Effect of iron glycine chelate supplementation on egg quality and egg iron enrichment in laying hens

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ABSTRACT This study was conducted to evaluate the effects of iron glycine chelate (**Fe-Gly**) on egg quality of laying hens. A total of 810 laying hens (HyLine Variety White, 26 wk old) were randomly assigned to 6 groups, and each group consisting of 135 hens (5 replicates of 27 hens each). Hens in the control group received a diet supplemented with 60 mg Fe/kg as $FeSO_4$, whereas hens in the other 5 groups received diets supplemented with 0, 20, 40, 60, and 80 mg Fe/kg from Fe-Gly, respectively. The study showed that dietary Fe-Gly treatments influenced ($P < 0.05$) the internal egg quality (egg weight, Haugh unit, albumen height), compared with the control group. However, dietary Fe-Gly supplementation showed few effects on the ultrastructure of eggshell in this study. The group of 60 mg Fe/kg as Fe-Gly was promoted ($P < 0.05$) in succinate dehydrogenase levels of liver and spleen compared with the 0 mg Fe-Gly/kg group, whereas the control (Fe/kg as $FeSO_4$) group has no differences compared with the 0 mg Fe-Gly/kg group. The concentrations of Fe in the eggshell, yolk, and albumen were increased with increasing concentrations of Fe-Gly, where Fe-Gly (60, 80 mg Fe/kg) had higher ($P < 0.01$) Fe concentration than the control in yolk and albumen. The Fe-Gly groups (60, 80 mg Fe/kg) were influenced ($P < 0.05$) in transferrin, divalent mental transport 1, and ferroportin 1, compared with the control ($FeSO_4$). In conclusion, Fe-Gly (60 mg Fe/kg) improved egg quality and egg iron enrichment. In general, there were no significant differences between Fe-Gly (40) and the control group in albumen height, Haugh unit, Fe concentration in eggshell and yolk. It revealed that $FeSO_4$ could be substituted by a lower concentration of Fe-Gly and Fe-Gly may be superior to $FeSO_4$ for egg quality in laying hens.

Key words: egg quality, eggshell ultrastructure, iron enrichment, iron transport, laying hen

INTRODUCTION

Egg quality is regarded as an essential concern by consumers and manufacturers (Ahmad and Balander, 2003; Arazi et al., 2009; Li et al., 2017), and egg quality characteristics are essential certifications for table eggs (Cherian, 2008). As living standards rise, people not only care for edible characters of foods, but also safety and trophism (Cen and He, 2007). In our daily life, egg is one of the traditional dishes of people from which we usually uptake protein (Stadelman and Cotterill, 1990), and it has been reported that high-quality animal protein from eggs is usually cheaper than meat and milk (Caner, 2010). However, with the development of the commercial egg industry, quality has become a big business in many countries, while manufacturers aspire to higher production, quality of eggs has decreased, which will threaten the further development of layer industry (Jones and Musgrove, 2005).

For the sake of improving egg quality, researchers have made efforts, and from their observations, it seems that adding trace elements into the diets to ameliorate production performance and deposition of microelement is a necessary approach to improve egg quality and nutrition. Iron (**Fe**) is one of the most essential trace elements for poultry (Abbasi et al., 2015), Fe takes part in many essential reactions, including transportation and storage of oxygen, participating in energy supply, protein metabolism, antioxidant activity and immunity (Drygalski and Adamson, 2013; Abbaspour et al., 2014). It has been reported that trace elements could enrich in eggs through dietary addition (Stadelman, 1999). Bess et al. (2012) found that supplying a suitable concentration of Fe could improve egg iron concentration. So, Fe supplement is vital for egg quality, but to our knowledge, there were few reports about the effects of dietary microelement in organic forms such as iron

glycine chelate (**Fe-Gly**) on egg quality and its mechanism. Also, the main iron supplement used in feed industry now is ferrous sulfate ($FeSO_4$) which has been found low absorption, causing waste and pollution (Ma et al., 2014). Recently, microelement in organic forms has been reported to have a better biological value than trace elements in inorganic forms (Kegley et al., 2002; Creech et al., 2004; Mohammadi et al., 2015). Alternatively, other researchers found that organic source (amino acid complexes) had no differences with the inorganic one in mineral retention and eggshell quality (Mabe et al., 2003; Xie et al., 2019). Sun et al. (2012) pointed out the reason of different results in the biological value of minerals might be different chelation strength (Li et al., 2004; Zhang et al., 2016) or ligands (House et al., 1997). Yi et al. (2007) and Ma et al. (2013) have proved that the organic trace mineral with an organic ligand has a better biological value than sulfate forms for broilers, because mineral absorption from inorganic trace mineral might be limited by their tendency to form complexes with dietary constituents or interfere with each other.

The main objectives of this study were to investigate the effects of Fe-Gly supplementation on egg quality and iron enrichment, at the same time, to examine whether Fe-Gly could substitute inorganic iron at a lower concentration.

MATERIALS AND METHODS

All experimental procedures were conducted following the Animal Welfare Committee guidelines and approved by the Animal Science College of Zhejiang University (Hangzhou, China). No. ZJU2013105002.

Bird Management

A total of 810 laying hens (HyLine Variety White, 26 wk old) were randomly assigned to 6 treatment groups, and each group with 5 replicates (n = 27 laying hens), and 3 hens were allocated to each cage (50 × 50 × 50 cm). Temperature and lighting were supplied according to commercial operations. The laying hens were housed in an enclosed, ventilated, and standard room. Feed and water were provided ad libitum. The experiment lasted for 9 wk and included a 1 wk as acclimation period and 8 wk of the test period.

