Production of Iron Enriched Eggs of Laying Hens

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ABSTRACT : An experiment was conducted to investigate the efficiency of transfer of dietary iron sources to eggs of laying hens. Eighty ISA-Brown laying birds of 30 wk old were housed in 40 cages of 2 birds each. Eight birds in four cages were assigned to one of the following ten treatments: T1; control, T2; 100 ppm iron supplementation with iron-methionine chelate (Fe-Met-100), T3; Fe-Met-200, T4; Fe-Met-300, T5; 100 ppm iron supplementation with iron sulfate ($FeSO_4$-100), T6; $FeSO_4$-200, T7; $FeSO_4$-300, T8; 100 ppm iron supplementation with Availa-Fe® (Availa-Fe-100), T9; Availa-Fe-200 and T10; Availa-Fe-300. Results of 40 d feeding trial showed that there were no consistent responses in laying performance by source and level of iron supplementation. However, eggshell strength and color were improved by Fe supplementation. Egg iron content was maximized at 10-15 days after feeding supplemental Fe. Fe- Met was the most effective source in enriching Fe of eggs followed by Availa-Fe and $FeSO_4$. Increasing supplementary Fe level more than 100 ppm was not effective in Fe-Met and Availa-Fe treatments. Average Fe enrichment of 18% was achieved after feeding Fe-Met-100 for 15 d. In conclusion, enrichment of Fe in egg could be effectively achieved by supplementation of Fe-Met-100 for 15 d. ***(Asian-Aust. J. Anim. Sci. 2004. Vol 17, No. 12 : 1725-1728)***

Key Words : Egg Iron, Iron-methionine Chelate, Eggshell Color, Eggshell Strength

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 carcass and egg 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. Of the major constituents of the egg, only its lipid component is easily changed by dietary manipulation of the laying hen. Iron content of the egg 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).

The present study was conducted to determine the optimum level and duration of feeding of supplementary iron from different sources to produce iron-enriched eggs.

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Received May 5, 2004; Accepted August 26, 2004

MATERIALS AND METHODS

Eighty 30 week-old ISA Brown laying hens were allotted to one of ten dietary treatments (Table 1) based on completely random design. Each treatment had 4 replicate cages of 2 birds each. The basal diet (Table 2) was supplemented with iron-methionine chelate (Fe-Met), $FeSO_4 \cdot 7H_2O$ ($FeSO_4$) or Availa-Fe® at the graded levels of 100, 200 and 300 ppm in Fe, respectively. Fe-Met was made at author's laboratory by reacting $FeSO_4 \cdot 7H_2O$ and D,L-methionine at a molar ratio of 1:2. It contained 15% Fe. Availa-Fe® is a commercial iron complex with amino acid containing 6% Fe (Alltech Inc. Nicholasville, Kentucky, USA). Birds were subjected to 16 h of light per day and were given experimental diets for 40 days. Feed and water was available for *ad libitum* consumption. Egg production and egg weight were recorded daily and feed consumption was recorded weekly. Eight eggs from each treatment was collected randomly at two day interval till 10 days of feeding and then, five day interval till the end of experiment for measuring egg shell strength, Haugh unit, eggyolk color, and eggshell color. 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 02254, USA). Eggshell strength was measured by Compression Test Cell of Texture Systems (Model T2100C, Food Technology Corp., Rockville, MD 20852 USA). Eggshell thickness was measured by Dial Pipe Gauge (Model 7360, Mitutoyo Corp., Kawasaki 213, Japan). Color fans were used to measure egg yolk color (Roche Color Fan) and eggshell color (Color Fan of Samyang Feed Co.Ltd., Korea). Iron content of egg yolk was analyzed by ICP (Inductively Coupled Plasma Spectroscopy, Jovon Yvon, JY-24, France) after wet ashing

Table 1. Dietary treatments

Treatments	Supplemental iron sources	Supplementation level of Fe, ppm
1 Control		
2 Fe-Met-100	Fe-methionine chelate[1]	100
3 Fe-Met-200	Fe-methionine chelate	200
4 Fe-Met-300	Fe-methionine chelate	300
5 $FeSO_4$-100	$FeSO_4 \cdot 7H_2O$	100
6 $FeSO_4$-200	$FeSO_4 \cdot 7H_2O$	200
7 $FeSO_4$-300	$FeSO_4 \cdot 7H_2O$	300
8 Availa-Fe-100	Availa-Fe[2]	100
9 Availa-Fe-200	Availa-Fe	200
10 Availa-Fe-300	Availa-Fe	300

[1] Fe-Methionine chelate was made at author's laboratory by reacting $FeSO_4 \cdot 7H_2O$ and D,L-methionine at a molar ratio of 1:2.
[2] Alltech Inc. Nicholasville, Kentucky, USA.

