UNCOOKED GRAIN CORN AS A SOURCE OF IRON FOR NORMAL AND ANEMIC RATS

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ABSTRACT

Hemoglobin and packed-cell volume were normal in rats fed for six weeks from weaning with four types of uncooked grain corn supplemented to provide adequate levels of all known nutrients. Iron content of the livers and spleens of the rats reflected the concentration of iron in the samples of corn. Corn was also evaluated as a source of iron for restoring hematological parameters in anemic rats. Relative biological value of iron in the grain was about half that of the ferrous sulfate used in the control diets. Hemoglobin and packed-cell volume were highest in rats fed waxy corn, lowest in those fed normal corn, and intermediate in animals given diets containing high-lysine and amylose corn. These differences may be related to iron content of the corn samples.

Since the introduction of corn with the *opaque*-2 gene in 1964 (1), many studies comparing the nutritive value of this corn with normal corn or with other grains have been reported. Most of these studies have been concerned with growth performance of animals of economic importance such as the chick (2–4) or pig (4,5). However, there have been some evaluations of the nutritive value of the *opaque*-2 corn for humans (6), and rats have been used as subjects to determine the most limiting amino acid in the mutant corn (7) and to estimate the availability of lysine (8). These reports have been concerned primarily with the improved protein and amino acid composition of *opaque*-2 corn in comparison with normal corn, and have contained little information on effects of genetic lines on other nutrients.

There are conflicting reports in the literature on the efficacy of corn as a dietary source of iron for man. Siderosis among Bantus has been attributed to a combination of the high iron content of their diets and "certain properties of maize," which forms a major portion of their food (9). Kinney *et al.* (10) showed that iron accumulated in liver of rats fed a diet of corn grits and lard, and that liver iron deposition was markedly increased when the dietary iron content was increased. These diets were inadequate in several nutrients, and it was demonstrated that pathological accumulation of iron in the liver could be largely prevented by correcting the phosphorus deficiency of the diets (11). More recently, the prevalence of iron deficiency anemia in countries where cereals provide the bulk of calories has been attributed to poor absorption of iron from grains such as corn (12,13).

In this paper, results are reported from two studies undertaken to assess the effects of corn on iron metabolism in rats. The first trial was designed to determine whether a corn diet, supplemented to meet most of the animal's nutrient requirements, would support normal iron utilization. The primary objective of the second study was to measure the availability of iron in corn for hemoglobin regeneration in anemic rats. In both experiments, four different grain types were used in order to evaluate the effects of these characteristics of the corn on iron metabolism.

MATERIALS AND METHODS

Weanling male rats of the Sprague-Dawley strain were obtained from ARS/Sprague-Dawley (Madison, Wisconsin) for the first experiment and from Charles River Breeding Laboratory (Wilmington, Massachusetts) for the second study. Corn of four grain types (normal, high-lysine, amylose, and waxy) was evaluated and each grain type consisted of a mixture of several breeding lines. Corn produced in 1973 was used in the first study and that grown in 1974 was used in the second experiment. Shelled corn was ground in a hammer mill to a particle size not greater than 1.5 mm.

Samples of the ground corn were wet-ashed for iron determination as described below. Since the values for iron content of corn of the 1973 crop were higher than expected, shelled corn which had not been through the hammer mill was ground in a small laboratory mill and analyzed. Values obtained for iron content of the samples (mg/kg) ground in the laboratory mill and the hammer mill were, respectively, normal, 15.3–26.5; high-lysine, 17.1–30.2; amylose, 17.3–66.6; and waxy, 15.9–23.8. Thus, all of the samples were contaminated with iron from the hammer mill, and the amylose sample, which was the first one ground, received the heaviest load. Total nitrogen content of the ground corn was measured by Kjeldahl analysis, and amino acids were determined on a Durrum 500 analyzer.

The experimental diets (Table I) used in the first study were formulated to contain the maximum amount of corn possible while at the same time providing enough of the animal's nutrient requirements to support an adequate rate of growth. Supplements were added to all experimental diets at the same rate with no attempt to account for the differences in composition among the grain types. The diets contained about 13% protein from corn and/or casein and l-methionine. The salt mixture added to the corn diets supplied 10 mg of iron per kg of diet in the form of ferrous sulfate. Rats with normal iron stores were fed these diets for six weeks beginning when they were four weeks old.

