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The Effect of Level and Period of Fe-methionine Chelate Supplementation on the Iron Content of Boiler Meat

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ABSTRACT : A broiler experiment was conducted to compare the effects of duration and level of iron-methionine chelate (Fe-Met) supplementation on the iron, copper (Cu) and zinc (Zn) content of broiler meat. Two hundred and fifty hatched Ross broiler chickens were randomly assigned to 5 dietary treatments. Each treatment had 5 replicates of 10 birds (5 males and 5 females) each. Birds were housed in raised floor batteries and fed traditional broiler diets *ad libitum* for 5 weeks. Dietary treatments were as follows: Control and two levels of Fe-Met (100 or 200 ppm in Fe) supplemented for either the whole period (0-5 wk) or grower period (4-5 wk). Production performance was not significantly affected by treatments. Iron content in the muscles (breast, leg and wing) and organs (liver and spleen) were significantly ($p<0.05$) increased as the level and duration of Fe-Met supplementation increased. The highest concentration of iron was shown in Fe-Met 200 fed for the whole period. Liver contained the highest amount of iron followed by spleen, leg muscle, wing muscle and breast muscle. Supplementation of Fe-Met 200 for the grower period resulted in higher iron concentration in liver and spleen than supplementation of Fe-Met 100 for the whole period. However, the same treatment resulted in lower iron concentration in muscles (breast, leg and wing) than the treatment of Fe-Met 100 for the whole period. In order to achieve the highest iron enrichment in the muscles, Fe-Met should be supplemented at 200 ppm in Fe for the whole period (5 wks). Fe-Met supplementation increased copper concentration in all muscles and organs except wing muscle. Zinc concentration decreased in breast and wing muscle but tended to increase in leg muscle, liver and spleen by Fe-Met 200 supplementation. Color of muscle was not significantly affected by Fe-Met treatments. However, redness of leg and breast muscle, and yellowness of leg and breast muscle tended to increase by supplementation of Fe-Met for the whole period. It was concluded that iron content of broiler meat can be effectively enriched by supplementation of 200 ppm of Fe as Fe-Met for 5 wks. (**Key Words :** Fe-methionine Chelate, Iron Enriched Meat, Broiler, Copper, Zinc)

INTRODUCTION

Iron (Fe) is an essential element for livestocks as well as human beings. NRC (1994) suggested that the Fe requirement should be 50-120 ppm for poultry, and Fe toxicity appears at a very high level over 2,000 ppm. It is well known that white meats, such as breast meat of broilers, are low in Fe content than red meats, such as beef. Extra supplementation of Fe in addition to meeting nutritional requirement of chicken would enable enrichment of Fe in white meat. Iron enriched broiler meat may meet the demand of niche market customers looking for such functional products.

Since a few years ago, researches on organic minerals have been actively undertaken because chelate minerals can be more effectively absorbed into the intestines than inorganic oxide and sulfate (Wedekind et al., 1992; Aoyagi and Baker, 1993). In the same way, Fe may have big difference in bioavailability according to the form of supply. As it was found that chelate minerals which are new organic compounds of the metal enhanced the productivity of livestock because they have a higher bioavailability than inorganic minerals, intensive researches on this issue have been conducted (Kratzer and Vohra, 1986). Organic minerals, in particular, amino acids and low molecule peptide (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 is because chelation of metal ions with organic substances such as amino acids or low molecule peptide makes metal ions electrically neutral and chemically stable, thereby allowing easy passage through the small intestinal wall. Actually, 95% of them are absorbed (Kratzer and Vohra, 1986). Paik (2001) reported

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Table 1. Composition and nutrient content of broiler diets