Experimental Design and Diets

The laying hens in the control group were fed the basal diet (formed according to the feeding standard of laying hens in China) supplemented with 60 mg Fe/kg in the form of $FeSO_4$ (inorganic Fe); the hens in other 5 experimental groups were fed diets supplemented with Fe-Gly (organic Fe, Fe content: 17%, glycine content: 73%; 0, 20, 40, 60, 80 mg Fe/kg, respectively, formed according to the basal diet), which

Table 1. Feed ingredients and nutrient composition of the basal diet.[1]

Items	Composition
Ingredients	Content (%)
Soybean meal	23.50
Wheat bran	2.00
Corn	61.00
Premix[2,3]	5.00
Limestone	8.50
Total	100.00
Nutrient[4]	
Metabolizable energy (MJ/kg)	10.58
Crude protein (%)	16.44
Methionine (%)	0.39
Lysine (%)	0.80
Calcium (%)	3.62
Total phosphorus (%)	0.55

[1]The actual content of Fe analyzed in the 6 treatment diets (mg/kg): dietary 0 mg Fe/kg as Fe-Gly = 2.65 mg Fe/kg; dietary 20 mg Fe/kg as Fe-Gly = 21.98 mg Fe/kg; dietary 40 mg Fe/kg as Fe-Gly = 42.46 mg Fe/kg; dietary 60 mg Fe/kg as Fe-Gly = 62.79 mg Fe/kg; dietary 80 mg Fe/kg as Fe-Gly = 83.41 mg Fe/kg; dietary 60 mg Fe/kg as $FeSO_4$ (control) = 63.78 mg Fe/kg.
[2]The premix provided the following per kilogram of the diet: vitamin E, 15 IU; vitamin A, 7,600 IU; vitamin D3, 2,000 IU; 2 mg; thiamine, vitamin K, 1 mg; vitamin B12, 5 mg; riboflavin, 8.5 mg; niacin, 32.5 mg; calcium pantothenate, 50 mg; folic acid, 5 mg; choline, 500 mg; pyridoxine, 8 mg; biotin, 2 mg; Se, 0.12 mg; Zn, 66 mg; I, 1 mg; Cu, 10 mg; Mn, 65 mg.
[3]The premix in 5 treatments provided per kg of diet: Fe-Gly, 0, 20, 40, 60, 80 mg, respectively, and in the control provided 60 mg $FeSO_4$/kg diet.
[4]Values were calculated from data provided by the Feed Database in China (2018).

were provided by Beijing Alltech Biological Products Co., Ltd (Beijing, China) as shown in Table 1. $FeSO_4$ and Fe-Gly were mixed with the premixes first and then with the other ingredients to make sure that the feed has been mixed more uniformly. The different contents of glycine brought by the addition of graded Fe-Gly were balanced by adding extra glycine in the premixes to make all nutrients, except for the Fe content, in diets were kept at the same levels.

Sample Collection

At 35 wk of age, after 12-h fasting (water offered ad libitum) (Miao et al., 2017), 60 layers (10 layers per treatment, 2 layers per replicate) were stunned and scarified by complete bleeding of the jugular vein and then evacuated for collecting duodenum samples. Samples were rinsed twice with ice-cold PBS and then dried with a filter paper to avoid blood contamination. All samples were kept at −80°C for analysis.

Determination of Egg Quality

Egg quality was measured at the end of the experiment. A total of 20 eggs from each treatment group (4 eggs per replication, 5 replications per treatment) were randomly collected to measure egg quality as reported previously (Bai et al. 2014; Miao et al., 2017). Egg quality (egg weight, eggshell strength, albumen height, Haugh unit, and yolk color) was measured by a

Table 2. Specific primers used for real-time PCR.

Gene name[1]	Accession No.	Primer	Sequence (5′-3′)	Size (bp)
TF	NM_205304.1	Forward	TTTCAAAGACTCTGCCATAATGC	174
		Reverse	TTGCTCTTCTCATCCTTGCCTAC	
DMT1	XM_025145317.1	Forward	CATGTACTTCGTGGTGGCCT	121
		Reverse	GATCAGACACAGCCACGTCA	
FPN1	XM_015289163	Forward	GATGCATTCTGAACAACCAAGGA	68
		Reverse	GGAGACTGGGTGGACAAGAACTC	
18S rRNA	AF173612.1	Forward	ATTCCGATAACGAACGAGACT	141
		Reverse	GGACATCTAAGGGCATCACA	

[1]TF, transferrin; DMT1, divalent metal transporter; FPN1, ferroportin; 18S rRNA, 18S ribosomal RNA.

digital egg tester (DET-6000, Nabel Co., Ltd, Kyoto, Japan). Eggshell thickness was measured (without shell membrane) with an Egg Shell Thickness Gauge (ESTG-1, Orka Food Technology Ltd., Ramat Hasharon, Israel).

Eggshell Ultrastructure

At the end of the experiment, 6 eggs were sampled randomly from each treatment group. These eggs were broken, and then washed with distilled water to get rid of dirt, following manual removal of inner shell membranes. After being dried in the air, shell samples (0.5 to 1 cm^2) of the sharp end of each egg were severally saved. Eggshell samples were prepared for scanning electron microscope analysis (JEOL JSMT330A, HITACHI Ltd., Tokyo, Japan). One sample was used for the analysis of the external shell surface, and the other was used in the assessment of the transversal surface. The samples were fixed to an aluminum support (stub) and coated with gold powder. The width of the mammillary cones in the eggshell was measured and calculated through the scanning electron microscope ruler according to the model of Dunn et al., (2012). Dates of mammillary cones widths were calculated as the average of 3 to 5 mammillary cones per scanning electron photograph.

Determination of SDH Activity in Tissues

The succinate dehydrogenase (**SDH**) activity in liver, kidney, spleen, and serum was assayed and calculated followed by the protocols of commercial kits (Nanjing Jiancheng Bioengineering Institute, Nanjing, China).

Determination of Fe Content in Eggshell, Albumen, and Yolk

Flame atomic absorption spectrophotometry (Perkin-Elmer, Norwalk, CT) was used to determine the content of Fe in feed, eggshell, albumen, and yolk, according to the previous methodology (Huang et al., 2007; Ma et al., 2018). The results are shown as mg/kg dry weight.

