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A Comparison of Iron Bioassay Diets[1]

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ABSTRACT Two broiler chick experiments were conducted to evaluate four basal diets for iron bioassay suitability. The test basal diets, identified according to their principal ingredients and iron content, were: (1) starch-skim milk—15 p.p.m., (2) degerminated corn-skim milk—18 p.p.m., (3) degerminated corn-fish meal-isolated soy—45 p.p.m., and (4) degerminated corn-fish meal-dehulled soy—59 p.p.m. Significant differences between an iron source with known low availability (ferric oxide) and a highly available iron source (ferrous sulfate) were not detected with the degerminated corn-fish meal-isolated soy or the degerminated corn-fish meal-dehulled soy diets. Likewise, there were no significant differences found between supplemental iron levels, 10 and 20 p.p.m. The corn-skim milk and starch-skim milk diets were both found to be satisfactory for iron bioassays. However, the sample size needed to estimate the population mean was almost twice as great for the starch-skim milk fed groups than was needed for the corn-skim milk fed groups which indicates the corn-skim milk diet obtained greater sensitivity in testing iron sources and levels. Mortality was excessively high in the starch-skim milk fed group. Ferrous sulfate was superior to ferric oxide as a source of iron.

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INTRODUCTION

SKIM milk-sugar type basal diets have generally been employed in iron bioassays. A skim milk-sucrose basal diet was used by Hill and Matrone (1961), Washburn (1969), Willingham and Hill (1970), Kubena *et al.* (1972a, b), and McNaughton *et al.* (1974). Pla and Fritz (1971) used a more practical low iron basal diet, which contained degerminated corn, skim milk and glucose.

Because of the excessive mortality (25-70%) in some of the studies of the above workers, four basal diets were tested in an attempt to find a more suitable iron and copper bioassay diet. Stein's test (Stein,

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[1] Journal article number 3125 from M.A.F.E.S.

1945) has been used successfully to determine the least number of samples required to estimate a population mean. Dilworth *et al.* (1965) with the use of Stein's test reported that diets critically deficient in phosphorus required more samples of bones for ashing to determine the treatment mean than were needed for less deficient diets.

MATERIALS AND METHODS

Day-old commercial broiler cockerels were wing-banded and randomly placed in electric heated battery brooders. The batteries were coated with a plastic spray to decrease metal contact. Composition of the basal diets is shown in Table 1. Either reagent grade ferrous sulfate or reagent grade ferric oxide were added to the basal to supply 10 and 20 p.p.m. of supplemental iron. Added iron and copper ratios were held constant at 20:1. Each test diet was fed to quadruplicate lots of ten broilers from 1 to 21 days of age. Test diets and distilled water were furnished *ad libitum.* Individual body weights were obtained at 21 days of age. Mortality was recorded daily.

Ferrous sulfate was used as a standard since 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

Table 1.—*Composition of basal diets*

Ingredient	Starch-skim milk diet, %	Corn-skim milk diet, %	Corn-fish-isolated soy diet, %	Corn-soy-fish diet, %
Degerminated yellow corn[1]	—	48.75	66.05	57.30
Soybean meal, 50% protein	—	—	—	29.00
Fish meal, 65% protein	—	—	3.00	3.00
Isolated soy protein	—	—	20.00	—
Skim milk	71.59	38.00	—	—
Starch	15.38	—	—	—
Alpha cel	1.62	—	—	—
Vegetable oil	4.00	4.00	4.00	4.00
Vitamin and mineral premix[2]	6.50	6.50	6.50	6.50
L-Arginine-HCl	0.83	0.96	0	0.01
Methionine hydroxy analogue—Ca, 93%	0	0.36	0.27	0.17
Glycine	0	1.00	0	0
L-Lysine-HCl	0	0.25	0.04	0
Choline Chloride	0.08	0.18	0.14	0.02
Total, %	100.0	100.0	100.0	100.0
Calculated analysis:				
Crude protein, %	22.01	18.88	22.28	21.59
Energy, M.E. Cal./kg.	2664	2721	2971	3212
Calcium, %	1.00	1.00	1.00	1.00
Phosphorus, %	0.70	0.70	0.70	0.70
Methionine + Cystine, %	0.86	0.86	0.86	0.86
Lysine, %	1.25	1.25	1.25	1.25
Iron chemical analyses, p.p.m.	15	18	45	59

[1] Approximately 70% degerminated.

[2] A vitamin premix and mineral premix were added to each diet to meet minimum N.R.C. (1971) requirements. Alpha cel was the carrier for these premixes.

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 and hematocrit (packed cell volume).

Blood was taken from the brachial vein from five birds per lot at 21 days of age for hemoglobin and hematocrit analysis. Hematocrit analyses were determined by a micro-capillary centrifuge technique (Johnson, 1955) and hemoglobin was determined by the oxy-hemoglobin method (Sunderman *et al.*, 1953). Iron content of each basal diet was determined with the Beckman Atomic Absorption Spectrophotometer.[2] The hemoglobin data obtained in Experiment 2 were used to calculate the number of observations necessary to estimate the population mean in accordance with the procedure of Stein (1945).

