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# Effects of Dietary Fe-soy Proteinate and MgO on Egg Production and Quality of Eggshell in Laying Hens

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**ABSTRACT :** This study investigated the effects of Fe-soy proteinate (Fe-SP) and magnesium oxide (MgO) dietary supplements on eggshell quality in laying hens. A total of 800 26-wk-old Hy-Line Brown hens were assigned to four dietary treatments: control (C), Fe-SP 100 (100 ppm Fe-soy proteinate), MgO (3 g MgO/kg diet), and Fe-SP 100+MgO. Each treatment had five replicates of 40 hens. The FT-IR (Fourier transform infrared) and XRD (X-ray diffraction) spectra of Fe-soy proteinate were different from those of $FeSO_4$ and soy digest. There were no significant differences among treatments in hen-day egg production, hen-house egg production, broken and soft egg rate, feed intake, or feed conversion. The MgO and Fe-SP 100+MgO treatments showed significantly ($p<0.05$) higher egg weights than the control group. Eggshell strength and thickness were significantly ($p<0.05$) higher in the MgO supplemented groups. The lightness and yellowness of the eggshells decreased, and the redness increased significantly in the Fe-SP treated groups. There were no significant differences among treatments in leukocyte level, but hemoglobin (Hb) concentrations were higher with Fe-SP treatments. In conclusion, supplementation with Fe-SP significantly affects eggshell color and hemoglobin concentration, whereas MgO supplementation increases eggshell strength and thickness. Egg weight and egg shell quality can be improved by supplementation of 100 ppm Fe in the form of Fe-SP and 3 g MgO/kg diet. (**Key Words :** MgO, Fe-soy Proteinate, Eggshell Quality, Layer, Hemoglobin)

## INTRODUCTION

Iron (Fe) is an essential biological element for livestock as well as human beings. Iron content shows minimum variability with dietary change (Naber, 1979). Absorbability of minerals in monogastrics can increase when mineral supplements are provided in the form of chelates (Kratzer and Vohra, 1986; Paik, 2001). Organic materials, in particular, amino acids and low molecule peptides (Miller et al., 1972; McNaughton et al., 1974; Zoubek et al., 1975; Spears, 1992) in the state of chelation with metal ions, are more effectively absorbed into the body (Fouad, 1976; Ashmead, 1993). It was demonstrated that the Fe content of eggs and egg shell color can increase due to supplementation with organic Fe supplements (Park et al., 2004; Paik et al., 2009).

Magnesium (Mg) is involved in many biochemical processes, including activation of phosphates and participation in carbohydrate metabolism, and its function is closely associated with calcium and phosphorus (McDonald et al., 1971). Magnesium supplementation at high levels has been shown to improve egg weight (Adams, 1976; Atteh et al., 1982). Atteh et al. (1982) reported that Mg supplementation improves egg shell strength when Ca levels are sufficient.

Since earlier studies have demonstrated that eggshell color and strength were improved by organic Fe and Mg supplementation, an experiment was conducted to confirm the effects of supplementary Fe-soy proteinate (Fe-SP), MgO, and a combination of these on eggshell quality in laying hens.

## MATERIALS AND METHODS

### Animals and diets

A total of 800 26-wk-old Hy-Line Brown laying hens were assigned to one of four dietary treatments. Each treatment consisted of five replications of 20 cages (two hens/cage). Diets were formulated in mash form, and feed and water were given *ad libitum* during the experimental

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period. Birds were provided with programmed lighting and ventilation, in which the lighting program started with 14:30 h of light on the initiation of the experiment and then increased 30 min every week until 16:00 h of light was achieved. The feeding trial period was 5 wks, and the average indoor temperature during the test period was 21°C. The composition of the control basal diet is shown in Table 1. The dietary treatments were control, Fe-SP 100 (100 ppm Fe-soy proteinate), MgO (3 g MgO/kg diet), and Fe-SP 100 +MgO.