Quantification of mRNA with Real-Time PCR

The expressions of transferrin (**TF**), divalent metal transporter 1 (**DMT1**), ferroportin 1 (**FP1**), and 18S rRNA in the duodenum were determined by a StepOne Plus Real-Time PCR System (ABI 7500, Applied Biosystems, Foster City, CA). First, the total RNA was extracted from duodenal samples (stored at –80°C) by using Trizol reagent (Invitrogen, Carlsbad, CA) and other reagents; the detailed operational processes were described by other researchers (Sambrook and Russell 2001; Sun et al., 2015). One microgram of the total RNA was reverse-transcribed to cDNA by using HIScript II QRT SuperMix for qPCR (Vazyme, Nanjing, China). The detailed operational processes were conducted referring to the manufacturer's instructions. Then real-time PCR analysis was carried out. Samples were amplified in the ChamQTM Universal SYBR qPCR Master Mix (Vazume, Nanjing, China), according to the protocol of ChamQTM Universal SYBR qPCR Master Mix (Vazume, Nanjing, China). Forward and reverse primers for each gene are shown in Table 2. The PCR reaction was performed within the following thermal procedure: 95°C for 30 s, followed by 40 cycles of that 95°C for 10 s and 60°C for 30 s, then go with that 95°C for 15 s, 60°C for 60 s, and 95°C for 15 sec. There were 6 samples for every group, each sample was conducted in duplicate, and no template control was included. The mRNA levels were standardized as the ratio to 18S rRNA in arbitrary units by using the $2^{-\Delta\Delta Ct}$ method (Livak and Schmittgen, 2001).

Statistical Analyses

The data were expressed as means and SEM and analyzed statistically by 1-way analysis of variance (**ANOVA**) using SPSS 20. Differences among all treatments were separated by the Tukey test for multiple comparisons. Values of $P < 0.05$ were considered significant.

The model of the statistical analysis was as follows:

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

where Y_{ij} = the observed value, μ = the overall mean, I_i = treatment effects and e_{ij} = the random error.

Table 3. Effect of Fe-Gly supplementation on egg quality.[1]

Items	Control[2] (mg/kg) 60	Fe-Gly (mg/kg) 0	20	40	60	80	SEM	P-value
Egg weight, g	52.09[b]	53.41[a,b]	54.31[a]	54.02[a]	53.56[a]	53.39[a,b]	0.45	< 0.01
Albumen height, mm	6.20[a,b]	5.35[b]	6.67[a,b]	6.97[a]	6.90[a]	6.92[a]	0.50	0.02
Yolk color	7.67	7.33	7.33	7.17	7.17	7.50	0.51	0.91
Haugh unit	83.47[a,b]	77.02[b]	84.38[a,b]	86.05[a,b]	86.90[a]	87.05[a]	3.18	0.03
Eggshell strength, kgf/m^2	3.09	3.10	3.32	4.01	3.45	3.34	0.44	0.36
Eggshell thickness, mm	0.26	0.27	0.30	0.31	0.28	0.27	0.02	0.19

[1]Results are the mean and SEM of 5 replicates, with 4 eggs per replicate. Means within a row with no common superscripts (a, b) differ significantly ($P < 0.05$). SEM = pooled standard error of the mean.
[2]Control birds were fed the diet (60 mg Fe/kg) supplemented with FeSO$_4$.

RESULTS

Effect of Fe-Gly Supplementation on Egg Quality of Laying Hens

As shown in Table 3, the groups of 60 and 80 mg/kg Fe-Gly were improved ($P < 0.05$) in the Haugh unit, compared with the 0 mg/kg Fe group, whereas there were no significant differences between the group of 0 mg/kg Fe and the control group (FeSO$_4$). Moreover, compared with the 0 mg/kg Fe group in albumen height, the groups of 40, 60, and 80 mg/kg Fe-Gly were increased ($P < 0.05$), whereas the control and 20 mg/kg Fe-Gly groups had no significant differences compared with the 0 mg/kg Fe group. Egg weight was also affected ($P < 0.05$) in the groups of 20, 40, and 60 mg/kg Fe-Gly, compared with the 0 mg/kg Fe group, whereas there were no differences between the group of 0 mg/kg Fe and the control group.

Effect of Fe-Gly Supplementation on the Mammillary Cone of Eggshell

Scanning electronic microscopy photographs showed that Fe-Gly supplementation showed no significant effects on the outer surfaces (Figure 1A-F) and transverse profiles (Figure 1G-L) of eggshells of laying hens among all experiment groups. Furthermore, from Figure 2, no differences were shown among all groups in the mammillary cone width.

Effect of Fe-Gly Supplementation on SDH Activity in Serum, Liver, Kidney, and Spleen

The SDH activity in serum, liver, kidney, and spleen is shown in Table 4. The serum SDH level in the group of 80 mg/kg Fe as Fe-Gly was increased significantly ($P < 0.05$), compared with the group of 0 mg/kg Fe, whereas other groups showed no significant differences. The SDH levels of liver and spleen in groups of 40, 60, and 80 mg/kg Fe-Gly had remarkable increases ($P < 0.05$) when compared to the group of 0 mg/kg Fe, whereas the control group showed no marked differences compared with the 0 mg/kg Fe group. There are no significant differences between all groups in kidney SDH level.

Effect of Fe-Gly Supplementation on Fe Concentration in Eggshell, Albumen, and Yolk

As shown in Table 5, the Fe concentrations of eggshell in groups of 60 and 80 mg/kg Fe-Gly were increased ($P < 0.01$), compared with the 0 mg/kg Fe group, whereas other groups (control, 0, 20, 40 mg/kg Fe-Gly) have no significant differences. Compared with the 0 mg/kg Fe group, the iron concentrations of yolk in all other groups were increased remarkably ($P < 0.01$). Albumen iron concentrations in the groups 60 and 80 mg/kg Fe-Gly were significantly higher ($P < 0.01$) than the control group.

