared with those for other interaction values. The best improvement of FCR was detected for both young and older chickens supplemented with 80 mg Fe-Gly/kg diet (T_4 x Y and T_4 x O) compared with the other interactions values.

Table 2: Effect of dietary organic iron (Fe-Gly) supplementation, chicken age and their interactions on productive performance of Silver Sabahia chickens

Traits/ factors	Egg weight (g)	Egg production (%)	Egg mass (g/ hen / day)	Feed consumption (g/hen/ day)	Feed conversion ratio (g feed /g egg mass)
Iron supplementation					
$FeSO_4$, 40 mg/kg diet (T_1)	53.88^c	60.08^b	32.34^b	114.03^a	3.58^a
Fe-Gly, 40 mg/kg diet (T_2)	53.82^c	60.56^b	32.58^b	110.67^c	3.46^a
Fe-Gly 60 mg/kg diet (T_3)	54.93^b	64.84^a	35.57^a	112.33^b	3.25^b
Fe-Gly 80 mg/kg diet (T_4)	55.76^a	64.52^a	37.06^a	112.30^b	3.05^c
SEM	0.17	0.82	0.55	0.29	0.05
P Value	0.001	0.001	0.001	0.001	0.001
Chicken age					
Young (Y)	53.46^b	64.68^a	34.57	112.53	3.32
Old (O)	55.74^a	61.31^b	34.20	112.13	3.34
SEM	0.17	0.75	0.42	0.24	0.4
P Value	0.001	0.001	0.53	0.71	0.54
Interaction between iron supplement and chicken age					
T_1 x Y	52.74^e	61.98^{bcd}	32.69^d	114.73^a	3.55^a
T_2 x Y	53.28^{ef}	62.14^{bcd}	33.11^{cd}	109.86^d	3.37^{ab}
T_3 x Y	53.98^e	67.06^a	36.14^b	113.33^b	3.26^{bc}
T_4 x Y	53.83^{de}	67.54^a	36.33^{ab}	112.20^{bc}	3.11^{cd}
T_1 x O	55.03^{bc}	58.17^d	31.99^d	113.33^b	3.61^a
T_2 x O	54.36^{cd}	58.97^{cd}	32.04^d	111.47^c	3.54^a
T_3 x O	55.88^b	62.62^{bc}	34.99^{bc}	111.33^c	3.23^{bcd}
T_4 x O	57.70^a	65.48^{ab}	37.78^a	112.40^{bc}	3.00^d
SEM	0..22	1.12	0.55	0.31	0.06
P Value	0.001	0.001	0.05	0.001	0.01

a, b. c and d means within each column for each item with different superscripts are significantly different (P≤0.05)

Table 3: Effect of dietary organic iron (Fe-Gly) supplementation, chicken age and their interactions on some egg quality traits of Silver Sabahia chickens.

Traits/ factors	Albumen height (mm)	Eggshell thickness (mm)	Eggshell strength (N)	Yolk (%)	Albumen (%)	Yolk color
Iron supplementation						
$FeSO_4$, 40 mg/kg diet (T_1)	6.06^{b}	31.76^{c}	4038.83^{d}	60.11^{b}	26.47^{c}	5.17^{c}
Fe-Gly, 40 mg/kg diet (T_2)	6.09^{b}	32.53^{b}	4409.83^{c}	60.81^{b}	26.81^{c}	6.50^{b}
Fe-Gly 60 mg/kg diet (T_3)	6.42^{ab}	35.25^{a}	4527.50^{b}	61.02^{b}	27.50^{b}	7.00^{a}
Fe-Gly 80 mg/kg diet (T_4)	7.04^{a}	35.33^{a}	4658.00^{a}	62.28^{a}	28.59^{a}	7.33^{a}
SEM	0.21	0.28	37.09	0.25	0.22	0.28
P Value	0.05	0.01	0.003	0.001	0.001	0.001
Chicken age						
Young (Y)	6.80^{a}	34.08^{a}	4480.92^{a}	60.55^{b}	26.72^{b}	6.25^{b}
Old (O)	6.17^{b}	33.36^{b}	4336.17^{b}	61.56^{a}	27.97^{a}	6.75^{a}
SEM	0.27	0.24	26.62	0.33	0.29	0.23
P Value	0.001	0.001	0.001	0.001	0.004	0.001
Interaction between iron supplement and chicken age						
T_1 x Y	5.93^{bdc}	32.57^{b}	4097.33^{d}	59.65^{c}	25.52^{e}	5.33^{e}
T_2 x Y	6.23^{bc}	33.23^{b}	4522.67^{b}	59.94^{c}	25.94^{e}	6.00^{d}
T_3 x Y	6.97^{ab}	35.26^{a}	4615.33^{a}	60.36^{bc}	27.08^{d}	6.67^{c}
T_4 x Y	7.40^{a}	35.27^{a}	4688.33^{a}	62.22^{a}	28.33^{ab}	7.00^{bc}
T_1 x O	5.17^{d}	30.96^{d}	3980.33^{e}	60.57^{bc}	27.42^{cd}	5.00^{e}
T_2 x O	5.92^{dc}	31.83^{cd}	4297.00^{c}	61.67^{ab}	27.67^{bcd}	7.00^{bc}
T_3 x O	6.68^{abc}	35.23^{a}	4439.67^{b}	61.67^{ab}	27.92^{bc}	7.33^{ab}
T_4 x O	6.91^{abc}	35.40^{a}	4627.67^{a}	62.33^{a}	28.85^{a}	7.67^{a}
SEM	0.25	0.26	20.53	0.31	0.34	0.35
P Value	0.001	0.05	0.001	0.001	0.001	0.001

