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Effects of Organic Iron Supplementation on the Performance and Iron Content in the Egg Yolk of Laying Hens

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An experiment was conducted to determine the efficacy of dietary iron-soy proteinate (Fe-SP) and iron-methionine chelate (Fe-Met) on the performance of laying hens and iron content in egg yolk. Eight hundred Hy-Line Brown laying hens of 68 wk old were housed in 400 cages of 2 birds each. Two hundred birds (10 cages × 10 replicates) were assigned to one of the following four treatments: Control; non supplementation with Fe-SP or Fe-Met, Fe-Met 100; 100 ppm iron supplementation with Fe-Met, Fe-SP 100; 100 ppm iron supplementation with Fe-SP, Fe-SP 200; 200 ppm iron supplementation with Fe-SP. Results of 35d feeding trial showed that there were significant differences in egg production, egg weight, feed conversion ratio and Haugh unit among the treatments. Egg weight and Haugh unit of Fe-SP 200 were significantly higher than the control. Hen-day egg production and feed conversion ratio of Fe-SP 100 and Fe-SP 200 were not significantly different from those of the control. Eggshell color was significantly improved in the Fe supplementation treatments compared to the control. The iron content of egg-yolk was maximized 5wk after feeding supplemental Fe and that of Fe-SP 100 was highest being 16.6% higher than the control. There were no significant differences in iron content of egg-yolk between source and level of iron supplementation. Copper content in the egg-yolk was not significantly affected by treatments but zinc content was significantly increased in iron supplemented treatments at 5th week after feeding. In conclusion, Fe content of egg-yolk could be effectively increased by supplementing 100 ppm iron as iron-soy proteinate for 5 wks. No significant difference was found in Fe content of egg yolk between Fe-SP and Fe-Met.

Key words: egg shell color, iron content of egg-yolk, iron-methionine chelate, iron-soy proteinate

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Introduction

In the past, poultry nutritionists had been interested in establishing nutrient requirements of poultry to support maximum performance of laying hens. In White Leghorn hens, the iron requirement was 35 to 45 ppm for maintenance of hematocrit and 55 ppm for maximum hatchability (Morck and Austic, 1981). NRC (1994) recommended 50-120 ppm of iron for poultry and 2,000 ppm for tolerance limit. Recently, nutritionists have been interested in enriching or altering the amount of certain nutrients in poultry products such as chicken meat and eggs in relation with recently increased consumer's interest in the nutritive value of foods. It is well known that the nutritive composition of egg can be changed by the nutritional composition of diet fed to chicken. Of the major constituents of the egg, only its lipid component is easily changed by dietary manipulation in the laying hens. Iron content showed minimum variability with dietary change while some variation was possible in other trace minerals (Naber, 1979). Absorbability of minerals in monogastrics could be increased by providing them in the form of chelates (Kratzer and Vohra, 1986; Paik, 2001). Among the chelating agents, amino acids and low molecular peptides have been known to be effective in animal production (Fouad, 1976; Ashmead, 1993). In an earlier experiment, Park *et al.* (2004) demonstrated that iron content of eggs can be increased by supplementing organic iron supplements. Fe-Met was the most effective source in enriching Fe in eggs followed by Availa-Fe® and $FeSO_4$. However, supplementation of iron at the level of 100, 200 or 300 ppm in each form did not significantly influence the iron level in the egg after reaching plateau. Several commercial organic iron supplements such as iron methionine chelate (Spears *et al.,* 1992), iron proteinate (Close, 1999) and iron amino acids complex (Yu *et al.,* 2000) are available. As the earlier experiment (Park *et al.,* 2004) tested the efficacy of Fe-Met, present study was conducted to compare Fe-SP and Fe-Met in increasing Fe content in egg yolk and to determine the optimal level and duration of Fe-SP supplementation.