(AOAC, 1995). Iron content expressed in mg/100 g egg was calculated based on the average yolk weight being 17 g and iron content of egg white being 5% of that of egg yolk. The results obtained from experiments were analyzed by ANOVA and contrast using GLM procedure of SAS® (SAS Institute, 1985). Significant differences among treatment means were determined using Duncan's multiple range test at $p<0.05$.

RESULTS AND DISCUSSIONS

The performance of laying hens fed different levels of Fe from various sources is shown in Table 3. Supplementary Fe did not improve the performance of laying hens although hens fed different sources of Fe showed different responses to the supplementation levels of each of Fe sources. In Fe-Met groups, highest performance was observed when hens were fed 100 ppm Fe diet, whereas higher performances were observed with hens fed 200 ppm Fe diets in ferrous sulfate or Availa-Fe groups. However, they were not significantly different from the control. Egg weight and feed intake were significantly different but there was no consistency by source or level of Fe supplementation. Feed conversion ratio of the control tended to be lower than those of Fe supplemented groups.

Table 2. Formula and composition of basal (control) diet

Ingredients	Percent	Calculated composition	
Corn	54.382	ME, kcal/kg	2,730
Soybean meal	17.390	Crude protein, %	17.00
Limestone	9.633	Arginine, %	1.13
Rice bran	4.000	Lysine, %	0.86
Rapeseed meal	3.000	Methionine, %	0.36
Corn gluten feed	3.000	Meth and cyst, %	0.58
Wheat bran	2.077	Calcium, %	3.90
Animal fat	3.000	Non-phytate P, %	0.38
Corn germ	1.000	Total P, %	0.50
Sesame seed	1.000	Salt, %	0.25
Additives[1]	0.597		
Calcium phosphate	0.290		
Salt	0.200		
Corn gluten meal	0.165		
Baymix-151[2]	0.100		
Baymix PL[3]	0.070		
Choline-Cl	0.047		
D,L-methionine	0.043		
Natuphos[4]	0.006		
Total	100.000		

[1] A complex of electrolytes, Pumkito, oyster shell and sarsaponin.
[2] Provides per kg of 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 μg, Pantothenic acid: 8 mg, niacin: 25 mg, folic acid: 0.5 mg.
[3] Provides per kg diet; Zn: 52.5 mg, Mn: 52.5 mg, Fe: 52.5 mg, I: 1.155 mg, Cu: 5.25 mg, Co: 0.315 mg, Se: 0.315 mg.
[4] Phytase provided by BASF Korea Ltd.

In the laying performance, consistency in response is lacking even with statistically significant differences. This may be due to the small sample size of experimental birds in present trial. Thus, the differences of individual birds at the beginning of the experiment influenced the overall performance during the experimental period. On the other hand, contrast analysis results showed that eggshell strength ($p=0.04$) and eggshell color ($p<0.0001$) were significantly improved by Fe supplementation. Eggshell strength was highest in eggs from hens fed $FeSO_4$-200 and eggshell color was deeper for hens fed Availa-Fe compared with those of other Fe supplemented treatments. It is well known that

Table 3. Effect of supplementary Fe source and level on the performance and egg quality of laying hens