Ingredients	Starter	Grower
	------------------- % -------------------	
Corn	53.02	58.79
SBM-44	34.60	33.4
Corn gluten	4.79	-
Animal fat	4.00	4.89
Calphos-18	1.75	1.22
Limestone	0.80	0.97
Salt	0.39	0.26
DL-methionine (98%)	0.26	0.17
Vitamin premix[1]	0.15	0.12
Mineral premix[2]	0.10	0.08
Lysine-HCl (78%)	0.14	0.10
Total	100.00	100.00
Calculated nutrient composition		
Me (kcal/kg)	3,050	3,100
Crude proein (%)	23.0	20.0
Lysine (%)	1.10	1.00
Met+cys (%)	0.86	0.70
Calcium (%)	1.00	0.90
Total P (%)	0.73	0.62

[1] Provided per kg of diet: vitamin A, 10,000 IU; vitamin D_3, 2,500 IU; vitamin E, 20 mg; vitamin K_3, 2 mg; vitamin B_1, 1 mg; vitamin B_2, 4 mg; vitamin B_6, 3 mg; vitamin B_{12}, 10 μg; biotin, 100 μg; niacin, 25 mg; pantothenic acid, 9 mg; folic acid, 0.5 mg.

[2] Provided per kg of diet: Zn, 90 mg; Mn, 96 mg; Fe, 50 mg; Cu, 24 mg; I, 1.2 mg; Se, 0.36 mg.

the results of application of chelated minerals in animal production. The results demonstrated that chelated minerals, e.g. Cu and Zn, are effective in improving the performance of animals at the lower supplementary levels compared to inorganic minerals, especially those used at pharmacological levels. Park et al. (2004) used iron-methionine chelate to produce iron-enriched eggs. It was demonstrated that enrichment of Fe in egg could be effectively achieved by supplementation of 100 ppm of Fe as Fe-methionine chelate. Seo et al. (2008) reported that iron-methionine chelate is more efficient than iron sulfate and 200 ppm iron supplementation as iron-methionine chelate is recommended for maximum iron enrichment in broiler meat.

The experiment was conducted to examine the productivity of broiler meat according to the level and period of iron-methionine chelate supplementation, to find out the extent of Fe accumulation within broiler meat and ultimately to develop effective means of producing iron-enriched broiler meat.

MATERIAL AND METHOD

Experimental diet

The composition of the basal diet (mash type) used as the control is shown in Table 1. Control diet as the basal diet contained 50 mg of supplementary Fe from $FeSO_4{\cdot}7H_2O$ per kg of diet. Fe-Met 100 diet and Fe-Met 200 diet contained additional 100 mg and 200 mg of Fe from iron-methionine chelate (Fe-Met), respectively. Supplemental Fe-Met at respective level was mixed with the basal diet by 200 kg vertical mixer. Fe-Met was made by reacting $FeSO_4{\cdot}7H_2O$ and methionine at 1:2 molar ratio following the principle of Cu-methionine chelate manufacturing method used by Lim and Paik (2003). Fe-Met was supplemented for the whole period of experiment (0 to 5th weeks) or for the growing period (4th to 5th weeks) only.

Experimental design and feeding

Two hundred fifty hatched Ross broiler chickens (125 male and 125 female) were randomly assigned to the 5 dietary treatments: Control, Fe-Met 100 diet fed for whole period, Fe-Met 200 diet fed for whole period, Fe-Met 100 diet fed for grower period and Fe-Met 200 diet fed for grower period. Each treatment had 5 replicates of 10 birds (5 males and 5 females) each. Birds were housed in two tier raised floor batteries (width: 76 cm, length 76 cm, height: 50 cm) located in the concrete experimental building. Birds were fed experimental diets and water *ad libitum* for 5 weeks using trough type feeder and waterer made with stainless steel. The room temperature was maintained at 28°C at 1st week, 26°C at 2nd week and 24°C at 3rd week and thereafter. Other environmental conditions (ventilation and humidity) were maintained at optimum standard. Weight gain and feed intake were measured at 3rd and 5th wk by pen by flat bottomed electronic scale with sensitivity of 0.01 kg.

