Iron and Copper Availability from Various Sources

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ABSTRACT A total of 1110 broiler cockerels were used in three experiments to determine the availability of iron and copper from various sources. A skim milk basal diet was fed in each experiment using chicks maintained in battery brooders beginning at one day of age. Four organic iron compounds (sequestered iron A[1] and ferric choline chloride[2] in Experiment 1 and sequestered iron B and C[1] in Experiment 2), ferrous sulfate and ferric oxide were the test iron compounds used in Experiments 1 and 2. Added dietary iron levels furnished by the test compounds were 10 and 20 p.p.m. (Experiment 1) and 5 and 10 p.p.m. (Experiment 2). The test copper compounds fed in Experiment 3 were added to furnish 1 and 2 p.p.m. copper using cupric sulfate, cuprous oxide and cuprous iodide.

No consistent differences in hemoglobin content, hematocrit (packed cell volume), chick weight and mortality were observed between ferrous sulfate and the four organic iron compounds; however, these compounds were significantly superior to ferric oxide. Hemoglobin content, hematocrit and liver copper content were significantly increased when cupric sulfate was fed as compared to results obtained with cuprous iodide or cuprous oxide. Increased chick weight gain and decreased mortality were observed with chicks fed cupric sulfate or cuprous iodide as compared to those fed cuprous oxide.

Chick availability values for ferric oxide (71 and 82%) were approximately three-quarters that of ferrous sulfate. The organic sources of iron gave high availability values (91-131%), although ferric choline chloride was not as available to the chick as the sequestered iron compounds (93-131%) as determined by regression analysis method and relative biological availability. Cuprous iodide and cuprous oxide have essentially equal availability, but the availability of these copper sources is only three-quarters that of cupric sulfate.

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INTRODUCTION

IRON and copper have received moderate attention in the literature in recent years. Fritz (1970) reported the need for attention of dietary iron in feed formulations. He, also, reported that care must be used in selection of supplemental iron sources that are biologically available. Elvehjem and Hart (1929) clearly demonstrated that anemia in chickens fed a milk diet could not be corrected with ferric oxide, but the feeding of ferric chloride or ferrous sulfate resulted in an immediate hemoglobin synthesis. Also, Willingham and Hill (1970) conducted an experiment to compare various inorganic forms of iron and copper commonly used in the poultry industry. These workers concluded the sulfate forms of iron and copper were superior to either the oxide or the carbonate form as measured by hemoglobin content and hematocrit (packed cell volume). Also, Fritz *et al.* (1970) found that the relative biological value of ferric oxide is considerably lower than that of ferrous sulfate for chicks. Nelson and Walker (1964) demonstrated a regression analysis technique used for biological evaluation of phosphate compounds. A similar method was used by Shah and Belonje (1973) for assessing iron availability. Also, Pla and Fritz (1971) proposed a relative biological availability procedure in which a comparison of iron from various supplements is made

1. Ocean Labs., Inc., Irvine. Calif. 92664
2. Naremco, Inc., Springfield, Mo. 65805

TABLE 1.—*Composition of semi-purified basal diets used in all experiments*

Ingredient	Percent
Dried skim milk	63.00
Starch	15.38
Mineral premix[1,3]	6.07
Vitamin premix[2]	1.00
Vegetable oil	10.00
DL-methionine	0.30
Glycine	0.50
L-arginine-HCl	0.50
Choline chloride (70%)	0.20
Butylated hydroxy toluene (25%)	0.05
Alpha cel	3.00

[1]The mineral premix provided the following reagent minerals in g./kg. of diet: $CaCO_3$, 25.73; K_2HPO_4, 11.20; NaCl, 6.0; $MgSO_4$, 2.50; $ZnCO_3$, 0.18; $MnSO_4 \cdot H_2O$, 1.51; $CuSO_4 \cdot 5H_2O$, 0.015; KI, 0.04; and $Na_2MoO_4 \cdot 2H_2O$, 0.0025.

