THREE STUDIES OF NUTRITIONAL QUALITIES OF FORTIFIED FLOUR

by

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I. INTRODUCTION

According to a survey by the Food and Nutrition Board of the National Academy of Science, there is evidence of potential risk of deficiency of vitamin A, thiamin, iron, calcium, magnesium, and zinc among significant segments of the U. S. population (1). Since 26% of the daily caloric intake in the U. S. diet comes from cereal-grain products, e.g. wheat flour, the Board has proposed increased nutrient fortification of cereal-grain products to decrease the risk of nutritional deficiencies. The proposal increases the iron level and adds vitamin A, vitamin B_6 (pyridoxin), folic acid, magnesium, zinc, and calcium to the present fortifications of cereal-grain products (2).

The overall objective of this project[1] is to determine the technological feasibility of implementing the proposed fortification policy for cereal-grain products (2). The studies reported in this thesis are those with fortified wheat flour.

The purpose of experiment I was to test whether use of diets containing flour with the proposed 1974 fortification levels (2) fed to growing rats to supply about 30% of calories would result in any measurable changes in animal performance, such as growth and increased levels of the nutrients used for fortification in blood and tissues.

In experiment II, diets containing fortified flour stored at 40°C for 6 months were fed to rats to test whether there was a decrease in

[1]Kansas Agricultural Department Station, 0969.

the relative biopotency of the stabilized vitamin A used for fortification that remained in the flour. When originally prepared, the fortified flour contained 5481 units vitamin A/lb, but after 6 months storage under accelerated conditions (40°C), vitamin A content had decreased to 3169 IU/lb as determined by the Carr-Price method (3).

The objective of experiment III was to determine whether increased levels of cellulose or added calcium phytate in flour-based diets fed to rats affected utilization of supplemental iron added either as $FeSO_4$ or reduced iron.

It is generally recognized that minerals bound by phytate are poorly available to man and monogastric animals, and also that the presence of undegraded phytate in the intestine may decrease the absorption of calcium, iron, magnesium, and zinc (4). There are other reports (5, 6, 7) which suggest that phytate does not interfere with the availability of iron, but, however, fiber may affect availability. The dietary fiber referred to included those plant constituents resistant to digestion by secretions of the human gastrointestinal tract (8, 9). It is composed of a heterogenous group of carbohydrate compounds (cellulose, hemicellulose, mucilages, pectin, gums) and the non-carbohydrate lignin (10).

We added pure cellulose and calcium phytate to the diets to study the effects of those individual components on iron absorption in flour-based diets.

II. REVIEW OF LITERATURE

Cereal Grain Products Fortification

In the 1930's, following the discovery of certain vitamin-deficiency diseases in the United States, efforts were initiated to improve the nutritional status of the American people. Cereal-grain products are appropriate vehicles for adding nutrients to the diet, both on the basis of suitability as a carrier and broad usage by almost everyone in the United States (2, 11). In November, 1940, the Committee on Food and Nutrition (now the Food and Nutrition Board) of the National Research Council endorsed a program favoring additions of thiamin, niacin, riboflavin and iron to flour (2). The supplementation program became mandatory during World War II, and many states passed laws requiring enrichment of flour and bread. Today 34 states have a mandatory requirement for flour and bread enrichment (2). In many states, particularly in the south, the enrichment program has extended to other cereal-grain products, such as corn meal (12). It is difficult to assess thoroughly the success of the enrichment program (2, 13). It is reported, however, that cereal enrichment programs have effectively reduced the incidence of pellagra and other B-vitamin deficiencies in the United States, but they have not had such a favorable effect upon the incidence of anemia (14). In the 1966 symposium in recognition of 25 years of the enrichment program, Sebrell said, "There was little doubt in the minds of those making the study that the disappearance of vitamin deficiency disease was due to the enrichment program..." (11). In another report it was stated that, "Since the initiation on a national scale of flour

and bread enrichment, food deficiency diseases arising from a lack of B-vitamins have virtually disappeared. Symptoms of beri-beri, pellagra, and riboflavin deficiency--common in the 1930's--are rarely seen today, and cases of simple anemia have been greatly reduced..." (15). Thus, fortified flour and bread apparently have made a significant contribution to the daily intake of those nutrients used for fortification.