Determination of meat color and analysis of Fe, Cu and Zn content in muscle and organ

On the termination of feeding, 10 birds (5 males and 5 females) of median weight of each treatment were chosen and sacrificed by cervical dislocation according to Animal Care Standard of Chung Ang University. Skin was peeled off. Colors of three spots in each muscle were measured with a Chromameter (Minolta CM-508i, Japan) and the means were calculated. Muscle (breast, leg and wing) and organ (liver and spleen) samples were taken for analysis. For the analysis of Fe, Cu and Zn concentration, samples were ashed with HNO_3 and HCl (AOAC, 1990) and assayed with ICP Emission Spectrometer (Model ULTIMA2, Jobin Yvon, France).

Chemical and 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).

Table 2. Effects of different level and duration of Fe-Met supplementation on weight gain, feed intake, feed/gain and mortality in broiler chickens

Item	Week	Control	Whole period*		Grower period**		SEM
			Fe-Met 100[1]	Fe-Met 200[2]	Fe-Met 100	Fe-Met 200	
Weight gain	0-3	718.7	682.9	704.7	677.2	730.4	19.14
(g/bird)	4-5	821.8	868.8	826.7	895.4	812.8	35.91
	0-5	1,540.5	1,551.7	1,531.4	1,572.5	1,543.2	48.48
Feed intake	0-3	1,008.0	884.4	912.3	952.1	986.5	38.91
(g/bird)	4-5	1,363.4	1,432.5	1,334.4	1,429.3	1,320.7	51.85
	0-5	2,371.4	2,316.8	2,246.7	2,381.4	2,307.2	74.58
Feed/gain	0-3	1.40	1.30	1.30	1.40	1.35	0.05
(g/g)	4-5	1.66	1.65	1.61	1.60	1.63	0.04
	0-5	1.54	1.49	1.47	1.51	1.50	0.03
Mortality	0-3	8.0[ab]	4.0[ab]	0.0[b]	2.0[ab]	10.0[a]	2.97
(%)	4-5	2.2	2.0	0.0	0.0	2.5	1.74
	0-5	10.0[ab]	6.0[ab]	0.0[b]	2.0[ab]	12.0[a]	3.58

[1] Fe-Met 100; supplemented 100 ppm Fe as Fe-methionine. [2] Fe-Met 200; supplemented 200 ppm Fe as Fe-methionine.
[a-b] Values with different superscripts in the same row are different ($p<0.05$).
* Chickens were fed treated diets during the whole feeding period (0-5 weeks).
** Chickens were fed control diet during starter period (0-3 weeks) and then switched to treated diets during the grower period (4-5 weeks).

RESULTS AND DISCUSSION

Weight gain, feed intake, feed/gain ratio and mortality for the period of experiment are shown in Table 2. There were no significant differences among treatments in weight gain, feed intake and feed/gain ratio over the whole period of experiment. The mortality was significantly lower in the Fe-Met 200-whole period treatment than in the Fe-Met 200-grower period treatment. Paik (2001) reported that growth performances of broilers and pigs were improved by supplementation of chelated Cu and Zn at pharmacological level. The findings were supported in the subsequent experiments (Lim et al., 2006). In the present study, however, Fe-methionine chelate supplementation at pharmacological level had no beneficial effects on the growth performance of broilers. The basal diet of the experiment contained 130 ppm Fe which is well over NRC (1994) requirement for broilers. In an earlier experiment, it was found that the supplementation of Fe-methionine chelate and $FeSO_4·7H_2O$ at 100 ppm or 200 ppm as Fe does not have adverse or positive effect on the production performance (Seo et al., 2008). As the mortality of Fe-Met 200-whole period treatment in the present experiment and earlier experiment (Seo et al., 2008) showed zero mortality, it may be worthwhile to confirm whether supplementing Fe-Met 200 from the starter period has influence in reducing mortality.