**Preparation of Fe-soy proteinate**

Soy digest was produced by hydrolysis of 100 g soybean meal (44% CP) in 500 ml distilled water with 2 ml 28% hydrogen peroxide (DC Chemical Co. Ltd., Korea) for 2 h. Two milliliters Alcalase 2.4 L (NovoNordisk, Denmark) was then added for further hydrolysis at pH 8, 60°C for 2 h. An iron solution was prepared by dissolving 100 g $FeSO_4 \cdot 7H_2O$ in 200 ml distilled water. Fe-soy proteinate was produced by mixing soy digest and Fe solution with 50 ml 50% NaOH pH buffer. The precipitate was separated, dried in an oven at 30°C for two days and then crushed into powder. The prepared Fe-SP was verified to contain approximately 20% Fe by analysis with an ICP spectrometer (Optima 5300DV, PerkinElmer, USA).

**Table 1.** Composition and nutrient content of the basal diet

| Item | Amount |
|---|---|
| Ingredients (%) | |
| Corn, ground | 41.45 |
| Wheat, ground | 15.00 |
| Soybean meal, 44% CP | 25.00 |
| DDGS[1] | 5.00 |
| Canola meal | 2.00 |
| Animal fat | 0.50 |
| Molasses | 0.50 |
| Granular ark shell | 1.00 |
| Dicalcium phosphate | 0.70 |
| Limestone | 8.70 |
| Premix[2] | 0.15 |
| Nutrient | |
| ME[3] (kcal/kg) | 2,750 |
| CP (%) | 16.5 |
| Fat (%) | 2.90 |
| Ash (%) | 13.40 |
| Ca (%) | 3.90 |
| Available P (%) | 0.33 |
| Lys (%) | 0.90 |
| Met (%) | 0.45 |
| Mg (%) | 0.17 |
| Fe (g/kg) | 0.15 |

[1] DDGS: Distiller's dried grains with soluble.
[2] Premix contains followings per kg: vitamin A, 7,000,000 IU; vitamin $D_3$, 1,500,000 IU; vitamin E, 10,000 IU; vitamin $K_3$, 1,000 mg, vitamin $B_1$, 1,200 mg; vitamin $B_2$, 3,000 mg; vitamin $B_6$, 6,000 mg; vitamin $B_{12}$, 18 mg; folic acid, 400 mg; biotin, 40 mg; Mg, 150 mg; Zn, 60,000 mg; Mn, 90,000 mg; Fe, 40,000 mg; Cu, 8,000 mg; Co, 100 mg; I, 1,000 mg; Se, 250 mg.
[3] Calculated value according to NRC (1994).

**Measurement of FT-IR and XRD spectra**

In order to determine the iron complex formation, FT-IR (Fourier transform infrared) and XRD (X-ray diffraction) spectra of soy digest, $FeSO_4$, and Fe-soy proteinate were collected. The FT-IR spectra were obtained on a Shimadzu spectrometer (FT-IR 8400S, Shimadzu Co. Ltd., Kyoto, Japan) with a resolution of 4 $cm^{-1}$, and XRD scattering spectra were measured by an X-ray diffractometer (Dmax 2000, Rigaku Co., Tokyo, Japan). For the XRD measurements, the wavelength of the Mo X-ray was 1.5418 Å with a scan range of $5° < 2\theta < 70°$ at 30 mA and 35 kV (Han et al., 2006).

**Chemical analysis**

The chemical composition of basal diet was analyzed using AOAC (1990) methods. Metabolizable energy (ME) was calculated based on ME values of ingredients (NRC, 1994).