TABLE I
Composition of Diets Used

	Experiment I		Experiment II		
	Control	Test	Control g/kg	Test	
Corn	...	878	...	400	800
Casein	143	50	143	120	98
l-Methionine	2	2	2	2	2
Vitamin mix	22	22	10	10	10
Salt mix	35[a]	27[b]	40[c]	40[c]	40[c]
Corn oil	65	20	50	50	50
Cellulose	15	...	15	15	...
Dextrin	718	...	740	363	...

[a]Williams and Briggs salt mix, modified (14).
[b]Provided per kg of diet: Ca, 5.8 g; Na, 555 mg; Cu, 3.5 mg; Mn, 49 mg; I, 0.19 mg; and Fe, 10 mg.
[c]Williams and Briggs salt mix, modified (ferric citrate omitted).

For the first three weeks of the second study, weanling rats were fed the basal diet (Table I) to which no iron was added. At the end of this period their hemoglobin content averaged 6.2%, and the rats were divided into 12 dietary groups on the basis of their hemoglobin level. One group was continued on the iron deficient diet, and three other groups were given this diet supplemented with 6, 12, or 24 mg/kg of iron as ferrous sulfate. The other groups were given diets containing either 40 or 80% of one of the types of corn as the source of iron. These diets were supplemented with vitamins and minerals, other than iron, as indicated for the first study. Sufficient casein and l-methionine were added to raise the lysine content of the diets to approximately 1%, and the methionine content of the diets was approximately 0.6%. These iron repletion diets were fed for 11 days.

Animals were housed individually in stainless steel cages and provided with food and deionized water ad libitum. Food intake was measured every second day and the animals were weighed twice weekly. The animals were anesthetized with ether (experiment I) or with sodium pentobarbital (experiment II) and exsanguinated by heart puncture. Blood was collected in a plastic syringe and allowed to clot under refrigeration. Serum was obtained after centrifugation and stored at −40° C until used. The liver and spleen were removed and washed in deionized water for separate mineral analysis.

All of the tissues, including serum, were wet-ashed with nitric, perchloric, and sulfuric acids for mineral analyses. Iron was determined with ortho-phenanthroline or ferrozine (15) after reduction with hydroxylamine hydrochloride. Copper was measured with bis(1-piperidyl thiocarbonyl) disulfide as described by Carter (16). Total iron-binding capacity of the serum was determined after saturation with iron and removal of the excess with magnesium carbonate. Total hemoglobin was determined as the cyanmethemoglobin complex and packed-cell volume (PCV) was measured in heparinized microtubes.

Data from the first experiment were subjected to analysis of variance, and treatment differences were evaluated by Duncan's multiple range test (17). In the

TABLE II
Effects of Corn of Several Grain Types on Growth and Tissue Iron Content of Rats. Experiment I[a]

	Weight Gain g	Feed Eff.[b] g/g	Kidney Weight g	Kidney % of Body wt.	Total Iron Liver µg	Total Iron Spleen µg	Dietary Composition Protein %	Dietary Composition Lysine %	Dietary Composition Methionine %	Dietary Composition Iron mg/kg
Normal corn	197b	.268b	1.77b	0.68b	909b	169b	12.4	0.65	0.48	33
High-lysine corn	212ab	.276b	1.86b	0.67b	1013b	166b	13.0	0.77	0.48	36
Amylose corn	216ab	.280b	1.85b	0.66b	1170a	242a	13.6	0.71	0.51	69
Waxy corn	206ab	.277b	1.79b	0.66b	910b	186b	13.3	0.68	0.49	31
Casein control	225a	.332a	2.08a	0.72a	1060ab	190b	13.4	1.15	0.65	38

[a]Values in a column having different letters are different at $P<0.01$.
[b]Feed efficiency: g of weight gained/g of feed consumed.