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Materials and Methods

Experimental Diet

The composition of the basal diet used as the control is shown in Table 1. Basal diet contained Fe at the level of 121.0 mg/kg (ppm). Fe-Met 100 diet contained additional 100 ppm of Fe as Fe-Met. Fe-SP 100 or 200 diet contained either additional 100 ppm or 200 ppm Fe as Fe-SP. Fe-Met was made by reacting $FeSO_4 \cdot 7H_2O$ and DL-methionine at 1: 2 molar ratio following the principle of Cu-methionine chelate manufacturing method used by Lim and Paik (2003). It contained approximately 15% Fe, 53% mono or dipeptide form of methionine and the rest was considered sulfate and water. Fe-SP was made by the procedure developed by Animal Nutrition Laboratory of Chung-Ang University. First, soybean meal digest was prepared by hydrolysis of soybean meal with mixed industrial enzyme (Alkalase 2.4 L Novozymes, Denmark) under an aqueous condition of pH 8 and 60℃ for 8 hr. Then, $FeSO_4 \cdot 7H_2O$ and soybean meal digest were reacted at 1: 1 weight ratio at pH 9.6. The precipitate was separated and dried. The final product was named iron-soy proteinate (Fe-SP) which contained 19.3% Fe. Basal diet was procured from a commercial feed mill in mash form. Each treatment diet was prepared using 200kg vertical mixer.

Table 1. **Formula and composition of basal diet used for the control**

Ingredient composition	(%)
Corn	51.58
Soybean meal	20.24
Wheat bran	3.00
Rice bran	1.50
Wheat	3.00
Lupin kernel	4.00
Corn gluten feed	2.07
Tallow	2.36
Salt	0.21
Limestone	9.69
Oyster shell	0.80
Dicalcium phosphate	0.82
Electrolytes[1]	0.15
Additives[2]	0.15
Vitamin premix[3]	0.13
Mineral premix[4]	0.12
Choline-Cl (50%)	0.07
Methionine Hydroxyl Analogue (88%)	0.08
Phytase[5]	0.03
Total	100.00
Calculated composition	
ME, kcal/kg	2,750
Crude protein, %	17.50
Crude fat	5.17
Crude fiber, %	3.30
Lysine, %	0.83
Methionine, %	0.37
Met.+Cys., %	0.64
Calcium, %	3.95
Total P, %	0.50
Avail P, %	0.38
Fe, mg/kg[6]	121.0

[1] Consists of KCl, 35%; $NaHCO_3$, 40%; Na_2SO_4,25%.
[2] Provides per kg diet; Cyromazine, 5 ppm; vitamin E, 15 IU; vitamin C, 100 ppm per kg.
[3] Provides per kg diet; vitamin A, 10,000 IU; vitamin D_3, 2,500 IU; vitamin E, 15 IU; vitamin K_3, 2 mg; vitamin B_1, 1.5 IU; vitamin B_2, 4 mg; vitamin B_6, 3 mg; vitamin B_{12}, 3 ug; pantothenic acid, 8 mg; niacin, 25 mg; folicacid, 0.5 mg per kg.
[4] Provideds per kg diet; Zn, 52.5 mg; Mn, 52.5 mg; Fe, 52.5 mg; Cu, 52.5 mg; I, 1.155 mg; Co, 0.315 mg; Se, 0.315 mg.
[5] Phytase provided by BASF Korea Ltd.
[6] Assayed value.

Experiment Animals and Design

Eight hundred Hy-Line Brown laying hens of 68 wk old were housed in 400 cages of 2 birds each. Two hundred birds (10 cages × 10 replicates) were assigned to one of the following four dietary treatments: Control; non supplementation with Fe-SP or Fe-Met, Fe-Met 100, Fe-SP 100 and Fe-SP 200. Since the peak iron level in the egg showed at 15, 30 and 35 days iron supplementation with 30 wk old ISA Brown (Park *et al.,* 2004) and 25 and 30 days with 85 wk old ISA Brown (Park *et al.,* 2005) and then plateaued, water and diets were provided *ad libitum* for 35 days feeding trial. Birds were subjected to 16 h of light per day.

Egg Productivity, Egg Quality and Analysis of Fe, Cu and Zn Content in Egg Yolk

To assess egg productivity, hen-day egg production, mean egg weight, soft & broken eggs production were recorded daily and weekly average was calculated. Feed intake was measured weekly and feed conversion ratio (feed intake/100 g egg mass) was calculated. One hundred egg samples (10 eggs per replicate) from each treatment were randomly collected each week to measure egg qualities such as, Haugh unit, egg shell strength, egg shell thickness, egg-yolk color, and egg shell color. Five week averages of the measurements were calculated. Haugh unit was calculated using the HU formula (Eisen *et al.,* 1962) based on the height of albumen determined by a micrometer (Model S-8400, AMES, Waltham, MA, USA). Egg shell strength was measured by Compression Test Cell of Texture Systems (Model T2100C, Food Technology Corp., Rockville, MD, USA). Eggshell thickness was measured by Dial Pipe Gauge (Model 7360, Mitutoyo Corp., Kawasaki, Japan). Color fans were used to measure egg-yolk color (Roche Color Fan, Roche, Swiss) and egg shell color (Egg Shell Color Fan of Samyang Feed Co. Ltd., Korea). Both color fans have color scale 1 (light) to 15 (dark orange for egg yolk and dark brown for egg shell). Since egg-yolk is by far richer source of minerals than egg white (albumen), e.g., 1.117 vs 0.033 mg Fe per fresh raw egg (Cook and Briggs, 1972), yolks were separated from egg white, freeze-dried (Vacuum Freeze