$^{a, b, c, d \text{ and } e}$ means within each column for each item with different superscripts are significantly different (P≤0.05).

Egg quality

Effects of dietary organic iron concentrations besides chicken age and their interactions on some egg quality traits of Silver Sabahia chicken are shown in Table 3. Irrespective of chicken age, supplementing the diets with 80 mg Fe-Gly/kg diet (T_4) induced significant increase of albumen height compared with those fed 40 mg Fe-Gly/kg diet (T_2) and inorganic group (T_1) with no statistical change with those for T_3 group. Regardless of iron addition, albumen height had significantly decreased (P≤0.001) for eggs of older chickens compared with those for younger ones regardless of the dietary concentrations. The interaction analysis reveals that highest record of albumen height was recorded for eggs of younger chickens which supplemented with the highest dose of Fe-Gly (T_4 x Y). Also, irrespective of chicken age, supplementing the diets with 80 mg Fe-Gly/kg diet (T_4) significantly increased (P≤0.001) each of eggshell strength, yolk and albumen percentages compared with those for all other groups. Moreover, eggshell thickness and egg yolk color had been significantly increased (P≤0.001) for eggs of T_3 and T_4 groups compared with those of T_1 and T_2 groups.

Regardless of dietary iron addition, eggs of older chickens represented significant increase (P≤0.001) for each of yolk, albumen percentages and yolk color. Whereas, eggshell thickness and shell strength had significantly increased (P≤0.001) for younger chickens compared with those for older ones. The interaction demonstrates that the highest records of eggshell thickness were observed for T_3 and T_4 groups for both younger and older chickens compared with those for other interactions. Generally, the interactions revealed that the best values of shell strength, yolk and albumen percentages were detected for both younger and older chickens supplemented with 80 mg Fe-Gly/kg diet.

Hatch output

Dietary organic iron concentration and chicken age besides their interactions on some hatching traits of Silver Sabahia chicken eggs are shown in Table 4. Supplementing the diet with 80 mg Fe-Gly/kg diet (T_4) substantially increased (P≤0.01) each of fertility and hatchability of fertile eggs percentages compared with the other studied concentrations (T_2 and T_3) and inorganic iron as T_1 group. This treated concentration of 80 mg Fe-Gly/kg diet decreased total embryonic mortality% compared with those for other groups. Moreover, irrespective of dietary iron addition, younger chickens realized significant enhancement of fertility and hatchability of fertile eggs percentages compared with those for older ones accompanied with significant diminish of embryonic mortality percentage. The highest significant records of hatched chick weights were observed for chickens supplemented with 60 mg Fe-Gly/kg diet compared with the other rest groups. While, $FeSO_4$ group (T_1) represented the worst significant values of hatched chick weight compared with the others. Regardless of dietary iron supplementation, hatched weight at pull out substantially heavier for chicks produced from old chickens compared with those from younger ones.

Table 4: Effect of dietary organic iron (Fe-Gly) supplementation, chicken age and their interactions on some hatching traits of Silver Sabahia chickens.