The Fe, Cu and Zn contents in muscles (breast, leg and wing) and organs (liver and kidney) of birds by treatment group are shown in Table 3. There were significant differences among treatment groups in Fe content in all muscles and organs. The Fe contents of the liver and spleen were almost same and they were about 10 times of those of muscles. Among the muscles, leg had the highest Fe content followed by wing and then breast. The Fe content of muscles and organs increased as the level and period of Fe-Met supplementation increased. The Fe-Met 200-whole period treatment group showed the highest Fe content in all muscles and organs. Compared to the control group, maximum Fe enrichments were 75% (10.05 vs. 17.59 mg/kg) in breast muscle, 34% (30.09 vs. 40.21 mg/kg) in leg muscle, 27% (22.58 vs. 28.73 mg/kg) in wing muscle, 48.7% (255.1 vs. 379.4 mg/kg) in the liver and 28.7% (252.6 vs. 325.2 mg/kg) in the spleen. Supplementation of Fe-Met 200 for grower period resulted in higher iron concentration in liver and spleen than supplementation of Fe-Met 100 for whole period. However, the same treatment resulted in lower iron concentration in muscles (breast, leg and wing) than the treatment of Fe-Met 100 for whole period indicating that iron concentration in the liver and spleen increased within a short period of time (2 wks) but enrichment of iron in the muscle took more time. It was also found that Fe can be more effectively accumulated in the muscle when Fe-Met is supplemented over the whole period of feeding than only in the later part of feeding (4^{th} to 5^{th} weeks). Present results are in well agreement with the results of Seo et al. (2008). They reported that iron-methionine chelate is more efficient than iron sulfate and 200 ppm iron supplementation as iron-methionine chelate was recommended for maximum iron enrichment in broiler meat. Regarding the effectiveness of iron-methionine chelate, Park et al. (2004) reported that Fe-methionine chelate is much more effective than $FeSO_4·7H_2O$ in enriching Fe content of eggs. Supplementing Fe-methionine at the level of 100 ppm Fe for 15 days resulted in satisfactory iron enrichment but increasing the supplementary Fe level to 200 or 300 ppm did not show any further response in iron enrichment. In the present broiler

Table 3. Effects of different level and duration of Fe-Met supplementation on the content of Fe, Cu, Zn breast, leg and wing muscle and liver and spleen of broiler chickens

Items		Control	Whole period*		Grower period**		SEM[3]
			Fe-Met 100[1]	Fe-Met 200[2]	Fe-Met 100	Fe-Met 200	
		mg/kg (wet tissue)					
Breast muscle	Fe	10.05[d]	14.08[b]	17.59[a]	11.73[c]	11.98[c]	0.27
	Cu	0.91[c]	1.40[b]	2.27[a]	1.28[b]	2.04[a]	0.09
	Zn	6.43[a]	6.37[a]	5.60[b]	6.24[a]	5.11[c]	0.12
Leg muscle	Fe	30.09[c]	34.28[b]	40.21[a]	30.53[c]	31.76[c]	0.63
	Cu	2.87	2.68	2.71	2.69	2.84	0.09
	Zn	27.35[b]	28.69[ab]	30.09[a]	29.25[ab]	27.56[b]	0.63
Wing muscle	Fe	22.58[c]	25.27[b]	28.73[a]	22.88[c]	23.62[bc]	0.75
	Cu	2.21[a]	1.94[ab]	1.88[b]	2.03[ab]	1.90[b]	0.09
	Zn	24.27[a]	19.73[c]	22.05[b]	20.52[bc]	22.31[ab]	0.71
Liver	Fe	255.10[c]	320.12[b]	379.35[a]	263.24[c]	352.89[ab]	13.20
	Cu	20.36[b]	21.08[ab]	21.97[ab]	22.73[a]	21.16[ab]	0.74
	Zn	104.12	120.62	118.63	103.58	113.98	6.04
Spleen	Fe	252.62[d]	269.42[c]	325.21[a]	271.18[c]	285.73[b]	4.80
	Cu	6.05[b]	7.05[ab]	7.36[a]	6.86[ab]	7.41[a]	0.35
	Zn	52.52[d]	58.91[b]	64.19[a]	56.79[bc]	55.03[cd]	1.13