Parameters	Control	Fe- Met			$FeSO_4 \cdot 7H_2O$			Availa Fe-60			SEM
		100	200	300	100	200	300	100	200	300	
Hen-day egg production (%)	91.4^{ab}	91.4^{ab}	82.5^{b}	87.1^{ab}	83.2^{b}	96.4^{a}	90.0^{ab}	83.9^{b}	84.6^{b}	82.9^{b}	8.12
Egg weight (g)	60.3^{ab}	60.4^{ab}	58.5^{bcd}	57.8^{cd}	61.1^{a}	57.3^{d}	60.0^{ab}	59.0^{bcd}	59.5^{abc}	59.2^{abcd}	1.45
Feed intake (g/hen/day)	115.3^{ab}	122.4^{a}	116.9^{ab}	124.4^{a}	115.7^{ab}	125.9^{a}	125.5^{a}	110.1^{b}	120.3^{ab}	109.3^{b}	8.01
Feed conversion (feed/egg mass)	2.13^{c}	2.23^{bc}	2.46^{abc}	2.66^{a}	2.32^{abc}	2.25^{abc}	2.39^{abc}	2.28^{abc}	2.52^{ab}	2.15^{bc}	0.29
Soft and broken egg (%)	2.28	1.19	2.77	2.13	3.42	2.63	3.48	0.87	3.10	5.68	2.35
Eggshell strength (kg/cm^2)[1]	0.52^{c}	0.53^{bc}	0.53^{bc}	0.58^{abc}	0.58^{ab}	0.60^{a}	0.57^{abc}	0.55^{abc}	0.55^{abc}	0.55^{abc}	0.04
Haugh unit	74.1	71.0	73.8	72.9	71.8	74.4	73.8	72.5	74.5	68.3	2.57
Egg york color	7.2^{ab}	7.0^{b}	7.3^{ab}	7.4^{ab}	7.4^{ab}	7.4^{ab}	7.3^{ab}	7.2^{ab}	7.5^{ab}	7.6^{a}	0.36
Eggshell color[2]	8.2^{c}	9.1^{b}	9.4^{b}	8.2^{c}	9.2^{b}	8.9^{bc}	8.7^{bc}	10.6^{a}	10.5^{a}	9.4^{b}	0.57

[a-d] Values in the same row with no common superscripts are significantly different ($p<0.05$).
[1] Contrast T1 vs. T2-T10; $p=0.04$. [2] Contrast T1 vs. T2-T10; $p<0.0001$.

Table 4. Effect of supplemental Fe source and level (ppm in Fe) on Fe content of egg

Day	Control	Met-Fe			$FeSO_4 \cdot 7H_2O$			Availa Fe-60			SEM
		100	200	300	100	200	300	100	200	300	
	---------- (mg/100 g egg) ----------										
0	1.87^{ab}	1.92^{a}	1.84^{ab}	1.89^{ab}	1.82^{ab}	1.96^{a}	1.87^{ab}	1.74^{b}	1.81^{ab}	1.8^{ab}	0.03
2	-	1.76^{bc}	1.88^{ab}	1.74^{c}	1.95^{a}	1.82^{abc}	1.84^{abc}	1.76^{bc}	1.77^{bc}	1.93^{a}	0.03
4	-	1.89^{abc}	1.81^{bc}	2.00^{ab}	1.76^{bcd}	1.91^{ab}	1.75^{bcd}	1.50^{d}	1.63^{cd}	2.12^{a}	0.06
6	-	1.89	2.08	1.91	2.00	1.89	2.04	2.06	2.06	1.80	0.09
8	-	1.91	1.83	1.99	2.20	2.14	1.96	2.04	1.97	2.08	0.10
10	-	2.07^{a}	2.21^{a}	2.25^{a}	1.92^{b}	2.06^{ab}	2.22^{a}	2.16^{ab}	2.07^{ab}	2.05^{ab}	0.06
15	-	2.43^{a}	2.31^{abc}	2.01^{d}	2.08^{d}	2.39^{ab}	2.40^{a}	2.20^{bcd}	2.17^{cd}	2.18^{cd}	0.04
20	1.98^{c}	2.38^{a}	2.21^{ab}	2.16^{abc}	2.11^{bc}	2.23^{ab}	2.06^{bc}	2.37^{a}	2.18^{abc}	2.27^{ab}	0.05
25	2.08	2.21	2.23	2.25	2.21	2.07	2.10	2.13	2.14	2.23	0.05
30	2.02^{c}	2.22^{abc}	2.41^{a}	2.39^{a}	2.11^{bc}	2.17^{abc}	2.27^{ab}	2.34^{ab}	2.27^{ab}	2.29^{ab}	0.04
35	1.98^{c}	2.45^{a}	2.26^{b}	2.39^{a}	1.99^{c}	2.15^{b}	2.27^{b}	2.21^{b}	2.23^{b}	2.27^{b}	0.03
40	1.97^{d}	2.42^{a}	2.28^{b}	2.36^{ab}	2.15^{c}	2.16^{c}	2.11^{c}	2.26^{b}	2.15^{c}	2.27^{b}	0.02
Overall average	1.98^{d}	2.13^{a}	2.13^{a}	2.12^{a}	2.03^{cd}	2.08^{abc}	2.07^{abc}	2.07^{abc}	2.04^{bcd}	2.11^{ab}	0.05
Average of 15-40 d	2.01^{e}	2.37^{a}	2.28^{b}	2.26^{bc}	2.11^{d}	2.19^{c}	2.20^{c}	2.25^{bc}	2.19^{c}	2.25^{bc}	0.04