Effects of Fe-Gly Supplementation on the TF, DMT1, and FPN1 mRNA Expression in the Duodenal of Laying Hens

The expressions of TF, DMT1, and FPN1 mRNA in the duodenum quantified by real-time PCR are shown in Figure 3. The DMT1 mRNA expression of duodenum was decreased ($P < 0.01$) in the groups of 60 and 80 mg/kg Fe-Gly and the expression of FPN1 mRNA in the duodenum has also been decreased ($P < 0.01$) in the group of 80 mg/kg Fe-Gly, compared with the control group. In addition, the expression of duodenum TF mRNA has also been decreased in groups of 60 and 80 mg/kg Fe-Gly, compared with the group of 0 mg/kg Fe, whereas the control group showed no significant differences compared with the group of 0 mg/kg Fe. Moreover, the group of 40 mg/kg Fe-Gly has a similar performance with the control group in expressions of TF, DMT1, and FPN1 mRNA.

DISCUSSION

The hen's egg forms a staple part of the poultry industry. Egg quality is of great interest to egg producers and poultry breeders. Thus, there has been considerable research effort focusing on ways of enhancing the egg
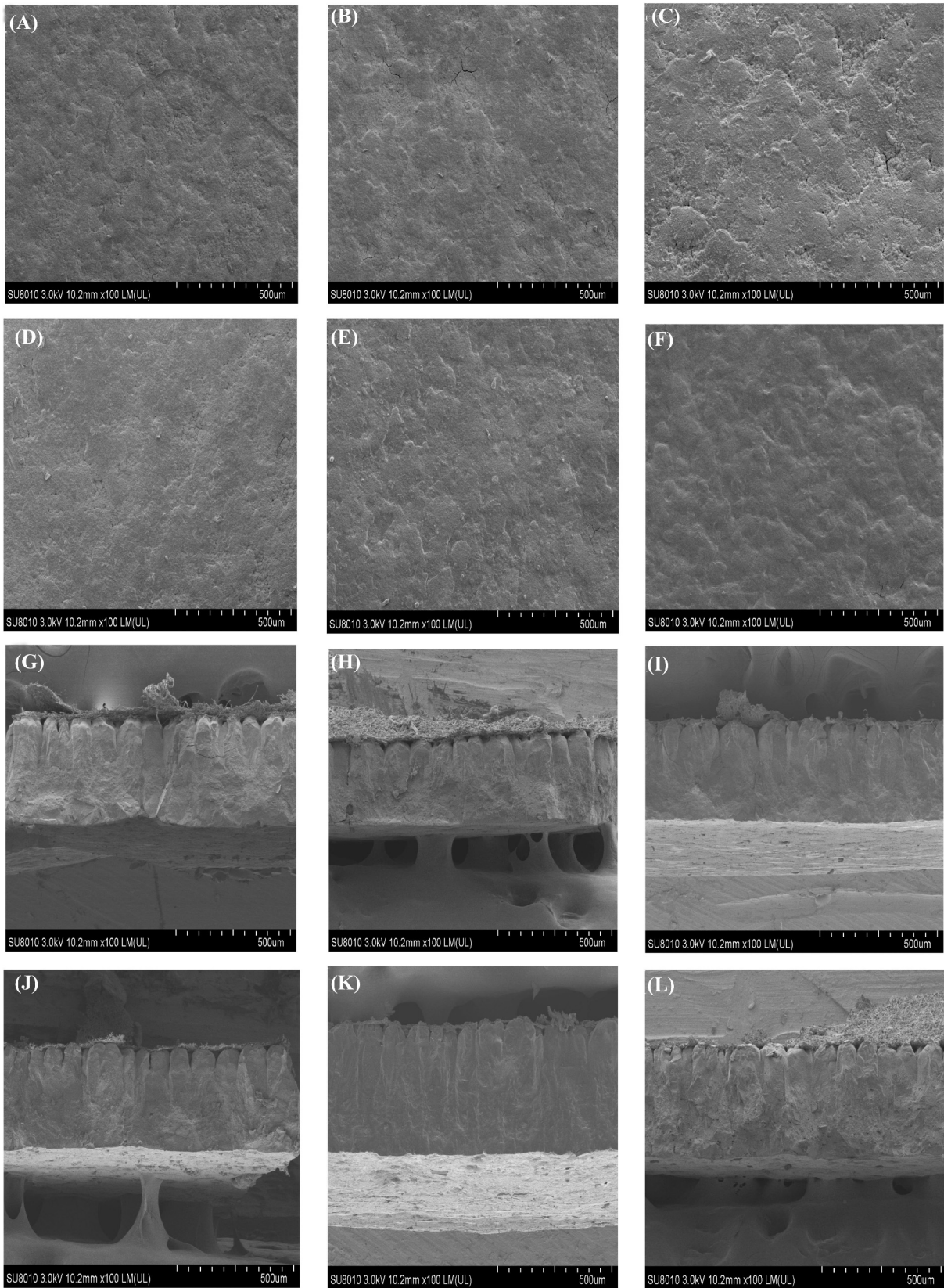
Figure 1. Scanning electron microscope photographs of transverse surface and external surfaces of eggshells. The transverse ultrastructure of group of control (A), 0 mg/kg Fe (B), 20 mg/kg Fe-Gly (C), 40 mg/kg Fe-Gly (D), 60 mg/kg Fe-Gly (E), 80 mg/kg Fe-Gly (F), The transverse ultrastructure of group of control (G), 0 mg/kg Fe (H), 20 mg/kg Fe-Gly (I), 40 mg/kg Fe-Gly (J), 60 mg/kg Fe-Gly (K), 80 mg/kg Fe-Gly (L). Scale bars: 500 μm. A sample of 6 eggs per treatment was used to observe eggshell ultrastructure.
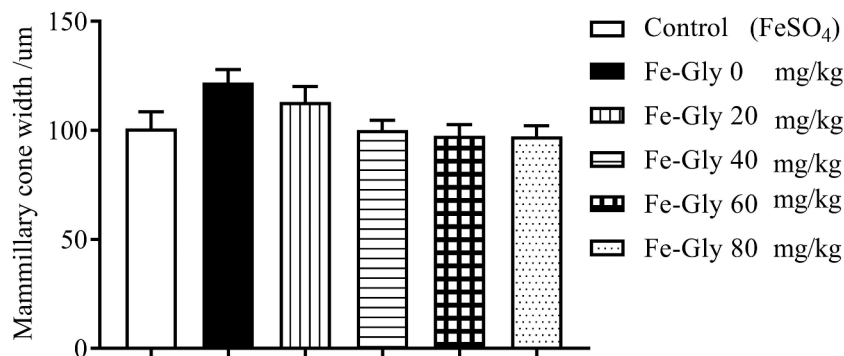
Figure 2. Effects of Fe-Gly supplementation in mammillary cone width in the eggshell of laying hens. Data are means $\pm$ SEM (n = 6).