Traits/ Factors	Fertility (%)	Hatchability of Fertile eggs (%)	Total embryonic mortality (%)	Hatched chick weight at pull out (g)
Iron supplementation				
$FeSO_4$, 40 mg/kg diet (T_1)	76.85^c	85.83^b	14.16^a	34.67^c
Fe-Gly, 40 mg/kg diet(T_2)	81.28^b	86.22^b	13.78^a	36.50^b
Fe-Gly 60 mg/kg diet (T_3)	83.33^b	87.32^b	12.68^a	40.33^a
Fe-Gly 80 mg/kg diet (T_4)	87.61^a	91.01^a	8.99^b	37.33^b
SEM	1.78	1.18	1.10	0.77
P value	0.01	0.01	0.01	0.01
Chicken age				
Young (Y)	84.42^a	88.28^a	11.27^b	36.75^b
Old (O)	80.12^b	86.91^b	13.09^a	38.67^a
SEM	0.76	0.66	0.66	0.29
P Value	0.002	0.05	0.05	0.01
Interaction between iron supplement and chicken age				
T_1 x Y	80.36^{ed}	86.68^c	13.32^a	34.33^d
T_2 x Y	85.00^{abc}	87.28^{bc}	12.72^{ab}	36.00^{cd}
T_3 x Y	84.44^{bc}	88.03^{abc}	11.97^b	39.33^b
T_4 x Y	87.88^a	91.13^a	8.87^c	37.33^c
T_1 x O	73.33^f	85.00^c	15.00^a	35.00^d
T_2 x O	77.58^e	85.16^c	14.84^a	37.00^c
T_3 x O	82.22^{cd}	86.60^c	13.40^a	37.33^c
T_4 x O	87.33^{ab}	90.89^{ab}	9.12^c	41.33^a
SEM	0.89	1.09	1.09	0.78
P Value	0.01	0.01	0.01	0.01

a, b, c, d, e and f means within each column for each item with different superscripts are significantly different (P≤0.05).

The interaction between iron concentrations and chicken age revealed that the best significant improvement (P≤0.01) of each for fertility and hatchability of fertile eggs set are observed for interactions of T_4 x Y and T_4 x O compared with the other interaction values. The interaction analysis reveals significant reduction of embryonic mortality for groups of T_4 x Y and T_4 x O compared with the other interactions. Regarding hatched chick weight, highly significant value of interaction is observed for older chickens supplied with 80 mg Fe-Gly (T_4 x O) compared with the other interactions.

Table 5: Effect of dietary organic iron (Fe-Gly) supplementation, chicken age and their interactions on iron concentration in egg yolk, eggshell and serum of Silver Sabahia chickens.

Traits/ Factors	Fe yolk (mg/100g)	Fe eggshell (mg/100g)	Fe serum (mg/L)
Iron supplementation			
$FeSO_4$, 40 mg/kg diet (T_1)	2.90^b	0.689^c	2.307^c
Fe-Gly, 40 mg/kg diet (T_2)	3.00^a	0.723^b	2.693^b
Fe-Gly 60 mg/kg diet (T_3)	3.00^a	0.721^b	2.859^a
Fe-Gly 80 mg/kg diet (T_4)	3.06^a	0.782^a	2.985^a
SEM	0.50	0.135	0.48
P value	0.01	0.01	0.01
Chicken age			
Young (Y)	4.05^a	0.746^a	2.819^a
Old (O)	2.92^b	0.709^b	2.603^b
SEM	0.62	0.175	0.97
P Value	0.01	0.01	0.01
Interaction between iron supplement and chicken age			
T_1 x Y	3.01^b	0.696^e	2.542^c
T_2 x Y	3.02^b	0.763^c	2.784^b
T_3 x Y	4.03^b	0.752^d	2.910^{ab}
T_4 x Y	3.14^a	0.775^b	3.040^a
T_1 x O	2.78^e	0.672^g	2.072^d
T_2 x O	2.95^d	0.682^f	2.602^c
T_3 x O	2.96^d	0.690^{ef}	2.808^b
T_4 x O	2.98^c	0.789^a	2.930^{ab}
SEM	0.79	0.153	0.48
P Value	0.01	0.01	0.01

a, b. c, d, e, f, and g means within each column for each item with different superscripts are significantly different (P≤0.05).

Egg and serum iron concentration

Impacts of dietary iron supplementation, chicken age and their interactions on iron concentration in egg yolk, eggshell and serum are presented in Table 5. It appears from data of this table that all supplementations of organic iron significantly (P≤0.01) increased iron yolk compared with those of inorganic iron. Whereas, iron had been significantly increased (P≤0.01) in eggshell for chickens supplemented with concentration of 80 mg Fe-Gly/kg diet only and in serum with concentrations of 60 mg and 80 mg Fe-Gly/kg diet compared with those of other experimental supplementations. Moreover, younger chickens represented significant increase of iron in egg yolk, eggshell and serum compared with those for older chickens. Also, the interaction between iron supplementation and chicken age revealed that the highest significant values of iron in egg yolk was observed for group of T_4 x Y. It means that the highest significant concentration iron in egg yolk was detected for group of younger chickens supplemented with 80 mg Fe-Gly/kg diet. While, iron in eggshell represented significant (P≤0.001) increase for group of older chickens supplemented with 80 mg Fe-Gly (T_4 x O) compared with other values of interactions. Generally, the group of younger chickens supplemented with 80 mg Fe-Gly/kg diet (T_4 x Y) represented high significant values of iron in serum flowed by T_4 x O and T_3 x Y without any statistical change.