[2]The vitamin premix provided the following amounts of vitamins per kg. of diet: (in milligrams) riboflavin, 9; thiamin·HCl, 6; Ca d-pantothenate, 20; niacin, 50; pyridoxine·HCl, 8; folic acid, 2; biotin, 0.2; menadione sodium bisulfate, 2; inositol, 100; vitamin B_{12}, 20 μg.; vitamin A, 25,000 U.S.P. units; vitamin D_3, 1200 I.C.U.; and Vitamin E, 17.6 I.U.

[3]$CuSO_4 \cdot 5H_2O$ was excluded in Experiment 3. The added cobalt content was maintained at 0.6 p.p.m. in all experiments. This was in addition to cobalt present in vitamin B_{12}.

to that of iron from a highly available source such as ferrous sulfate.

Much concern is expressed to find a more economical and more biologically available form of iron and copper than the established reference compounds, ferrous sulfate and cupric sulfate. Although the initial economic aspects of iron and copper feed grade supplements are not important, the availability of these compounds is important when considering the chance of deficiencies occurring. The experiments reported herein were conducted to evaluate the availability of iron and copper from various sources.

MATERIALS AND METHODS

Broiler strain cockerels obtained from a commercial hatchery were used in three experiments. At one day of age the chicks were wingbanded and randomly assigned to decks with raised wire floors in electrically heated battery brooders. Test diets and distilled water were furnished *ad libitum* in each experiment.

Composition of the skim milk basal diet used in all three experiments is shown in Table 1. Copper and cobalt content were held constant in all diets in Experiments 1 and 2 at 2 p.p.m. copper and 0.6 p.p.m. cobalt. The cobalt added was in addition to cobalt found in vitamin B_{12} to avoid cobalt deficiency. In Experiment 3, iron and cobalt were held constant in all diets at 80 and 2 p.p.m., respectively. Cupric sulfate was excluded from the skim milk basal diet in Experiment 3.

Test materials were added to the skim milk basal diet in the quantities indicated in each summary table. In Experiment 1, two commercial organic iron compounds (ferric choline chloride and sequestered iron A), ferrous sulfate and ferric oxide were fed to chicks to furnish 10 and 20 p.p.m. added dietary iron. Each diet was fed to triplicate lots of 10 broiler cockerels for a three week test beginning at one day of age. Two organic iron compounds (sequestered iron B and C) and ferrous sulfate were the test iron compounds used in Experiment 2. Added dietary iron levels furnished by the test compounds were 5 and 10 p.p.m. Each diet was fed to quadruplicate lots of 10 broiler cockerels for a four week test beginning at one day of age. Copper compounds tested in Experiment 3 were added to furnish 1 and 2 p.p.m. copper using cupric sulfate, cuprous oxide and cuprous iodide as the test compounds. Each diet was fed to quadruplicate lots of 20 broiler cockerels beginning at one day of age.

Blood was taken for hemoglobin content and hematocrit analysis from five birds per lot per dietary treatment at 21 days of age in Experiment 1 and 3; and in Experiment

TABLE 2.—*Composite data showing the effect of organic and inorganic iron compounds on hemoglobin content, packed cell volume (hematocrit) and chick weight (21 days of age) fed at 10 and 20 p.p.m. added iron (Experiment 1)*

	Hemoglobin, g. %			Hematocrit, %			Mean chick wt., g.		
Additive	10 p.p.m.	20 p.p.m.	Mean[1]	10 p.p.m.	20 p.p.m.	Mean[1]	10 p.p.m.	20 p.p.m.	Mean[1]
None	—	—	6.03[c]	—	—	26.81[c]	—	—	328[a]
Ferric choline chloride[2]	8.94	9.37	9.16[a]	33.19	34.76	33.98[a]	317	394	356[a]
$FeSO_4$	9.28	10.22	9.75[a]	32.28	35.38	33.83[a]	309	339	324[a]
FeO	7.12	8.83	7.98[b]	27.86	30.33	29.10[b]	244	261	252[b]
Sequestered Iron A[3]	8.42	10.11	9.26[a]	30.81	35.71	33.26[a]	280	385	333[a]

[1]Means within a column and without a common superscript are significantly different ($P < 0.05$).
[2]Ferric choline chloride contains 14.19% iron. Naremco, Inc., Springfield, Mo. 65805.
[3]Sequestered iron contains 10.20% iron. Ocean Labs., Inc., 1632 McGaw Ave., Irvine, Calif. 92664.