second experiment, data obtained from the corn diets were evaluated by analysis of variance and, in addition, two different calculations were used to estimate the availability of iron in corn in comparison to that supplied by ferrous sulfate in the standard diets. The slope-ratio test, which has been recommended (18,19) as a valid assay for such studies, was one of the comparisons used. A regression equation was calculated using data obtained from rats fed the standard diets and another using those derived from animals given the several corn diets. The ratio of the slope of the line described by the experimental diets to that of the line for the standard diets is a measure of the relative biological value of iron in corn. The second method used was similar to the graphical method described by Pla and Fritz (20), but was calculated mathematically rather than graphically. A regression equation was calculated with data obtained from animals on the standard diets and then solved for the theoretical iron intake that would give the response obtained for each experimental diet. The ratio of the theoretical iron intake to the actual amount of the mineral consumed by the animals is the relative biological value of iron in that diet.

RESULTS AND DISCUSSION

Experiment I

Feed efficiency (weight gained per gram of feed consumed) was lower in the animals fed the corn diets than in those given the casein control diet (Table II).

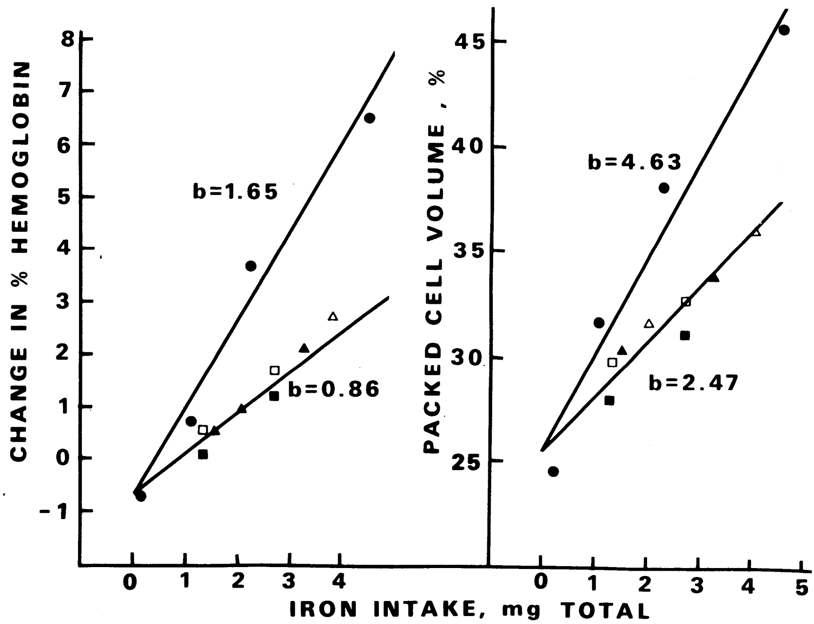

Fig. 1. Change in hemoglobin and final packed-cell volume after regeneration of iron status in anemic rats with control diets containing ferrous sulfate (●), or experimental diets containing two levels of corn of four grain types: normal (■), high-lysine (□), amylose (▲), or waxy (Δ).

The concentration of lysine and methionine in the corn diets was only about 70% of the amount recommended for growth of young rats and may account for the reduced feed efficiency. The animals grew fairly well on the corn diets and only those fed the normal corn gained significantly less weight than the controls. The diet containing the normal corn was lowest in protein and lysine content. Both the absolute weight of the kidneys and the weight of kidneys relative to body size were lower in animals fed the corn diets than in those fed the control diet.

There were no significant differences due to dietary treatment in the hematological parameters measured. The hemoglobin concentration averaged 15.9 g/100 ml and PCV was 52%. Liver and spleen iron contents were within normal ranges, and dietary differences were reflected only in the increased iron content of tissues of animals fed the diet containing the amylose corn, which contained twice as much iron as the other diets.

Experiment II

The data and regression equations calculated for the slope-ratio evaluation are plotted in Fig. 1. For both change in hemoglobin during the regeneration period and PCV, the standard and test equations have a common y-intercept for zero intake of iron. This is a requirement for the assay method to be valid. The ratios of the pairs of slopes for each parameter indicate a biological value of about 52% for iron in the several types of corn.