Blood parameters

All studied dietary concentrations of organic iron substantially increased most of the studied hematological parameters for chickens (RBC, PCV, Hb and WBC's) compared with those for inorganic iron group (Table 6). Also, studied concentrations of 60 mg and 80 mg Fe-Gly/kg diet showed significant increase of lymphocytes compared with those of inorganic iron ($FeSO_4$). Heterophil percent did not reveal any statistical difference among the all experimental groups. Irrespective of iron supplementation, the studied traits of RBC, PCV, Hb and WBC's recorded significant increase (P≤0.05) of values for younger chickens compared with the elder ones, whereas, lymphocyte and heterophil percentages represented opposite results. The data of interaction from Table 6 suggest that the best values of interaction are detected for group of younger chickens supplemented with highest concentration of Fe-Gly (T_4 x Y) among the studied parameters of RBC, PCV, Hb and WBC's.

Table 6: Effect of dietary organic iron (Fe-Gly) supplementation, chicken age and their interactions on hematological traits of Silver Sabahia chicken.

Traits/ Factors	Red blood cells ($RBC\times10^6/mm^3$)	Packed cells volume % (PCV)	Hemoglobin (g/dl) (Hb)	White blood cells ($WBC\times10^6/mm^3$)	Lymphocyte (%)	Heterophil (%)
Iron supplementation						
$FeSO_4$, 40 mg/kg diet (T_1)	2.45^b	28.19^b	10.39^b	24.25^c	40.90^b	25.47
Fe-Gly, 40 mg/kg diet (T_2)	2.71^a	32.40^a	11.09^a	24.91^{ab}	42.40^{ab}	25.61
Fe-Gly 60 mg/kg diet (T_3)	2.61^a	31.91^a	11.33^a	25.28^b	42.90^a	25.76
Fe-Gly 80 mg/kg diet (T_4)	2.72^a	32.99^a	11.44^a	25.68^a	43.40^a	26.01
SEM	0.03	0.50	0.15	0.22	0.52	0.37
P Value	0.008	0.001	0.001	0.001	0.01	0.17
Chicken age						
Young (Y)	2.69^a	32.89^a	11.59^a	25.54^a	41.25^b	24.89^b
Old (O)	2.55^b	29.85^b	10.53^b	24.52^b	43.55^a	26.63^a
SEM	0.2	0.36	0.0.9	0.15	0.36	0.23
P Value	0.001	0.001	0.001	0.001	0.001	0.001
Interaction between iron supplement and chicken age						
T_1 x Y	2.62^{ab}	29.36^d	10.56^c	24.71^b	40.20^b	24.46^b
T_2 x Y	2.75^a	34.56^a	11.54^b	25.73^a	41.00^b	24.53^b
T_3 x Y	2.66^{ab}	33.04^{ba}	12.08^a	26.01^a	41.40^b	24.99^b
T_4 x Y	2.74^a	34.62^a	12.21^a	25.71^a	42.40^{ab}	25.57^{ab}
T_1 x O	2.29^c	27.02^e	10.22^c	23.79^c	41.60^b	26.49^a
T_2 x O	2.67^{ab}	30.24^{cd}	10.64^c	24.09^{bc}	43.80^a	26.70^a
T_3 x O	2.56^b	30.78^{cd}	10.58^c	24.55^{bc}	44.40^a	26.54^a
T_4 x O	2.69^{ab}	31.37^{bc}	10.68^c	25.65^a	44.40^a	26.82^a
SEM	0.04	0.58	0.14	0.27	0.63	0.44
P Value	0.001	0.05	0.001	0.001	0.001	0.001

$^{a, b, c, d \text{ and } e}$ means within each column for each item with different superscripts are significantly different (P≤0.05)

Table 7: Effect of dietary organic iron (Fe-Gly) supplementation, chicken age and their interactions on some blood parameters of Silver Sabahia chickens.