Table 4. Effect of Fe-Gly supplementation on SDH activity in serum, liver, kidney, and spleen.[1]

	Control[2] (mg/kg)	Fe-Gly (mg/kg)						
Items	60	0	20	40	60	80	SEM	*P*-value
Serum, mg/kg	6.00[a,b]	4.83[b]	5.50[a,b]	6.17[a,b]	6.33[a,b]	7.00[a]	0.62	0.03
Liver, mg/kg	2.10[a,b]	1.69[b]	1.88[a,b]	2.22[a]	2.36[a]	2.19[a]	0.16	0.03
Kidney, mg/kg	2.11	1.98	2.00	2.18	2.49	2.42	0.25	0.21
Spleen, mg/kg	2.18[a,b]	1.70[b]	2.16[a,b]	2.63[a]	2.60[a]	2.84[a]	0.25	0.01

[1]Results are the mean and SEM of 6 replicates. Means within a row with no common superscripts (a, b) differ. significantly ($P < 0.05$). SEM = pooled standard error of the mean.
[2]Control birds were fed the diet (60 mg Fe/kg) supplemented with $FeSO_4$.

Table 5. Effect of Fe-Gly supplementation on Fe concentration in eggshell, albumen, and yolk.[1]

	Control[2] (mg/kg)	Fe-Gly (mg/kg)						
Items	60	0	20	40	60	80	SEM	*P*-value
Eggshell, mg/kg	2.28[a,b]	1.84[c]	2.03[b,c]	2.26[a,b]	2.30[a]	2.45[a]	0.09	<0.01
Yolk, mg/kg	55.97[c]	36.40[e]	45.59[d]	59.81[b,c]	63.81[a,b]	65.46[a]	1.57	<0.01
Albumen, mg/kg	7.57[b]	6.31[d]	6.72[c,d]	7.03[c]	8.16[a]	8.30[a]	1.45	<0.01

[1]Results are the mean and SEM of 5 replicates, with 4 eggs per replicate. Means within a row with no common superscripts (a, b, c, d) differ significantly ($P < 0.05$). SEM = pooled standard error of the mean.
[2]Control birds were fed the diet (60 mg Fe/kg) supplemented with $FeSO_4$.

quality. However, as manufacturers aspire to high production, egg quality has been decreased, affecting the further development of laying hen industry (Jones and Musgrove, 2005). In the recent years, organic microelements, especially, mineral-amino acid chelate, and complex have been focused on their roles in production performance and egg quality of laying hens (Favero et al., 2013; Li et al., 2017). Researchers have reported that as a kind of Fe supplementation, the amino chelated or proteinated source of iron is more advantageous than $FeSO_4$ (Kegley et al., 2002; Creech et al., 2004). This may be correlated to better absorption of Fe-Gly implicating that the bioavailability of Fe from Fe-Gly is higher than that of Fe from FeSO4. It has been suggested that the higher bioavailability of Fe-Gly is probably because of its chemical structure that partially prevents Fe-phytate interactions (Layrisse et al., 2000).

Moreover, iron is one of the components of materials found in bodies and takes part in many reactions,

substance metabolism, and energy transformation. Many studies have shown that chelated sources of iron have higher relative availability compared to the inorganic iron (Bovell-Benjamin et al., 2000; Feng et al., 2009). So, the use of Fe-Gly was more useful for hens, because of that the iron in the form of Fe-Gly was easily absorbed than iron in the form of $FeSO_4$ (Ma et al., 2013), and then the transfer of iron was promoted (Sun et al., 2015), and the activity of SDH enhanced (Ma et al., 2016), which would promote substance metabolism. The groups of 60 and 80 mg/kg Fe-Gly might enhance protein synthesis in egg and then improve the Haugh unit and egg weight.

Tu et al. (2004) reported that $FeSO_4$ and Fe-Gly as Fe supplements showed a positive effect on eggshell strength and decreased percent shell thickness. However, in our study, eggshell strength and thickness also showed a positive trend in Fe-Gly groups, but the differences between groups of Fe-Gly and $FeSO_4$ were not
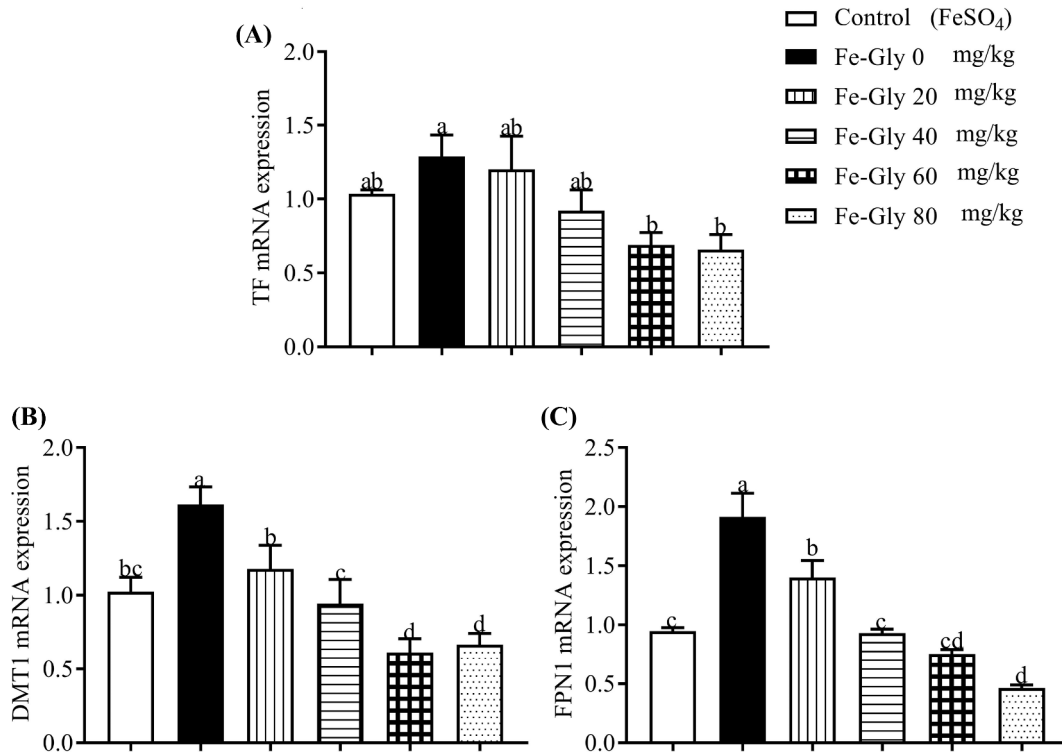
Figure 3. Effects of Fe-Gly supplementation in the duodenal TF, DMT1, and FPN1 mRNA expression (A–C) of laying hens. Values are the fold-change relative to that control group and expressed as mean $\pm$ SEM (n = 6).