A factorially arranged randomized complete block design was used in each experiment. Statistical examination of the data was performed using the 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 significance refer to the 5% level of probability.

RESULTS AND DISCUSSION

The first experiment was conducted primarily to find a diet more suitable for iron bioassays than the starch-skim milk diet which has previously resulted in extremely high chick mortality (Kubena *et al.*, 1972a, b; McNaughton *et al.*, 1974). The National Research Council's (N.R.C.) iron requirement for the chick is 80 p.p.m. (1971). If the chick does require this amount of iron, the possibility exists that basal diets containing much more iron than the skim milk-sugar type diet could be used for bioassay purposes. A basal diet which would support better growth and better livability than the skim milk-sugar type diets previously used and still respond to iron supplementation is needed. The diets presented in Table 1 were all supplemented with 10 and 20 p.p.m. of iron from either ferrous sulfate or ferric oxide. The results obtained from feeding each diet to quadruplicate groups of chicks are shown in Tables 2, 3, and 4. It is obvious that the corn-soy-fish diet and the corn-fish-isolated soy diets are unsuitable since these diets do not differentiate between the two sources of iron, nor do they show any difference in response due to differences in supplemental iron levels. Since the corn-fish-isolated soy diet contained by chemical analysis only 45 p.p.m. of iron, these results indicate that the NRC requirement level of 80 p.p.m. is much too high.

Both the starch-skim milk and corn-skim milk diets, which contained 15 and 18 p.p.m. of iron, respectively, according to chemical analysis, appear to be quite sensitive to iron supplementation (Tables 3 and 4). Feeding each of these diets resulted in significantly higher values for hemoglobin, hematocrit and body weight when supplemented with ferrous sulfate as compared to ferric oxide. Likewise, the response for all these criteria were significantly greater with 20 p.p.m. of supplemental iron *versus* 10 p.p.m., indicating that both diets are rather sensitive to iron supplementation.

The starch-skim milk basal fed birds had significantly lower hemoglobin levels than the corn-skim milk fed birds. In contrast, growth rate and livability were significantly improved with the corn-skim milk basal diets (Table 2). Birds fed the starch-skim milk basal diet grew significantly slower and had significantly poorer livability than all other groups. The overall results from Experiment 1 suggest that the corn-skim milk diet was superior to

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[2] Beckman Instruments, Inc. Fulton, Calif. Model No. 485.

Table 2.—*Effect of basal diets on chick response, Exp. 1*

Diet	Hemoglobin, g. %[1]	Hematocrit, %[1]	Chick weight g.[1]	Mortality %[1]
Starch-skim milk	4.83d	25b	118d	33.2b
Corn-skim milk	6.89c	26b	206c	5.9a
Corn-fish-isolated soy	9.41b	32a	403b	2.6a
Corn-soy-fish	10.59a	32a	460a	2.6a

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

Table 3.—*Effect of type of diet on the chick response to iron furnished by either ferrous sulfate or ferric oxide (hemoglobin content and hematocrit), Exp. 1*

		Hemoglobin, g. %			Hematocrit, %		
		Added Fe, p.p.m.			Added Fe, p.p.m.		
Diet	Iron source	10	20	Mean[1]	10	20	Mean[1]
Starch-skim milk	Ferric oxide	8.02	8.55	8.28b	28.6	30.1	29.4b
	Ferrous sulfate	9.35	10.36	9.86a	32.1	34.5	33.3a
Mean[1]		8.68b	9.46a		30.4b	32.3a	
Corn-skim milk	Ferric oxide	8.57	9.66	9.12b	29.6	32.0	30.8b
	Ferrous sulfate	9.95	10.98	10.46a	31.2	33.4	32.3a
Mean[1]		9.26b	10.32a		30.4b	32.7a	
Corn-fish-isolated soy	Ferric oxide	11.73	11.37	11.55a	34.3	36.6	35.4a
	Ferrous sulfate	10.80	9.66	10.23b	33.2	35.6	34.4a
Mean[1]		11.26a	10.52a		33.8b	36.1a	
Corn-soy-fish	Ferric oxide	11.56	11.09	11.32a	35.1	34.8	35.0a
	Ferrous sulfate	10.84	10.21	10.52a	36.0	35.2	35.6a
Mean[1]		11.20a	10.65a		35.6a	35.0a	

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

Table 4.—*Effect of type of diet on the chick response to iron furnished by either ferrous sulfate or ferric oxide (chick weight and mortality), Exp. 1*