Traits/ Factor	Total protein (g/dl)	Globulin (g/dl)	Uric acid (mg/dl)	Creatinine (mg/dl)	AST (U/L)	ALT (U/L)
Iron supplementation						
$FeSO_4$, 40 mg/kg diet (T_1)	4.684^b	2.002^a	37.194^a	0.9125^a	34.940^a	16.130^a
Fe-Gly, 40 mg/kg diet (T_2)	4.651^b	1.850^b	32.784^b	0.8630^b	33.380^b	14.750^b
Fe-Gly 60 mg/kg diet (T_3)	4.758^a	2.006^a	31.340^c	0.8290^c	33.400^b	14.080^c
Fe-Gly 80 mg/kg diet (T_4)	4.790^a	2.026^a	30.170^d	0.8085^c	32.020^c	14.060^c
SEM	0.01	0.02	0.19	0.009	0.36	0.17
P Value	0.001	0.05	0.001	0.001	0.001	0.001
Chicken age						
Young (Y)	4.795^a	1.931^b	32.909	0.8322^b	32.875^b	14.540
Old (O)	4.646^b	2.011^a	33.235	0.8744^a	33.995^a	14.970
SEM	0.01	0.01	0.37	0.008	0.29	0.16
P Value	0.001	0.002	0.172	0.001	0.001	0.071
Interaction between iron supplement and chicken age						
T_1 x Y	4.746^{bc}	1.978^{ab}	36.968^a	0.8998^{ab}	34.100^b	15.920^a
T_2 x Y	4.770^{bc}	1.752^c	32.268^c	0.8674^{bc}	33.180^{bcd}	14.500^{bc}
T_3 x Y	4.812^{ab}	1.944^b	31.100^{de}	0.7718^e	32.380^{cd}	13.920^c
T_4 x Y	4.852^a	2.050^a	29.700^f	0.7900^{de}	31.840^d	13.820^c
T_1 x O	4.622^d	2.026^{ab}	37.420^a	0.9252^a	35.780^a	16.340^a
T_2 x O	4.532^e	1.948^b	33.300^b	0.8586^{bc}	33.580^{bc}	15.000^b
T_3 x O	4.704^c	2.068^a	31.580^{cd}	0.8870^{ab}	34.420^{ab}	14.240^{bc}
T_4 x O	4.728^c	2.002^{ab}	30.640^e	0.8270^{cd}	32.200^{cd}	14.300^{bc}
SEM	0.02	0.02	0.25	0.006	0.46	0.23
P Value	0.001	0.001	0.001	0.01	0.01	0.01

[a, b, c, d and e] means within each column for each item with different superscripts are significantly different (P≤0.05). AST: aspartate amino transferase ALT: alanine amino transferase.

Supplementing the diet with both concentrations of 60 and 80 mg Fe-Gly/kg diet significantly raised total protein compared with those for 40 mg Fe-Gly/kg diet and 40 mg $FeSO_4$ /kg diet, while globulin value represented the worst significant value for birds fed basal diet with 40 mg Fe-Gly/kg diet compared with other studied groups (Table 7). Apparently, uric acid and creatinine had been substantially diminished due to supplementing the diet with each of 60 or 80 mg Fe-Gly compared with others. The same significant influence is observed for AST and ALT due to the same supplementations of 60 and 80 mg Fe-Gly/kg diet.

Regardless of dietary iron addition, younger chickens represented significant increase (P≤0.05) for total protein and significant decrease (P≤0.05) for globulin compared with those for older chickens. In addition younger chickens represented significant decrease (P≤0.05) in creatinine and AST enzyme compared with those for older ones. While, uric acid and ALT enzyme did not reveal any statistical differences between the studied ages.

The interaction between iron supplementation and chicken age revealed the response and the increase of total protein for younger chickens supplemented with higher concentrations of Fe-Gly (60 and 80 mg /kg diet) compared with the other interactions. Generally, the studied interactions in this table reveal that the studied blood parameters of uric acid and creatinine as kidney function and AST and ALT as liver function are responded and decreased with the highest concentration used of organic iron for younger chickens compared with the other interactions of iron supplementation and chickens age.

Antioxidant status

Table 8 shows the effect of dietary iron addition, chicken age and their interactions on antioxidant status. It is apparent from data of this table that TAC and SOD represented significant increase (P≤0.05) with increasing concentration of Fe-Gly compared with other concentrations. While, MDA value was significantly decreased (P≤0.05) with 60 and 80 mg Fe-Gly compared with those for 40 mg Fe-Gly and 40 mg $FeSO_4$ supplementations. Also, GSH-

PX values were significantly increased (P≤0.05) for all experimental supplementations of Fe-Gly compared with $FeSO_4$. Irrespective of dietary iron concentration, younger chickens represented significant increase (P≤0.05) of each TAC, SOD and GSHPX values compared with those for older ones. While, MDA represented opposite results and chicken age did not represent any statistical change. Interaction analysis revealed that all studied concentrations of Fe-Gly resulted in improved values of TAC, SOD, GSH-Px, and MDA compared to the $FeSO_4$ group across both chicken age groups.