The data used for the second method of estimating biological availability of iron in corn are the same as those shown in Fig. 1. In fact, the regression equation for the standard diet is the same for both methods of evaluation. Results of the single point calculations are shown in Table III. The values obtained from both hemoglobin and PCV at each of the levels of dietary corn indicate approximately the same relative biological value of iron contained in any one of the grain types. For normal corn, the biological value is about 40% of that obtained for diets containing ferrous sulfate. For the other three corn types, lysine, amylose, and waxy, iron has a relative biological value of approximately 55%. These estimates of biological value are quite similar to those obtained from the slope-ratio calculation. The lower availability of iron in normal corn may have been inferred from the slope-ratio graph, since the plotted points for hemoglobin and PCV of animals fed this type of corn were all below the regression line.

TABLE III
Relative Biological Value (%) of Iron in Corn of Several Grain Types.[a]
Experiment II

	Change in Hemoglobin		Packed-Cell Volume	
	Corn in the Diet		Corn in the Diet	
	40%	80%	40%	80%
Normal corn	39.9	44.9	33.9	43.6
High-lysine corn	62.0	53.3	63.3	55.7
Amylose corn	51.1	52.6	60.3	52.6
Waxy corn	51.2	51.7	61.1	54.9

[a]Calculated from the regression equations obtained from the control diets as described in the text.

Analysis of variance of data from the eight treatment groups indicated significant differences due to level of corn in the diet for all measurements of iron metabolism, except for iron content of the spleens (Table IV). Since corn was the primary source of iron in these diets, such differences would be expected. Only PCV and change in hemoglobin during the regeneration period showed differences associated with grain types of the corn, and these appear to be related to the iron content of the different lots of corn (Table V).

Indications of normal iron metabolism in rats fed diets containing about 88% corn in the first study differ from reports in the literature of excessive (9–11) or very poor (12,13) absorption of iron from this grain. The absorption of excessive amounts of iron in the cases reported were undoubtedly due to a combination of the poor quality of the diets used and the overload of iron that they contained.

In many references in the literature documenting poor absorption of iron from corn by adults (13) or infants (21), the grain, usually cooked as a porridge, is fed alone. The data reported in this paper of normal iron metabolism in rats fed diets of corn grain supplemented with essential nutrients suggest that the poor nutritional quality of corn, when fed alone, may contribute significantly to the lack of availability of its iron.

TABLE IV
Effect of Corn as a Source of Iron on Several Parameters of Iron Metabolism in Anemic Rats.[a] Experiment II

	% Corn in the Diet	
	40	80
Change in % hemoglobin	0.56	1.95[b]
Packed-cell volume, %	29.8	33.5
Serum iron, μg/100 ml	67.3	87.1
Serum iron-binding capacity, μg/100 ml	900	852
Liver iron, μg total	194	217
Liver copper, μg total	54.5	37.8

[a]See Table V for amount of iron in the corn samples used.
[b]Differences due to concentration of corn in the diet are significant at P<0.01 for all parameters listed.

TABLE V
Differences in Hemoglobin Change and Packed-Cell Volume of Anemic Rats Associated with Type of Grain in Corn.[a] Experiment II

	Change in % Hemoglobin	Packed-Cell Volume %	Iron in Corn mg/kg
Normal corn	0.69	29.6	16.0
High-lysine corn	1.12	31.3	16.3
Amylose corn	1.32	31.9	18.7
Waxy corn	1.84	33.9	23.3

[a]Differences associated with type of grain in corn were significant at P<0.01 for both change in hemoglobin and packed-cell volume.

In animals that have been subjected to the stress of iron deficiency, absorption of iron from raw corn is inefficient compared to absorption of the iron from ferrous sulfate in the control diets. Even though the corn diets used in experiment II contained sufficient casein and methionine to supply both lysine and methionine at approximately the levels recommended for growing rats, differences in protein quality between the control and experimental diets may have affected iron metabolism. On the other hand, raw corn itself may have an adverse effect on absorption of iron that is apparent only when the experimental subjects are already iron-deficient.

Differences in iron metabolism of rats associated with the type of grain in corn used in these studies appear to be related to iron content of the corn samples rather than to the characteristics of the grain.

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