$^{a-d}$ Values in the same row with no common superscripts are significantly different ($p<0.05$).

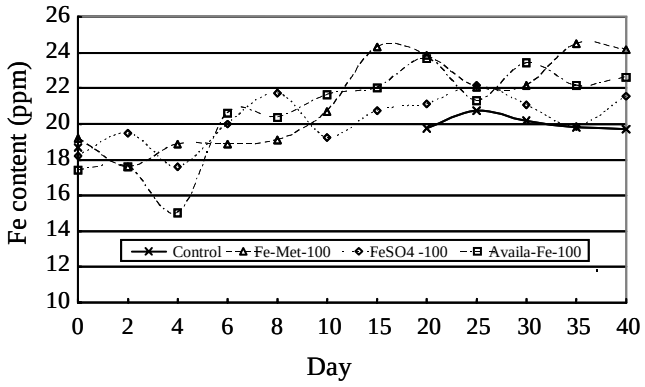

Figure 1. Change of Fe content in eggyolk by time of iron supplementation.

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. Depletion of Fe reduces Cu concentration in tissue of broiler (Ruiz et al., 2000) while Cu-methionine chelate supplementation increases eggshell strength (Lim and Paik, 2003). Increasing dietary Ca and P decreased hemoglobin, hematocrit and red blood cell number (Waddell and Sell, 1964). Injection of Fe resulted in reduction of serum Zn (Fleet et al., 1990) but increased accumulation of hepatic Zn-metallothionein in chicks (McCormic, 1987; Fleet et al., 1990). Zinc is a prosthetic part of carbonic anhydrase that plays impotant role in $CaCO_3$ formation of eggshell. Major pigment of brown eggshell is protoporphyrin (Lang and Wells, 1987). Kennedy and Vevers (1973) considered that the porphyrins of eggshells were derived from erythrocytes which are known to synthesis porphyrins (Dresel and Falk, 1953). If this theory is true, it is understandable that Fe supplementation increased erythrocyte formation resulting in improved eggshell color. Stevens et al. (1974), however, supported the hypothesis that avian shell gland is the site of biosynthesis of eggshell porphyrins.

Egg iron content increased significantly in all supplemental Fe treatments (Table 4). Egg Fe content was maximized 10-15 days after feeding supplemental Fe. At the level of 100 ppm Fe supplementation, Fe-Met was most effective in enriching Fe of egg followed by Availa-Fe and $FeSO_4$ (Figure 1). One hundred ppm Fe in organic form (Fe-Met or Availa-Fe) was enough to increase egg iron content, whereas ferrous sulfate needed 200 or 300 ppm of Fe to reach maximum egg iron content. The average Fe content of $FeSO_4$-200 during the period of 15-40 d was 2.19 mg/100 g. This value is 9% higher than that of the control but 8% lower than that of Fe-Met-100. Naber (1979) reported Fe content of the egg showed minimum variability among many other elements. But the present results show that Fe content of egg can be enriched by Fe supplementation and organic forms (Fe-Met or Availa-Fe) are more effective than sulfate form.

In conclusion, Fe supplementation improves eggshell strength and eggshell color, and enrichment of Fe in egg can be effectively achieved by supplementation of Fe-Met at the level of 100 ppm Fe for 15 d.

ACKNOWLEDGEMENT

The authors wish to acknowledge the financial support of Agribrands Purina Korea Inc.

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