Table 8: Effect of dietary organic iron (Fe-Gly) supplementation, chicken age and their interactions on antioxidant traits of *Sabahia siliver* chickens.

Traits/ Factors	MDA (Umol/l)	TAC (Umol/l)	SOD (U/ mg)	GSHPX (Umol/l)
Iron supplementation				
$FeSO_4$, 40 mg/kg diet (T_1)	1.2022[a]	406.400[c]	224.700[d]	842.280[b]
Fe-Gly, 40 mg/kg diet (T_2)	0.9473[b]	458.500[b]	255.000[c]	959.590[a]
Fe-Gly 60 mg/kg diet (T_3)	0.8108[c]	467.400[ab]	270.500[b]	952.160[a]
Fe-Gly 80 mg/kg diet (T_4)	0.7920[c]	474.000[a]	277.100[a]	973.380[a]
SEM	0.01	4.16	2.15	6.89
P Value	0.001	0.001	0.001	0.001
Chicken age				
Young (Y)	0.8968[b]	457.800[a]	262.850[a]	948.615[a]
Old (O)	0.9792[a]	445.350[b]	250.800[b]	915.090[b]
SEM	0.02	4.52	2.67	8.45
P Value	0.001	0.05	0.004	0.001
Interaction between iron supplement and chicken age				
T_1 x Y	1.2240[a]	415.800[c]	228.800[f]	860.280[d]
T_2 x Y	0.8178[c]	461.000[b]	257.800[de]	971.160[ab]
T_3 x Y	0.7856[cd]	474.000[ab]	278.000[b]	978.420[ab]
T_4 x Y	0.7598[d]	480.400[a]	286.800[a]	984.600[a]
T_1 x O	1.1800[a]	397.000[d]	220.600[g]	824.280[e]
T_2 x O	1.0768[b]	456.000[b]	252.200[e]	948.020[bc]
T_3 x O	0.8360[c]	460.800[b]	263.000[cd]	925.900[c]
T_4 x O	0.8242[c]	467.600[ab]	267.400[c]	962.160[ab]
SEM	0.01	5.06	2.38	8.11
P Value	0.025	0.05	0.05	0.029

[a, b, c,d,e and g] means within each column for each item with different superscripts are significantly different (P≤0.05). MDA: malondialdehyde, TAC: total antioxidant capacity, SOD: superoxide dismutase, GSHPX: glutathione peroxidase

DISCUSSIONS

Everal researchers have reported that supplementing diets with higher concentrations of Fe-Gly per kg of feed benefits egg weight (EW) and egg production percentage (EP%) in younger flocks. For example, Xie *et al.* (2019) demonstrated that chickens aged 26 weeks supplemented with up to 80 mg Fe-Gly/kg diet showed significantly increased EW and egg production rates compared to the control group. Also, Cao *et al.* (2023) showed that supplementing Fe-Lys-Gly in layers increased average daily EW and egg production rate of layers fed with 30 to 75 Fe/ kg diet as Fe-Lys-Gly compared to diet without adding extra iron. Our experimental levels of Fe-Gly especially with 60 or 80 mg / kg diet realized best significant results of EW and EP for both studied chicken ages. Kim *et al.* (2023) have drawn the same conclusion of improving EW only with high supplementation level of organic Fe in aged laying hens. The results of FCR improvement for young birds supplemented with 60 or 80 mg Fe-Gly/kg diet parallel earlier studies by Coa *et al.* (2023) who reported that FCR for layers in the groups supplemented with 45, 60 and 75 Fe-Lys-Gly had markedly improved comparable with the group without exogenous iron. The amino chelated or proteinated source of iron is more advantageous than $FeSO_4$ (Tang *et al.*, 2021). This may be correlated to better absorption of Fe-Gly im-plicating that the bioavailability of Fe from Fe-Gly is higher than that of Fe from $FeSO_4$. It has been suggested that the higher bioavailability of Fe-Gly is probably because of its chemical structure that partially prevents Fe-phytate interactions (Chen *et al.*, 2022).

The improvement of egg production for either younger or older chickens supplemented with the higher levels of Fe conjugated with glycine (80 mg) could be due to the higher bioavailability of Fe-Gly than that for $FeSO_4$ as stated by Kegley *et al.* (2002) and Ji *et al.* (2007). It is concluded from current results that supplementing the diet with 80 mg Fe-Gly/kg could be useful way for realizing better results of egg production particularly for old age chickens.