Drier, Beta-A, Germany), and defatted using Soxhlet Apparatus. Contents of Fe, Cu and Zn of egg yolk were analyzed by ICP (Inductively Coupled Plasma Spectroscopy, Jovon Yvon, JY-24, France) after wet ashing with HNO_3 and HCl (AOAC, 1995).

Statistical Analysis

The data were analyzed by ANOVA using General Linear Models (GLM) procedure of SAS (1995). Significant differences between treatment means were determined at $P<0.05$ using Duncan's new multiple range test (Duncan, 1955).

Results

Table 2 shows the effects of supplementing Fe-Met 100, Fe-SP 100 and 200 on the productivity and egg quality of laying hens. Five week feeding trial showed that hen-day egg production, egg weight and feed conversion among the productivity parameters were significantly affected. Hen-day egg production was not significantly different between Fe-SP treatments and the control but was lower in Fe-Met treatment than others. Egg weight was highest in Fe-SF 200 followed by Fe-SP 100, Fe-Met 100 and the control which was significantly lower than Fe-SP 200. Feed conversion ratios of Fe-SF 100, Fe-SP 200 and the control were not significantly different among them but were significantly lower than that of Fe-Met 100. Feed intake and soft & broken egg production rate were not significantly affected by treatments. Among the egg quality parameters, Haugh unit, eggshell color index were significantly different among treatments. Haugh unit was highest in Fe-SP 200 followed by Fe-SP 100. There was no significant difference between the control and Fe-SP treatments but Fe-Met 100 was significantly lower than Fe-SP treatments in Haugh unit. Egg shell color index was significantly higher in Fe supplemented treatments than the control. Egg shell strength, egg shell thickness and egg-yolk color were not significantly different among treatments.

Table 3 summarizes the effects of supplemental Fe-Met and Fe-SP sources on Fe, Cu and Zn content in egg yolk. Fe content in egg yolk was not significntly different among treatments until 4 weeks after feeding. At 5th week, however, there were significant differences among treatments in iron content. Iron content of egg yolk was highest in Fe-SP 100 followed by Fe-Met 100, Fe-SP 200 and the control. There were no significant differences among treatments in Cu content of egg yolk. The zinc contents in egg-yolk, however, were significantly different at 5th week. All of the Fe supplemented groups had higher Zn content than the control.

Discussion

Significant differences in hen-day egg production, egg weight, feed conversion and Haugh unit can not be properly explained. In an earlier expriment conducted by Park *et al.* (2004), there were no significant differences between the control and Fe-Met 100 in the productivity parameters of young (30 wk old) ISA Brown layers. The birds of present experiment were 68 wks old Hy-Line Brown layers. The difference of breed and age may be parts of the contributing factors of the present results. The minimum requirement of methionine for brown egg layer (NRC, 1994) is 0.30% of diet. The present control diet contained 0.37% methionine, which meets the minimum requirement well. Fe-Met 100 provides extra 0.053% D,L-methionine. This level of methionine alone would not significantly influence the production parameters. It is noteworthy that all of the Fe supplemented groups had significantly higher egg shell color index. This result is in well agreement with the result of Park *et al.* (2004). Solomon (1997) implicated that Fe, Zn, Cu and Mn may function as chelating carriers at the central position of porphyrins which are pigments of egg shell. Kennedy and Vevers (1973) considered that the porphyrins of egg shell are derived from erythrocytes. It is understandable that high level of Fe and Zn in the egg yolk by Fe supplementation indicates higher availabilty of these minerals in the birds resulting in increased porphyrins formation from

Table 2. **Effect of supplementary Fe sources on the performance and egg quality of laying hens**