**Egg production and quality**

To assess egg productivity, hen-day and hen-house egg productions, mean egg weight, and soft and broken egg productions were recorded daily and used to calculate a weekly average. Feed intake was measured weekly, and feed conversion (feed intake/100 g egg mass) was calculated. One hundred egg samples (20 eggs per replicate) from each treatment were randomly collected every week to measure egg quality such as Haugh index, egg shell strength, egg shell thickness, egg yolk and shell color. Five week averages were calculated for each of the measurements. Haugh units were calculated using the HU formula (Eisen et al., 1962) based on the height of the albumen as determined by a micrometer (Model S-8400, AMES, Waltham, MA, USA). Eggshell strength was measured by Compression Test Cell of Texture Systems (Model T2100C, Food Technology Corp., Rockville, MD, USA). Eggshell thickness was measured by a Dial Pipe Gauge (Model 7360, Mitutoyo Corp., Kawasaki, Japen), the Hunter Lab colors of the eggshells were measured using a Chroma Meter (Model CR-400, Minolta Corp., Japen), and egg yolk color was measured with a color fan (Roche Color Fan, Roche, Switzerland) on a color scale of 1 (light) to 15 (dark orange).

**Analysis of blood**

At the end of the experiment, blood samples were collected from the wing veins (ten birds per treatment) into

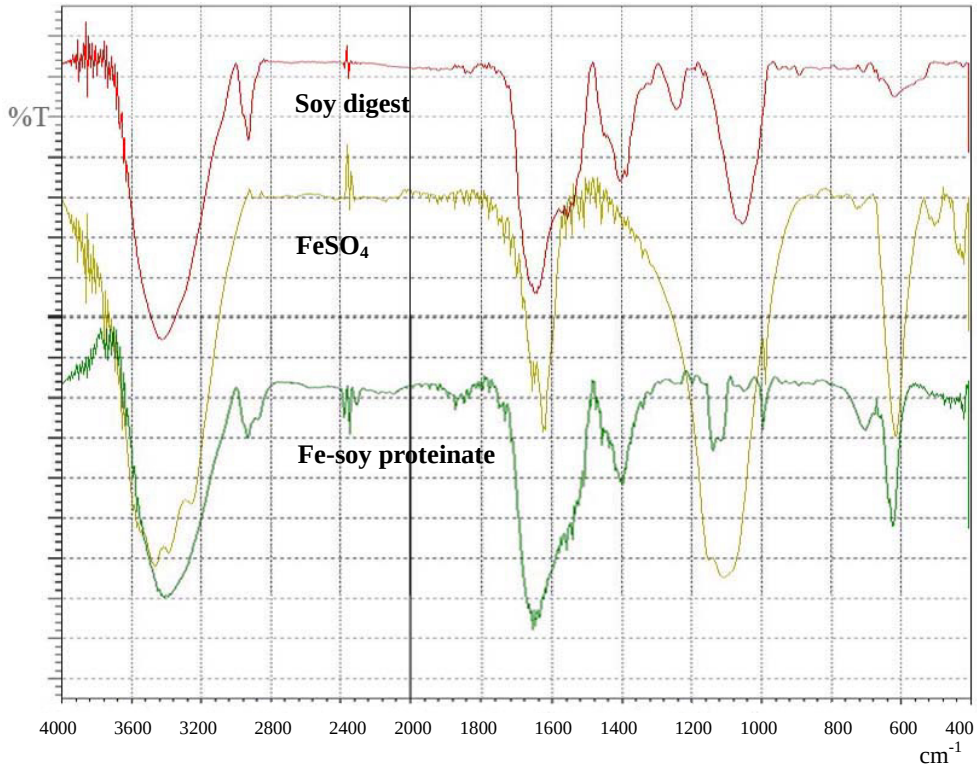


**Figure 1.** FT-IR spectra of soy digest, $FeSO_4$, and Fe-soy proteinate.

EDTA-treated vaccutainers (each 5 ml). Samples were analyzed using HEMACYTE (Oxford Science, Inc).

**Statistical analysis**

Data were subjected to analysis of variance using the GLM (SAS Institute, 2000). Significant differences among the treatment were measured using Duncan's multiple range test at $p<0.05$ (Steel and Torrie, 1980).