2, five birds per lot per dietary treatment were taken at 28 days of age. Body weight was determined in all experiments and mortality was checked in Experiments 2 and 3. Liver copper content was determined at 21 days of age in Experiment 3. Hematocrit values were measured by a micro-capillary centrifuge technique (Lucas and Jamroz, 1961) and hemoglobin content was determined by the oxy-hemoglobin method (Sunderman *et al.*, 1953). Liver copper content was determined on a wet weight basis by the Beckman Atomic Absorption Spectrophotometer (Model No. 485)[3]. Mineral availability was determined by the regression analysis method (Nelson and Walker, 1964) and relative biological availability (Pla and Fritz, 1971).

A factorially arranged randomize complete block design was used in each experiment. Statistical examination of the data was performed using analysis of variance (Steel and Torrie, 1960). Duncan's new multiple range test (1955) was used to separate significant differences between means. All statements of significant differences refer to the 5% level of probability.

3. Beckman Instrument, Inc., Fullerton, Calif. 92634

RESULTS AND DISCUSSION

Experiments 1 and 2. Essentially the same results were obtained in Experiments 1 and 2 and these results are shown in Tables 2 and 3, respectively. In Experiment 1, the addition of ferrous sulfate and the organic iron compounds (ferric choline chloride and sequestered iron A) resulted in significant improvements in chick performance (measured by hemoglobin content, hematocrit, and chick weight) when compared to ferric oxide. The basal diet resulted in significantly poorer hemoglobin contents and packed cell volumes when fed to chicks. This was not apparent when chick weight was used as the criterion. The organic iron sources fed in this experiment gave equal chick responses as measured by hemoglobin content, packed cell volume and chick weight when compared to ferrous sulfate.

Ferrous sulfate and the two commercial organic iron compounds (sequestered iron B and C) fed to chicks in Experiment 2 gave equal responses at corresponding levels of iron in the diet using hemoglobin content and packed cell volume as the criteria; however, ferrous sulfate gave a significantly increased body weight and a decreased mortality as compared to sequestered iron B compound. The basal diet was significantly poorer than the experimental diets containing either the

TABLE 3.—*Composite data showing the effect of organic and inorganic iron compounds on hemoglobin count, packed cell volume, chick weight and percent mortality (28 days of age) fed at 5 and 10 p.p.m. added iron (Experiment 2)*

	Hemoglobin, g. %			Hematocrit, %			Mean chick wt., g.			% Mortality		
Additive	5 p.p.m.	10 p.p.m.	Mean[1]	5 p.p.m.	10 p.p.m.	Mean[1]	5 p.p.m.	10 p.p.m.	Mean[1]	5 p.p.m.	10 p.p.m.	Mean[1]
None	—	—	4.19^{b}	—	—	14.00^{b}	—	—	119^{c}	—	—	98^{c}
Sequestered Iron B[2]	10.19	10.86	10.52^{a}	30.65	33.15	31.90^{a}	250	292	271^{b}	70	60	65^{b}
$FeSO_4$	9.70	10.46	10.08^{a}	30.25	32.50	31.38^{a}	299	308	304^{a}	50	28	39^{a}
Sequestered Iron C[2]	9.95	10.48	10.22^{a}	31.80	32.95	32.38^{a}	273	289	281^{ab}	60	45	52^{ab}
Mean[1]	9.95^{b}	10.60^{a}		30.90^{b}	32.87^{a}		274^{a}	296^{a}		60^{b}	44^{a}	

[1]Means within a column or row grouping and without a common superscript are significantly different ($P < 0.05$).
[2]Sequestered iron B and C contains 12.56% and 8.63% iron, respectively. Ocean Labs., Inc., 1632 McGaw Ave., Irvine, Calif. 92664.