[1] Fe-Met 100; supplemented 100 ppm Fe as Fe-methionine. [2] Fe-Met 200; supplemented 200 ppm Fe as Fe-methionine.
[a-b] Values with different superscripts in the same row are different ($p<0.05$).
* Chickens were fed treated diets during the whole feeding period (0-5 weeks).
** Chickens were fed control diet during starter period (0-3 weeks) and then switched to treated diets during the grower period (4-5 weeks).

study, however, supplementation of 200 ppm Fe as Fe-methionine resulted in better response than 100 ppm Fe in iron enrichment of broiler meat.

Cu and Zn contents in muscles and tissues showed significant differences among the treatment groups but there was no consistent tendency to evidence interactions between minerals. It is well known that there are interactions, competitive antagonisms, among Fe, Cu and Zn (Underwood, 1977). Such interactions become clearer when a certain mineral component is supplied at excessively high level while other mineral(s) is marginally deficient. In the present study, however, Fe supplementation at the level of 100 or 200 mg/kg did not show any consistent antagonistic interactions with Cu and Zn in the meat and organs of broilers. Probably, Fe supplementation level at 100 or 200 mg/kg was not too high while Cu and Zn level in the experimental diet were sufficient enough not to cause any consistent interactions.

Color measurements for the muscles of each treatment group are shown in Table 4. There were no significant difference among treatments in lightness (L), redness (a) and yellowness (b) over the whole period of experiment. However, redness (a) and yellowness (b) in the leg and breast muscle tended to increase in the Fe-Met 100 and 200-whole period groups than in the control group. Overall meat color evaluation indicated that redness of leg and breast muscle and yellowness of leg and breast muscle tended to increase when Fe was supplemented at the level of 100 or 200 mg/kg as Fe-Met for the whole feeding period. Increase of redness and yellowness and iron concentration in the meat may be caused by increase of transferrin and/or myoglobin concentration in the meat.

It was concluded that iron content of broiler meat can be effectively enriched by supplementation of 200 ppm of Fe

Table 4. Effects of different level and duration of Fe-Met supplementation on the color of breast and leg muscle of broiler chickens

Items		Control	Whole period*		Grower period**		SEM[3]
			Fe-Met 100[1]	Fe-Met 200[2]	Fe-Met 100	Fe-Met 200	
Leg muscle	L***	51.31	51.19	49.71	51.02	50.30	0.87
	a****	4.00	4.91	5.00	4.28	4.47	0.32
	b*****	8.46	9.43	9.02	8.83	8.46	0.42
Breast muscle	L	48.59	49.05	49.86	49.60	50.41	0.71
	a	2.00	2.56	2.42	1.98	1.86	0.24
	b	8.91	10.14	10.12	9.16	9.29	0.49

[1] Fe-Met 100; supplemented 100 ppm Fe as Fe-methionine. [2] Fe-Met 200; supplemented 200 ppm Fe as Fe-methionine.
* Chickens were fed treated diets during the whole feeding period (0-5 weeks).
** Chickens were fed control diet during starter period (0-3 weeks) and then switched to treated diets during the grower period (4-5 weeks).
*** L = Luminance or brightness (vary from black to white).
**** a; red-green component (+a = red, -a = green).
***** b; yellow blue component (+b = yellow, -b = blue).

as Fe-Met for 5 wks. Interactions between Fe and Cu or Zn concentration in the meat were not consistent or apparent at the present supplementation level. The meat color tended to increase in redness and yellowness by Fe-Met supplementation.

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