## RESULTS AND DISCUSSION

The infrared (IR) spectra of soy digest, $FeSO_4$, and Fe-soy proteinate are shown in Figure 1. The spectrum of Fe-soy proteinate was also reported by Chi and Han (2008). The soy digest was expected to have free carboxyl ($COO^-$) and amino groups in the middle and terminal positions of the peptide chain. The IR spectrum of soy digest exhibited peaks at around 1,650 $cm^{-1}$ and 1,400 $cm^{-1}$, corresponding to the amide I and amide II absorption bands of the peptide, respectively. The IR spectrum of $FeSO_4$ showed strong and broad S=O stretching peaks between 1,200 $cm^{-1}$ and 1,000 $cm^{-1}$, typical of sulfate. The peak near 610 $cm^{-1}$ corresponds to the Fe-O stretching frequency. In the IR spectra of Fe-soy proteinates, the C=O stretching frequency significantly shifted to a higher energy, indicating the coordination of the C=O group to the ferrous ion.

The X-ray diffractometer (XRD) scattering spectra of soy digest, $FeSO_4$, and Fe-soy proteinate are shown in Figure 2. The XRD spectra of soy digest and Fe-soy proteinate did not show diffraction, while that of $FeSO_4$ showed a strong diffraction pattern indicative of high crystallinity. These results seem to indicate that Fe-soy proteinate exists as amorphous Fe complexes and does not contain any simple Fe salts, which tend to form a microcrystalline complex.

Table 2 shows the effects of Fe-SP 100 and MgO supplementation on the productivity and egg quality of laying hens. There were no significant differences among treatments in hen-day or hen-house egg productions, broken or soft egg productions, feed intake, or feed conversion over the whole period of the experiment. The MgO and Fe-SP 100+MgO treatments showed significantly higher egg weight than did the control. The five wk feeding trial showed that eggshell strength, thickness, and color were significantly affected by the supplementation treatments. The strengths and thicknesses of the eggshells were significantly different among treatments. The MgO treatments (MgO and Fe-SP 100+MgO) had significantly greater eggshell strengths and thicknesses than did those without MgO supplementation. Colorimetric analysis showed that egg shell lightness and yellowness significantly decreased, while redness increased significantly in Fe-SP supplemented treatments (Fe-SP 100 and Fe-SP 100+MgO). The Haugh index and egg yolk color were not significantly different among treatments. The greater egg weights in the MgO treatments are in good agreement with the results of