TABLE 4.—*Composite data showing the effect of various copper compounds on hemoglobin content and packed cell volume (Hematocrit) of chicks (21 days of age) fed diets containing 1 and 2 p.p.m. copper (Experiment 3)*

Additive	Hemoglobin, g. %			Hematocrit, %		
	1 p.p.m.	2 p.p.m.	Mean[1]	1 p.p.m.	2 p.p.m.	Mean[1]
None	—	—	3.99[b]	—	—	20.4[b]
Cuprous oxide	3.63	4.13	3.88[b]	18.6	20.8	19.6[b]
Cupric sulfate	4.68	5.47	5.08[a]	20.2	23.1	21.7[a]
Cuprous iodide[2]	3.90	4.47	4.19[b]	18.4	19.6	19.0[b]
Mean[1]	4.07[b]	4.69[a]		19.0[b]	21.2[a]	

[1]Means within a column or row grouping and without a common superscript are significantly different ($P < 0.05$).
[2]Diamond Shamrock Chemical Company, Baltimore, Maryland 21226.

ferrous sulfate or the organic iron compounds in Experiment 2 as measured by hemoglobin content, packed cell volume, chick weight and mortality. In both experiments, a significant improvement was made in each criterion at the higher level of added iron except in Experiment 2 in which no improvement in chick weight was detected due to the level of added iron.

Experiment 3. Results of Experiment 3 are shown in Tables 4 and 5. The five criteria tested in this experiment included hemoglobin content, packed cell volume, liver copper content, chick weight and mortality. Cupric sulfate when fed to chicks resulted in significantly higher hemoglobin contents, packed cell volumes and liver copper contents as compared to either cuprous oxide or cuprous iodide; however, both cupric sulfate and cuprous iodide were superior as compared to cuprous oxide using mortality and chick weight as the criteria. Extremely low hemoglobin contents were found due to the low copper diets fed. In all criteria tested, an improved performance was noted at the higher level of added copper.

Ferrous sulfate and cupric sulfate were used as the standards in testing the availability of iron and copper sources (Table 6). As found by other researchers, the oxide forms of iron and copper gave low availability values in this study as determined by the regression analysis method and relative biological availability. The organic sources of iron (ferric choline chloride and sequestered iron A, B

TABLE 5.—*Composite data showing the effect of various copper compounds on liver copper content, % mortality, and weight of chicks (21 days of age) fed diets containing 1 and 2 p.p.m. added copper (Experiment 3)*

Additive	Liver copper, p.p.b.			% mortality			Mean chick wt., g.		
	1 p.p.m.	2 p.p.m.	Mean[1]	1 p.p.m.	2 p.p.m.	Mean[1]	1 p.p.m.	2 p.p.m.	Mean[1]
None	—	—	8.9[b]	—	—	54[b]	—	—	112[b]
Cuprous oxide	13.0	16.5	14.8[b]	51	47	49[b]	116	124	120[b]
Cupric sulfate	23.0	29.4	26.2[a]	26	24	25[a]	184	224	204[a]
Cuprous iodide	14.4	19.5	16.9[b]	28	22	25[a]	169	202	185[a]
Mean[1]	16.8[b]	21.8[a]		44[a]	39[a]		156[b]	183[a]	

[1]Means within a column or row grouping and without a common superscript are significantly different ($P < 0.05$).

Table 6.—*Mineral availability and relative biological availability of various iron and copper sources tested in Experiments 1, 2 and 3*

Iron or copper source	Mineral availability, %[1]	Relative biological availability, %[2]
Ferrous sulfate (standard)	100	100
Ferric choline chloride	91	94
Ferric oxide	71	82
Sequestered iron A	93	100
Sequestered iron B	106	104
Sequestered iron C	131	104
Cupric sulfate (standard)	100	100
Cuprous oxide	60	76
Cuprous iodide	60	82

[1]Regression analysis method by Nelson and Walker (1964).
[2]Relative Biological Availability method by Pla and Fritz (1971).

and C) gave very high availability values (91-131%), although ferric choline chloride was not as available to the chick as the sequestered iron compounds. Cuprous iodide and cuprous oxide have essentially equal availability for the chick.

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