significant, so the scanning electronic microscopy photographs of eggshell have been done to see ultrastructure of eggshell. Mammillary knobs layer following the outer shell membrane's outer surface is the first calcified layer in eggshell (Carnarius et al., 1996). Eggshell with a smaller mammillary cone width has been connected with higher shell quality (Bain, 1992; Ahmed et al., 2005; Li et al., 2017). In the present study, there are no significant differences in the mammillary cone width and the outer surface among all groups.

The previous studies have reported that iron is a vital element required for SDH (Ma et al., 2016; Zhang et al., 2016), which is a key enzyme of TCA (tricarboxylic acid cycle, the hinge of substance and energy metabolism). The improvement of SDH activity is beneficial for accelerating TCA and promoting substance metabolism. Zhang et al. (2016) found that as a Fe supplement, organic iron could enhance the levels of SDH in broilers. Feng et al. (2009) indicated that Fe-Gly had a better effect than $FeSO_4$ on improving levels of SDH. In the present study, SDH levels of liver and spleen in groups of 40, 60, and 80 mg/kg Fe-Gly were increased significantly, compared with the 0 mg/kg Fe-Gly group, whereas the control group had no marked differences compared with the 0 mg/kg Fe group. Serum SDH level has been increased remarkably in the group of 80 mg/kg Fe-Gly, compared with the group of 0 mg/kg Fe-Gly. This observation is essential for Fe-Gly use and supports the idea that it is required for the SDH activity. The result, which is supported by reports of Feng et al. (2009) and Zhang et al. (2016), means that Fe-Gly

can improve SDH activity and to accelerate substance metabolism, compared with $FeSO_4$.

It has been demonstrated that metal chelated with amino acid or protein has a better bioavailability to the animals (Kegley et al., 2002; Creech et al., 2004). Shinde et al. (2011) reported that iron in organic form (80 mg ferrous methionine chelate/kg diet) showed a higher relative availability than ferrous sulfate (80 mg FeSO4/kg diet) and was more efficient in depositing Fe in the hematopoietic organs of broiler. The accumulation of trace elements in eggs relied on their chemical forms and dosage (Stadelman and Pratt, 1989). In the present study, Fe concentrations in eggshell, yolk, and albumen were increased with the dosages of iron, which was in accordance with Cao et al. (1999). The iron concentrations of eggshell in groups of 60 and 80 mg/kg Fe-Gly were increased significantly, compared with 0 mg/kg Fe group, whereas the control group has no significant differences compared with the 0 mg/kg Fe group. Furthermore, the iron concentrations of yolk and albumen in groups of 60 and 80 mg/kg Fe-Gly were improved remarkably, compared with the control. It might because of that after chelating with amino acid, iron in the form of Fe-Gly has a better permeability to the membrane so that the iron absorption was increased, transfer of iron was enhanced, and the accumulation of iron was improved. Moreover, the group of 40 mg/kg Fe-Gly showed no significant differences with the control in iron concentrations of yolk and eggshell.

Transferrin, DMT1, and FPN1 play essential roles in iron transport (Bai et al., 2014). TF and DMT1 are

responsible for iron import (Andrews, 1999; Gammella et al., 2017). FPN1 plays a vital role in releasing cellular iron to blood circulation to provide other tissues for iron (Donovan and Andrews, 2004). Research indicated that higher iron concentration could decrease the expression of TF, and lower iron concentration induces body to increase TF expression level (Bai et al., 2014). Similarly, Canonne-Hergaux et al. (2000) and Sun et al. (2015) reported that the increased iron concentration could reduce the expression level of DMT1. The expression of FPN1 in duodenum negatively correlated with the storage iron in the organism (Knutson et al., 2003; Nemeth et al., 2004). In the present study, the expression of DMT1 and FPN1 mRNA in the duodenum was decreased significantly in groups of 60 and 80 mg/kg Fe-Gly, compared with the control group. In addition, the expression of duodenum TF mRNA has also been increased significantly in groups of 60 and 80 mg/kg Fe-Gly, compared with the group of 0 mg/kg Fe, whereas the control group showed no significant differences compared with the group of 0 mg/kg Fe. The results in accordance with previous researches indicated that Fe-Gly had better biological value than $FeSO_4$ for laying hens, and could promote iron transport, which might partly explain for the higher Fe concentration in the egg. Moreover, the group of 40 mg/kg Fe-Gly showed no significant differences compared with the control group in the expression of TF, DMT1, and FPN1 mRNA.

In summary, the present study indicated that dietary Fe-Gly supplementation could improve the internal egg quality (egg weight, albumen height, and Haugh unit), tricarboxylic acid cycle, iron transport, and iron accumulation of egg, compared with dietary $FeSO_4$ supplementation. The group fed Fe-Gly at the level of 60 mg/kg showed a better result than the control group ($FeSO_4$) in improving egg quality and egg iron enrichment. It revealed that $FeSO_4$ could be substituted by the lower concentration of Fe-Gly which may have better effects on egg quality and iron enrichment.