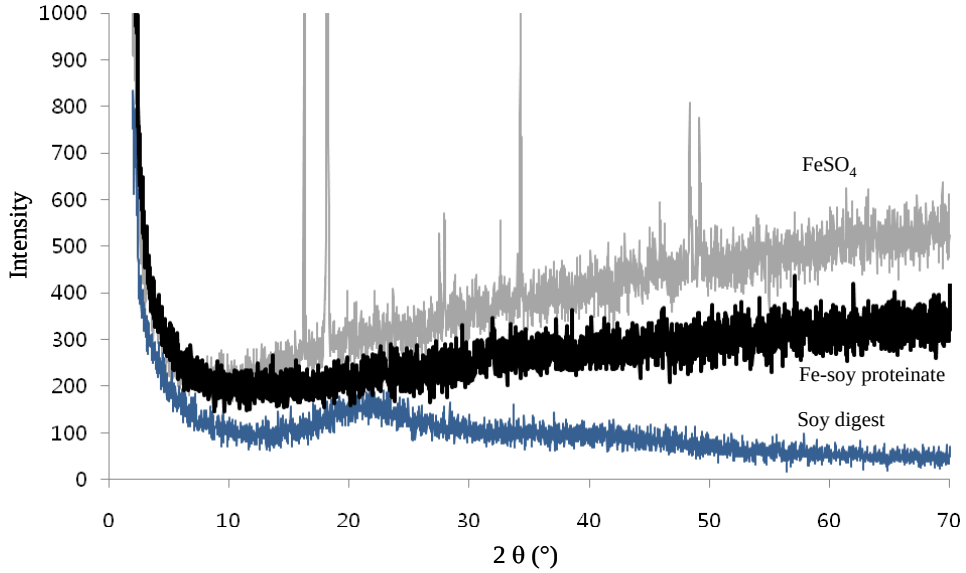


**Figure 2.** XRD spectra of soy digest, $FeSO_4$, and Fe-soy proteinate.

Stafford et al. (1972). Magnesium supplementation also increased eggshell strength and thickness, as reported in other research (Adams, 1976; Atteh et al., 1982). Atteh et al. (1982) reported that Mg supplementation improves egg shell strength when Ca levels are sufficient. Increased eggshell strength is beneficial to the quality of breeding eggs and improves hatching yield (Katalin et al., 2004).

It is well known that minerals have strong interactions. There are no directly applicable references but interactions between supplementary Fe and other minerals, such as Ca, P, Cu, Zn, and Mg, may have influenced eggshell strength. Eggshell color significantly improved due to supplementation with Fe-SP 100. This result is in good agreement with the results of Park et al. (2004) and Paik et al. (2009). Solomon (1997) implicated that Fe, Zn, Cu, and Mn may function as chelating carriers at the central positions of porphyrins. The major pigment of brown eggshells is protoporphyrin (Lang and Wells, 1987).

**Table 2.** Effects of supplementary Fe-soy proteinate (Fe-SP) and MgO on the performance and egg quality of laying hens

| Item | | Treatments[1] | | | | SEM (n = 5) |
|---|---|---|---|---|---|---|
| | | Control | Fe-SP 100 | MgO | Fe-SP 100+MgO | |
| Performance | | | | | | |
| Hen-day egg production (%) | | 94.4 | 94.9 | 94.5 | 94.7 | 0.75 |
| Hen-house egg production (%) | | 94.2 | 94.7 | 94.5 | 94.5 | 0.80 |
| Egg weight (g) | | 59.5$^{b}$ | 60.0$^{ab}$ | 61.8$^{a}$ | 61.8$^{a}$ | 0.69 |
| Broken or soft eggs (%) | | 0.12 | 0.11 | 0.12 | 0.11 | 0.02 |
| ADFI (g/hen) | | 117.0 | 118.0 | 116.1 | 116.9 | 0.83 |
| Feed conversion (feed g/100 g egg mass) | | 1.73 | 1.73 | 1.75 | 1.74 | 0.02 |
| Egg quality | | | | | | |
| Haugh index[2] | | 96.2 | 96.5 | 96.1 | 96.2 | 0.66 |
| Eggshell strength (g) | | 4,342.1$^{b}$ | 4,344.2$^{b}$ | 4,479.4$^{a}$ | 4,469.2$^{a}$ | 39.85 |
| Eggshell thickness (μm) | | 36.3$^{b}$ | 36.7$^{b}$ | 38.2$^{a}$ | 38.1$^{a}$ | 0.28 |
| Egg yolk color | | 8.88 | 8.98 | 8.94 | 8.96 | 0.12 |
| Eggshell color (Hunter Lab)[3] | L* | 52.2$^{a}$ | 49.6$^{b}$ | 51.9$^{a}$ | 50.0$^{b}$ | 0.20 |
| | a* | 14.7$^{c}$ | 15.7$^{a}$ | 14.8$^{c}$ | 15.3$^{b}$ | 0.07 |
| | b* | 19.8$^{a}$ | 19.5$^{b}$ | 19.9$^{a}$ | 19.3$^{b}$ | 0.06 |

[1] Control = Control diet; Fe-SP 100 = 100 ppm Fe as Fe-soy proteinate; MgO = 3 g/kg MgO; Fe-SP 100+MgO = 100 ppm Fe as Fe-soy proteinate+3 g/kg MgO.

[2] Haugh index: HU = 100 log (albumen height (mm)-(1.7×egg weight (g)$^{0.37}$+7.57))

[3] L*: 0 = black, 100 = white, a*: lower numbers = more green (less red), higher numbers = more red (less green); measurement range = (-60) to 60, b*: lower numbers = more blue (less yellow), higher numbers = more yellow (less blue); measurement range = (-60) to 60

[a-c] Values with different superscripts in the same row are significantly different ($p<0.05$).