Improvement results of studied egg quality traits such as albumen height, eggshell thickness and eggshell strength due to the diet treated with 80 mg Fe-Gly/kg diet added credence to the reported observations by different authors with different doses of supplementation in young layers as Tu (2004) reported that additional Fe-Gly represented valuable effect on eggshell thickness, Haugh unit and shell strength. Also, Li *et al.* (2017) showed that mineral-amino acid chelate had a vital role on egg quality traits. Moreover, Xie *et al.* (2019) mentioned that thickness and eggshell strength were improved due to Fe-Gly supplementation, but differences between Fe-Gly and $FeSO_4$ were not significant.

Also, the existing data of decreasing eggshell thickness and eggshell strength for elder chickens are coincided with the increase of egg weight and eggshell weight compared with those for younger ones as previously documented by Mona *et al*. (2016). Moreover, earlier than those Tumova *et al*. (2014) and Rizk *et al*. (2008) showed that flock age had negative impact on eggshell quality including shell strength.

The finding in our results regarding the egg weight increase with increase of flock age followed by increase of yolk and albumen corresponds to those reported earlier (Suk and Park, 2001). The related increase of yolk weight with breeder age could be due to the rate of deposition and synthesis of lipoproteins resulting in larger eggs with larger yolks (Iqbal *et al*., 2016). The egg weight increase with the hen age is due to the increase of yolk and albumen weight (Suk and Park, 2001). Impact of aging on production and reproduction in poultry is documented by Liu *et al*. (2018) and Ma *et al*. (2020). Aging is a natural and physiological process that can progressively produce harmful reactive oxygen species (Lee *et al*., 2009). As a result, when endogenous antioxidant and peroxides in the organisms, the disruption of redox homeostasis and oxidative stress would intevilably occur (Estevez, 2015). As a result, aged bird could be more responded to experimental treatment.

Limited results are available regarding the improvement of hatching results due using organic iron chelated with glycine, whereas Morok and Austic (1981) and Gou *et al*. (2020) who demonstrated the significant effect of inorganic ferrous on hatching results. The importance of adding iron on hatchability is supported by Mackenzie *et al*. (2008) who reported that iron is important for transportation of oxygen in embryos and deficiency can lead to hypoxia in embryos, impairing the growth and survival. Besides, ferric deficient could lead to malformations and mortality embryos as detected by Abbasi *et al*. (2015). The significant decrease in embryonic mortality observed in the bird group supplemented with 80 mg Fe-Gly/kg diet, compared to other groups may be explained by the findings of Khalid *et al*. (2023). They demonstrated that Fe-Gly supplementation increased iron levels in both serum and egg content comparable to $FeSO_4$ supplementation as observed in current research, which may account for the reduction in embryonic mortality. Different workers have asserted the same our conclusion of decreasing fertility and hatchability percentage due to the parental flock age. Almeida *et al*. (2008) and Yilmazd and Sahan (2009) mentioned that fertility and hatchability in general deteriorate with the progress of breeder age. Moreover, Lin *et al*. (2022) noted that fertility and hatchability are highly dependent on the breeder flock age. The diminish of fertility and hatchability percentages of older chickens is primarily due to the increase of embryonic mortality as appeared in our results. This statement was explained by Elibol *et al*. (2002) who revealed that the embryos of older parents generate more heat from day 16 till the incubation end.

From the foregoing results and discussion we recommend using 80 mg Fe-Gly/kg diet for improving fertility and hatchability percentage coincided with decreasing embryonic mortality for both younger and older chickens. In this study, adding all experimental concentrations of Fe-Gly to diets increased iron in egg yolk, eggshell and serum (Table 5). These results are keeping with those reported by Xie *et al*. (2019) who reported that supplementation the diet with 60 mg Fe-Gly increased iron concentration in egg yolk in comparison with those for $FeSO_4$. Moreover, Sarlak *et al*. (2021) reported that iron concentration in serum, eggshell and yolk had been increased by supplementation of Fe-Gly compared to $FeSO_4$ group. Also, Cao *et al*. (2023) revealed that iron concentration in serum and egg yolk had been elevated by Fe-Lys-Glu levels compared with $FeSO_4$. Numerous studies found that iron concentration in egg yolk was influenced by several sources of iron and they found increasing iron content of egg yolk due to adding Fe- amino acids to the diet compared with inorganic Fe (Sarlak *et al*., 2021). Moreover, Cao *et al*. (2023) confirmed that Fe-Lys-Glu has a higher biological efficacy compared to $FeSO_4$ and this is evident from the increase of phosphoerine percentage in the yolk which binds to iron strongly and prevents the formation of insoluble iron compounds and consequently increase the iron absorption rates and iron storage in eggshell and yolk. Also, the same pervious authors added that sufficient iron concentration in plasma is the power key to supply and balance of iron.