ACKNOWLEDGMENTS

REFERENCES

Abbasi, M., M. Zaghari, and M. G. S. Khalaji. 2015. Is dietary iron requirement of broiler breeder hens at the late stage of production cycle influenced by phytase supplementation? J. Appl. Anim. Res. 43:166–176.

Abbaspour, N., R. Hurrell, and R. Kelishadi. 2014. Review on iron and its importance for human health. J. Res. Med. Sci. 19:164–174.

Ahmad, H. A., and R. J. Balander. 2003. Alternative feeding regimen of calcium source and phosphorus level for better eggshell quality in commercial layers. J. Appl. Poult. Res. 12:509–514.

Ahmed, A. M. H., A. B. Rodriguez-Navarro, M. L. Vidal, J. Gautron, J. M. García-Ruiz, and Y. Nys. 2005. Changes in eggshell mechanical properties, crystallographic texture and in matrix proteins induced by moult in hens. Br. Poult. Sci. 46:268–279.

Andrews, N. C. 1999. The iron transporter DMT1. Int. J. Biochem. Cell Biol. 31:991.

Arazi, H., I. Yoselewitz, Y. Malka, Y. Kelner, O. Genin, and M. Pines. 2009. Osteopontin and calbindin gene expression in the eggshell gland as related to eggshell abnormalities. Poult. Sci. 88:647–653.

Bai, S., L. Huang, Y. Luo, L. Wang, X. Ding, J. Wang, Q. Zeng, and K. Zhang. 2014. Dietary manganese supplementation influences the expression of transporters involved in iron metabolism in chickens. Biol. Trace Elem. Res. 160:352–360.

Bain, M. M. 1992. Eggshell strength: a relationship between the mechanism of failure and the ultrastructural organisation of the mammillary layer. Br. Poult. Sci. 33:303–319.

Bess, F., S. L. Vieira, A. Favero, R. A. Cruz, and P. C. Nascimento. 2012. Dietary iron effects on broiler breeder performance and egg iron contents. Anim. Feed Sci. Technol. 178:67–73.

Bovell-Benjamin, A. C., F. E. Viteri, and L. H. Allen, 2000. Iron absorption from ferrous bisglycinate and ferric trisglycinate in wholemaize is regulated by iron status. Am. J. Clin. Nutr. 71:1563–1569.

Caner, C. 2010. Effect of edible eggshell coatings on egg quality and consumer perception. J. Sci. Food Agric. 85:1897–1902.

Canonne-Hergaux, F., M. D. Fleming, J. E. Levy, S. Gauthier, T. Ralph, V. Picard, N. C. Andrews, and P. Gros. 2000. The Nramp2/DMT1 iron transporter is induced in the duodenum of microcytic anemia *mk*mice but is not properly targeted to the intestinal brush border. Blood. 96:3964–3970.

Cao, S., L. Yang, M. Cheng, L. Chen, and K. Chen. 1999. Effects of feeding high-iron and high-iodine diet to hens on the iron and iodine content in egg and egg quality. J. Netw. Comput. Appl. 17:248–254

Carnarius, K. M., K. M. Conrad, M. G. Mast, and J. H. Macneil. 1996. Relationship of eggshell ultrastructure and shell strength to the soundness of shell eggs. Poult. Sci. 75:655–663.

Cen, H., and Y. He. 2007. Theory and application of near infrared reflectance spectroscopy in determination of food quality. Trends Food Sci. Technol. 18:72–83.

Cherian, G. 2008. Egg quality and yolk polyunsaturated fatty acid status in relation to broiler breeder hen age and dietary n-3 oils. Poult. Sci. 87:1131–1137.

Creech, B. L., J. W. Spears, W. L. Flowers, G. M. Hill, K. E. Lloyd, T. A. Armstrong, and T. E. Engle. 2004. Effect of dietary trace mineral concentration and source (inorganic vs. chelated) on performance, mineral status, and fecal mineral excretion in pigs from weaning through finishing. J. Anim. Sci. 82:2140–2147.

Donovan, A., and N. C. Andrews, 2004. The molecular regulation of iron metabolism. Hematol. J. 5:373–380.

Drygalski, A. V., and J. W. Adamson. 2013. Iron metabolism in man. J. Parent. Enteral Nutr. 37:599–606. doi:10.1177/0148607112459648

Dunn, I. C., A. B. Rodríguez-Navarro, K. Mcdade, M. Schmutz, R. Preisinger, D. Waddington, P. W. Wilson, and M. M. Bain. 2012. Genetic variation in eggshell crystal size and orientation is large and these traits are correlated with shell thickness and are associated with eggshell matrix protein markers. Anim. Genet. 43:410–418.

Favero, A., S. L. Vieira, C. R. Angel, A. Bosmikich, N. Lothhammer, D. Taschetto, R. F. A. Cruz, and T. L. Ward. 2013. Development of bone in chick embryos from cobb 500 breeder hens fed diets supplemented with zinc, manganese, and copper from inorganic and amino acid-complexed sources. Poult. Sci. 92:402–411.

Feng, J., W. Q. Ma, Z. R. Xu, J. X. He, Y. Z. Wang, and J. X. Liu. 2009. The effect of iron glycine chelate on tissue mineral levels, fecal mineral concentration, and liver antioxidant enzyme activity in weanling pigs. Anim. Feed Sci. Technol. 150:106–113.

Gammella, E., P. Buratti, G. Cairo, and S. Recalcati. 2017. The transferrin receptor: the cellular iron gate. Metallomics. 9:1367–1375.

House, W. A., D. R. V. Campen, and R. M. Welch. 1997. Dietary methionine status and its relation to the bioavailability to rats of

zinc in corn kernels with varying methionine content. Nutr. Res. 17:65–76.

Huang, Y. L., L. Lu, X. G. Luo, and B. Liu. 2007. An optimal dietary zinc level of broiler chicks fed a corn-soybean meal diet. Poult. Sci. 86:2582–2589.

Jones, D. R., and M. T. Musgrove. 2005. Effects of extended storage on egg quality factors. Poult. Sci. 84:1774–1777.