Parameters	Treatments				SEM
	Control	Fe-Met 100	Fe-SP 100	Fe-SP 200	
Hen-day egg production (%)	80.78[a]	78.65[b]	82.09[a]	82.06[a]	0.74
Egg weight (g)	67.79[b]	67.86[b]	68.17[ab]	68.53[a]	0.18
Feed intake (g/hen/day)	129.34	130.53	129.40	131.79	0.87
Feed conversion (feed/egg mass)	2.37[b]	2.46[a]	2.32[b]	2.35[b]	0.02
Soft & broken egg (%)	0.17	0.17	0.13	0.17	0.03
Haugh unit	82.04[bc]	81.32[c]	82.85[ab]	83.20[a]	0.44
Egg shell strength (g/egg)	2849	2869	2790	2890	43.6
Egg shell thickness (μm)	400	398	401	399	0.29
Egg yolk color	8.06	8.23	8.15	8.28	0.11
Egg shell color	10.94[b]	11.48[a]	11.58[a]	11.29[a]	0.13

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

Table 3. **Effects of supplemental Fe sources on Fe, Cu and Zn content of egg yolk**

Item	Wk	Treatments				SEM
		Control	Fe-Met 100	Fe-SP 100	Fe-SP 200	
		-------- ppm (DM basis, Fat free) --------				
Fe	0	229.3	228.7	228.5	230.4	4.98
	1	232.9	233.6	233.4	235.2	4.59
	2	232.8	234.1	247.0	239.4	5.73
	3	231.2	236.3	233.9	234.2	4.96
	4	228.0	230.7	231.9	236.4	4.91
	5	229.1[b]	259.0[ab]	267.1[a]	248.1[ab]	11.33
Cu	0	9.69	9.65	9.54	9.43	0.38
	1	9.57	9.55	9.78	9.65	0.14
	2	9.30	9.50	9.23	9.73	0.32
	3	9.13	9.21	9.23	9.13	0.18
	4	9.96	9.63	9.68	9.88	0.25
	5	9.45	9.96	9.61	9.67	0.20
Zn	0	100.1	102.1	100.5	102.8	2.02
	1	101.2	103.3	103.3	102.3	0.83
	2	107.2	107.8	108.3	108.0	1.99
	3	112.9	114.7	112.5	111.5	1.44
	4	109.1	111.8	112.6	112.4	2.24
	5	108.1[b]	120.1[a]	119.4[a]	121.2[a]	3.49

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

erythrocyte and improved egg shell color. Increase of Fe content in the egg yolk in the Fe supplemented treatments has been expected. In the present experiment, Fe content of egg yolk was increased by 16.6% in Fe-SP 100, 13.1% in Fe-Met and 8.3% in Fe-SP 200 treatment. In the previous experiment (Park *et al.,* 2004, 2005), Fe-Met 100 significantly increased Fe content of egg yolk. Increasing Fe supplementation from 100 ppm to 200 ppm as Fe-SP did not improve Fe enrichment in egg yolk. This result is also in agreement with the result of Park *et al.* (2004), in which supplementation of Fe at the level of 200 ppm and 300 ppm in different organic forms (Fe-Met or Availa-Fe®) or inorganic form ($FeSO_4$) did not improve Fe enrichment in the egg yolk compared to 100 ppm Fe supplementation. In the present experiment, Fe level in the egg yolk increased at 2nd wk but significant increase was only shown at 5th week after feeding supplemented diets. The Fe level in the previous trials peaked at 15, 30 and 35 days (Park *et al.,* 2004) with 30 wk old and 25 and 30 days with 85 wk old ISA Brown (Park *et al.,* 2005). Such a discrepancy may have been caused by age difference of layers, species of birds, experimental environment and season. Probably, older hens require more time to enrich Fe in the yolk than younger ones. Significant increase of Zn level in egg yolk is also noteworthy. It is well known that there are strong interactions among divalent minerals, such as Fe (II), Zn and Cu. Iron supplemented treatments showed increased Zn level while Cu level in the egg yolk was not affected. Similar interactive response of minerals was reported in pigs. Zn level was increased while Cu level was not affected in the liver and kidney when Fe-glycine chelate was supplemented to weanling pig diet (Feng *et al.,* 2008).

Fe-SP 100 is as good as, if not better, Fe-Met in enriching Fe in egg yolk. As the price of soybean meal is lower than methionine, the production cost of Fe-SP could be less than that of Fe-Met. It was concluded that Fe content of eggyolk can be effectively enriched by supplementing 100 ppm iron as iron-soy proteinate for 5 wks in 68 wks old Hy-Line Brown layers. Concomitant increase of Zn level and improvement in egg shell color can be expected as well.

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