		Chick weight, g.			Mortality, %		
		Added Fe, p.p.m.			Added Fe, p.p.m.		
Diet	Iron source	10	20	Mean[1]	10	20	Mean[1]
Starch-skim milk	Ferric oxide	201	214	208b	25.3	14.8	20.0a
	Ferrous sulfate	234	259	246a	31.0	6.8	18.9a
Mean[1]		218b	236a		28.2b	10.8a	
Corn-skim milk	Ferric oxide	246	270	258b	3.5	1.8	2.6a
	Ferrous sulfate	311	330	320a	3.5	5.3	4.4a
Mean[1]		278b	300a		3.5a	3.6a	
Corn-fish-isolated soy	Ferric oxide	419	437	428a	3.5	1.8	2.6a
	Ferrous sulfate	416	439	428a	1.8	6.8	4.3a
Mean[1]		418a	438a		2.6a	4.3a	
Corn-soy-fish	Ferric oxide	479	485	482a	3.5	5.3	4.4a
	Ferrous sulfate	477	488	482a	3.2	3.2	3.2a
Mean[1]		478a	486a		3.4a	4.2a	

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

TABLE 5.—*Effect of type of diet on the chick response to iron furnished by either ferrous sulfate or ferric oxide, Exp. 2*

Diet	Iron source	Hemoglobin, g. %[1]			Hematocrit, %[1]		
		Added Fe, p.p.m.			Added Fe, p.p.m.		
		10	20	Mean[1]	10	20	Mean[1]
Starch-skim milk	Ferric oxide	7.40	9.32	8.36b	30.5	33.4	32.0b
	Ferrous sulfate	9.48	10.83	10.16a	33.0	34.9	34.0a
Mean[1]		8.44b	10.08a		31.8b	34.2a	
Corn-skim milk	Ferric oxide	8.73	9.66	9.20b	30.9	33.2	32.0b
	Ferrous sulfate	9.77	11.62	10.70a	33.2	35.2	34.2a
Mean[1]		9.25b	10.64a		32.0b	34.2a	

Diet	Iron source	Chicken weight, g.[1]			Mortality, %[1]		
		Added Fe, p.p.m.			Added Fe, p.p.m.		
		10	20	Mean[1]	10	20	Mean[1]
Starch-skim milk	Ferric oxide	171	207	189b	37.5	20.0	28.8b
	Ferrous sulfate	214	253	234a	12.5	5.0	8.8a
Mean[1]		192b	230a		25.0b	12.5a	
Corn-skim milk	Ferric oxide	249	293	271b	10.0	5.0	7.5b
	Ferrous sulfate	305	338	322a	2.5	0	1.2a
Mean[1]		277b	316a		6.2a	2.5a	

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

TABLE 6.—*Effect of basal diets on the chick response, Exp. 2*

Diet	Hemoglobin, g. %[1]	Hematocrit, %[1]	Chick weight, g.[1]	Mortality, %
Starch-skim milk	5.91b	29.4a	114b	62.5b
Corn-skim milk	6.82a	30.1a	210a	15.0a

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

all other diets used, since it supported good growth, good livability and responded to iron supplementation. Since Dilworth *et al.* (1965) found with the use of Stein's test (1945) that phosphorus assay diets can be too deficient for maximum efficiency, a second experiment was conducted to determine if this was true with iron bioassay diets.

Experiment 2 was conducted using only the corn-skim milk and starch-skim milk diets to verify the data from Experiment 1. These results are shown in Tables 5 and 6. The hemoglobin data from this experiment were subjected to Stein's test (1945) to determine more precisely which diet was best for use in iron bioassay studies (Table 7). Data obtained in Experiment 2 with these two diets were essentially identical to the data obtained in Experiment 1. Significant improvements in hemoglobin, hematocrit and body weight were found with both diets using ferrous sulfate *versus* ferric oxide and 20 p.p.m. of added iron *versus* 10 p.p.m. (Table 5). Also, hemoglobin, weight, and mortality were significantly improved with the corn-skim milk basal diet, as compared to the starch-skim milk basal diet (Table 6).

The results from Stein's test in which the number of blood samples needed to estimate the hemoglobin population mean for each treatment with confidence limits of 95% are shown in Table 7. These data indicate that

Table 7.—*Hemoglobin samples required to estimate mean sample response to supplemental iron levels as affected by diet (Stein's Test), Exp. 2*

Diet	Added Fe level, p.p.m.	Deviation from mean[1] 5%	10%
Corn-skim milk	10	86	21
	20	67	17
Mean		76	19
Starch-skim milk	10	156	39
	20	120	30
Mean		138	34

[1] Confidence limits of 95%.

the corn-skim milk diet is approximately twice as efficient as the starch-skim milk diet. For example, 86 blood samples are needed to estimate the population mean using the corn-skim milk diet supplemented with 10 p.p.m. of iron; whereas, 156 blood samples are needed under identical conditions when the starch-skim milk diet is employed. These data also show that more blood samples are required to estimate the treatment mean where the diets were supplemented with 10 p.p.m. of iron than are needed with 20 p.p.m. of supplemental iron. The results in this study show that Stein's test could be used, as effectively as Dilworth *et al.* (1965) used it, in determining the blood samples required in determining a population mean.

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