The observed increase of iron in egg yolk and eggshell in this study could explain the improvement of hatching output as both traits are the main sources for transferring the iron to the embryo during incubation leading to hatching success. Generally, ferric increase in egg yolk, eggshell and serum as demonstrated in the current results could refer to the increase of absorption and utilization of iron inside the body and accordingly reducing the fecal excretion and environmental pollution. This statement is in line with those reported by Zeng *et al*. (2023) who found that chelated organic iron could reduce iron content in feces more than ferrous sulfate, indicating that organic iron is more bioavailable and causing less waste and pollution.

Hematocrit and hemoglobin are considering good indicator of Fe presence and low levels of them referring to the anemia (Kals *et al*., 2016; Taschetto *et al*., 2017). Also, Ebrahem *et al*. (2014) mentioned that lower percentage of hematocrit is related to lower hatchability. Also, iron is an important element of producing RBC and synthesis of hemoglobin (Trivedi and Barve., 2021). Current results regarding to RBC, hemoglobin and hematocrit as shown in Table 6 are in agreement with the conclusion of Li *et al.* (2018) who reported that adding Fe-Gly increased RBC count, hematocrit, hemoglobin content and serum iron of piglets compared with $FeSO_4$. Moreover, Cao *et al.* (2023) demonstrated that RBC count and hemoglobin content had increased in groups fed diet supplementation with 30 to 75 mg of Fe-Lys-Glu compared with $FeSO_4$ in Beijing white laying hens, so, organic iron had valuable biological value compared with those for inorganic iron. Supporting to our results referring to the significant increase of WBC's count, lymphocytes and heterophils percentages, Olgun *et al.* (2022) found that WBC's had a linear increase with the supplementation of Fe-Gly to the diet of quails.

The significant increase of serum total protein and globulin in this study due to adding Fe-Gly is in accordance with those previously reported by Chao et al. (2018) who came to the same conclusion and reveled that Fe-Gly may have better promoting impact on protein synthesis than $FeSO_4$. While, Olgun *et al.* (2022) indicated that supplementing the quail diet with organic iron did not represent statistical effect on serum biochemical constituents. In comparison with numerous studies, there is a paucity of information describing associated changes between organic iron concentrations kidney and liver functions of young and old chickens.

Our results of decreasing MDA and increasing of TAC, SOD besides GSHPX activity's as antioxidant capacity are consistent with the previous results reported by Xie *et al.* (2019) who found raising of SOD activity due to the increase of Fe-Gly concentration in the diet. Also, Sarlak et al. (2021) found that supplementation the diet with Fe-Gly increased SOD activity and decreased MDA content in the serum layers. Moreover, Cao *et al.* (2023) showed that supplementation the diet with Fe-Lys-Glu enhanced SOD activity and decreased MDA content in the serum. The benefits of organic iron to the chicken diet were also reported by Emerit *et al.* (2001) who stated that iron capable to donate and accept electrons readily, this capability makes it physiologically essential, as a useful compound of cytochromes and oxygen-binding molecules. In addition Cao *et al.* (2023) found that adding 45-60 mg Fe-Lys-Glu in the diet substantially improved antioxidant capacity of layers by improving SOD activities and lowering MDA content in serum.

CONCLUSION

This study suggests that supplementing chicken diets with 60 or 80 mg of organic iron chelated with glycine (Fe-Gly/kg diet) provides optimal improvements in hematological parameters, antioxidant status, and liver function. Such supplementation enhances both productive and reproductive performance, with particularly pronounced benefits in older chickens by helping to mitigate age-related metabolic deficiencies. These positive effects are comparable to those observed with inorganic iron sources currently used in poultry diets. Therefore, incorporating organic iron supplementation into chicken nutrition programs should be strongly considered. Additionally, the response of different chicken breeds to dietary Fe-Gly supplementation in terms of production and reproduction will be addressed in the second part of this research.

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NOVELTY STATEMENT

The authors have developed the composition of Silver Sabahia chickens (Egyptian developed breed) diets by organic iron at different ages .The results of this study are published for the first time.

AUTHORS CONTRIBUTION

REK, BMA and AMK: Experiment idea and design.
MRE, MRA and BMA: Performed the farm experiment.
BMA, MRE and MRA: Performed the laboratory analysis.
WAF and AMK: Statistical analysis.
REK, BMA, WAF and AMK: Wrote and revised the manuscript with approval of all authors.

Generative AI and AI-assisted technology statement

The authors declare that no generative AI was used in the creation of this manuscript.

Conflict of interest

The authors have declared no conflict of interest.

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