**Table 3.** The levels of leukocytes and erythrocytes in the blood of laying hens fed experimental diets

| Item | | Treatments[1] | | | | SEM (n = 10) |
|---|---|---|---|---|---|---|
| | | Control | Fe-SP 100 | MgO | Fe-SP 100+MgO | |
| Leukocytes[2] | WBC (K/μl) | 7.93 | 7.91 | 6.47 | 7.75 | 1.11 |
| | HE (K/μl) | 0.79 | 0.76 | 0.61 | 0.71 | 0.171 |
| | LY (K/μl) | 6.41 | 6.41 | 5.24 | 6.78 | 0.87 |
| | SI (HE/LY) | 0.012 | 0.017 | 0.031 | 0.017 | 0.009 |
| | MO (K/μl) | 0.11 | 0.11 | 0.12 | 0.10 | 0.016 |
| | EO (K/μl) | 0.67 | 0.66 | 0.52 | 0.62 | 0.125 |
| | BA (K/μl) | 0.059 | 0.058 | 0.079 | 0.070 | 0.019 |
| Erythrocytes[3] | RBC (M/μl) | 2.39 | 2.52 | 2.28 | 2.52 | 0.079 |
| | Hb (g/dl) | 7.38$^{ab}$ | 7.85$^{ab}$ | 7.10$^{b}$ | 7.96$^{a}$ | 0.27 |
| | HCT (%) | 22.88 | 23.55 | 21.92 | 24.10 | 0.86 |
| | MCV (fL) | 95.67 | 93.34 | 95.58 | 95.67 | 1.16 |
| | MCH (pg) | 31.71 | 31.18 | 31.11 | 30.90 | 0.66 |
| | MCHC (g/dl) | 33.23 | 33.40 | 32.59 | 32.25 | 0.76 |

[1] Control = Control diet; Fe-SP 100 = 100 ppm Fe as Fe-soy proteinate; MgO = 3 g/kg MgO; Fe-SP 100+MgO = 100 ppm Fe as Fe-soy proteinate+3 g/kg MgO.

[2] Leukocytes, WBC = White blood cell; HE = Heterophil; LY = Lymphocytes; MO = Monocyte; EO = Eosinophil; BA = Basophil; SI = Heterophil/lymphocytes.

[3] Erythrocytes, RBC = Red blood cell; Hb = Hemoglobin; HCT = Hematocrit; MCV = Mean corpuscular volume; MCH = Mean corpuscular hemoglobin; MCHC = Mean corpuscular hemoglobin concentration.

[a, b] Values with different superscripts in the same row are significantly different ($p<0.05$).

Kennedy and Vevers (1973) considered that the porphyrins of eggshells were derived from erythrocytes which are known to synthesize porphyrins (Dressel and Falk, 1953). If this theory is correct, it is understandable that Fe supplementation increased erythrocyte formation and breakdown, resulting in improved eggshell color in brown hens.

The levels of leukocytes and erythrocytes in blood are shown in Table 3. There were no significant differences among treatments with regard to leukocyte level; however, the concentrations of hemoglobin (Hb) were significantly different. The concentration of Hb was highest in Fe-SP 100 +MgO followed by those of the Fe-SP 100, control, and MgO, in that order. Other than Hb, there were no significant differences in the erythrocytes among treatments. The concentrations of red blood cell (RBC) in erythrocytes tended to increase in the Fe-SP treatments. Significant differences in Hb and in the tendency for higher RBC in Fe-SP treatments may have been caused by increased erythrocyte formation by Fe supplementation.

It was concluded that egg weight and eggshell qualities, such as strength, thickness, and color, can be effectively improved by supplementation with 100 ppm Fe in the form of Fe-SP and 3 g/kg MgO for five wks. Interactions between Fe and Mg in the laying hens were not apparent.

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