Kegley, E. B., J. W. Spears, W. L. Flowers, and W. D. Schoenherr. 2002. Iron methionine as a source of iron for the neonatal pig. Nutr. Res. 22:1209–1217.

Knutson, M. D., M. R. Vafa, D. J. Haile, and M. Wesslingresnick. 2003. Iron loading and erythrophagocytosis increase ferroportin 1 (FPN1) expression in J774 macrophages. Blood. 102:4191–4197.

Layrisse, M., M. N. Garcíacasal, L. Solano, M. A. Barón, F. Arguello, D. Llovera, J. Ramírez, I. Leets, and E. Tropper. 2000. Iron bioavailability in humans from breakfasts enriched with iron bis-glycine chelate, phytates and polyphenols. J. Nutr. 130:2195–2199.

Li, S., X. Luo, B. Liu, T. D. Crenshaw, X. Kuang, G. Shao, and S. Yu. 2004. Use of chemical characteristics to predict the relative bioavailability of supplemental organic manganese sources for broilers. J. Anim. Sci. 82:2352–2363.

Li, L. L., N. N. Zhang, Y. J. Gong, M. Y. Zhou, H. Q. Zhan, and X. T. Zou. 2017. Effects of dietary Mn-methionine supplementation on the egg quality of laying hens. Poult. Sci. 97:247–254.

Livak, K. J., and T. D. Schmittgen. 2001. Analysis of relative gene expression data using real-time quantitative PCR and the $2-\Delta\Delta$CT CT method. Methods. 25:402–408.

Ma, W. Q., W. Jing, Z. Zhao, S. Hong, Y. Min, and F. Jie. 2013. Comparison of absorption characteristics of iron glycine chelate and ferrous sulfate in Caco-2 cells. Int. J. Agric. Biol. 15:372–376.

Ma, X., X. Liao, L. Lu, S. Li, L. Zhang, and X. Luo. 2016. Determination of dietary iron requirements by full expression of iron-containing enzymes in various tissues of broilers. J. Nutr. 146:2267–2273.

Ma, X. Y., S. B. Liu, L. Lu, S. F. Li, J. J. Xie, L. Y. Zhang, J. H. Zhang, and X. G. Luo. 2014. Relative bioavailability of iron proteinate for broilers fed a casein-dextrose diet. Poult. Sci. 93:556–563.

Ma, Y., Y. Shi, L. Li, C. Xie, and X. Zou. 2018. Toxicological effects of mercury chloride on laying performance, egg quality, serum biochemistry, and histopathology of liver and kidney in laying hens. Biol. Trace Elem. Res. 185:465–474.

Mabe, I., C. Rapp, M. Bain, and Y. Nys. 2003. Supplementation of a corn-soybean meal diet with manganese, copper, and zinc from organic or inorganic sources improves eggshell quality in aged laying hens. Poult. Sci. 82:1903–1913.

Miao, L., L. Li, M. Qi, M. Zhou, N. Zhang, and X. Zou. 2017. Effects of excess dietary fluoride on serum biochemical indices, egg quality, and concentrations of fluoride in soft organs, eggs, and serum of laying hens. Biol. Trace Elem. Res. 4:1–7.

Mohammadi, V., S. Ghazanfari, A. Mohammadi-Sangcheshmeh, and M. H. Nazaran. 2015. Comparative effects of zinc-nano complexes, zinc-sulphate and zinc-methionine on performance in broiler chickens. Br. Poult. Sci. 56:486–493.

Nemeth, E., M. Tuttle, J. Powelson, M. Vaughn, A. Donovan, D. Ward, and J. Kaplan. 2004. Hepcidin regulates cellular iron efflux by binding to ferroportin and inducing its internalization. Science. 306:2090–2093.

Sambrook, J., and D. Russell. 2001. Molecular Cloning: A Laboratory Manual, 3rd ed. Cold Spring Harbor Laboratory Press, NY, USA.

Shinde, P. L., S. L. Ingale, J. Y. Choi, J. S. Kim, S. I. Pak, and B. J. Chae. 2011. Efficiency of inorganic and organic iron sources under iron depleted conditions in broilers. Br. Poult. Sci. 52:578–583.

Stadelman, W. 1999. The incredibly functional egg. Poult. Sci. 78:807–811.

Stadelman, W. J., and O. J. Cotterill. 1990. Egg Science and Technology. 3rd ed. Food Products Press, Binghamton, NY.

Stadelman, W. J., and D. E. Pratt. 1989. Factors influencing composition of the hen's egg. Worlds Poult. Sci. J. 45:247–266.

Sun, Q., Y. Guo, J. Li, T. Zhang, and J. Wen. 2012. Effects of methionine hydroxy analog chelated Cu/Mn/Zn on laying performance, egg quality, enzyme activity and mineral retention of laying hens. J. Poult. Sci. 49:20–25

Sun, J., D. Liu, and R. Shi. 2015. Supplemental dietary iron glycine modifies growth, immune function, and antioxidant enzyme activities in broiler chickens. Livest. Sci. 176:129–134.

Tu, Y. J., X. T. Zou, and S. Q. Tang. 2004. Effect of different iron from sources on laying performance and egg quality in Roman hens. Agric. Life Sci. 5:018.

Xie, C., H. A. M. Elwan, S. S. Elnesr, X. Dong, J. Feng, and X. T. Zou. 2019. Effects of iron glycine chelate on laying performance, antioxidant activities, serum biochemical indices, iron concentrations and transferrin mRNA expression in laying hens. J. Anim. Physiol. Anim. Nutr. 103:547–554.

Yi, G. F., C. A. Atwell, J. A. Hume, J. J. Dibner, C. D. Knight, and J. D. Richards. 2007. Determining the methionine activity of mintrex organic trace minerals in broiler chicks by using radiolabel tracing or growth assay. Poult. Sci. 86:877–887.

Zhang, L. Y., L. Lu, L. Y. Zhang, and X. G. Luo. 2016. The chemical characteristics of organic iron sources and their relative bioavailabilities for broilers fed a conventional corn–soybean meal diet. J. Anim. Sci. 94:2378–2396.