The Food and Nutrition Board reviewed the enrichment program in 1971 and found that since the current cereal-grain enrichment standards were adopted in the early 1940's, a number of significant changes have occurred in the food consumption habits of the U. S. population, new information has developed concerning nutritional requirements, and total energy requirements have declined as improved transportation systems and mechanization have reduced the physical work required for many occupations, etc. (2). A ten-state nutrition survey launched in 1968 by the Department of Health, Education and Welfare indicated that significant numbers of people in the population studied had intakes below the Recommended Daily Allowance (RDA) for calcium, iron, and vitamin A (16). The Board reviewed data on several additional nutrients to assess the importance of considering including them in the cereal-grain products fortification program. As a result of that review, they proposed to expand the standards and include several additional nutrients (Table 1). The nutrients recommended for the fortification of cereal-grain products were selected primarily on the basis of their roles in meeting the needs of significant population groups that face potential nutritional risk (2).

Table 1

Fortification Levels for Flour in mg/lb

Nutrient	Currently used	NAS 1974 proposed level
Thiamine	2.9	2.9
Riboflavin	1.8	1.8
Niacin	24	24
Iron	13-16.5[2]	40
Calcium	--	900
Vitamin A	--	2.2 (7300 IU)[1]
Pyridoxin	--	2
Folic acid	--	0.3
Magnesium	--	200
Zinc	--	10

[1] The originally proposed level of 7300 IU was lowered to 4472 IU.

[2] In the 1941 fortified flour 16 mg/lb was used.

Vitamin A Biopotency in Food Products

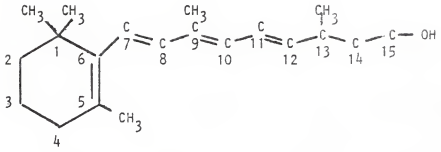
Due to the number of geometric isomers of vitamin A that might be present in vitamin A concentrates, the determination of vitamin A in various sources used for feed supplementation could be complicated (17, 18). The four isomers of practical importance in commercial vitamin A products are shown in Figure 1. All-trans retinol is the most biologically active form (17, 18). If cis isomers are present in the products, vitamin A biopotency will be lowered (17, 18, 19). Relative biopotencies of the cis-isomers, as percentages of the biopotency for all-trans-retinol, are shown in Table II.

No colorimetric or spectrophotometric procedure alone can be used to estimate the biopotency of the trans-cis types retinol. Ames, et al. found that two empirical equations based on formation of products with maleic anhydride and assay by either the blue color reaction or the USP procedure satisfactorily estimated the biopotency of trans-cis types of vitamin A products (17, 18).

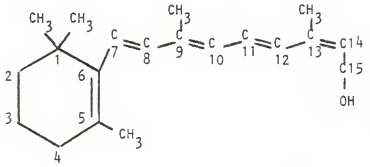
The relative biopotency values serves as a correction factor for the presence of isomers with biopotencies less than that of all-trans vitamin A. The relative biopotencies estimated from chemical measurements of maleic value agreed with bioassay data (17). Parrish and Aguilar found that storage of concentrates or feed containing vitamin A had a comparatively small effect on relative biopotencies even though total vitamin A content was lost in liquid feed supplement, dry supplement, or premix (19, 20).

FIGURE 1.

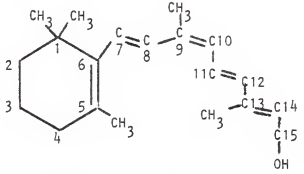
ISOMERS OF VITAMIN A



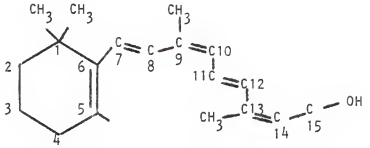
all-trans



13-mono-cis



9-mono-cis



9,13-di-cis

Figure 1

Table II

Biopotencies of Retinyl Acetate (18)

Isomeric Form	Relative Biopotency (%)
All-*trans*	100
13-mono-*cis*	75
9-mono-*cis*	21
9,13-di-*cis*	24

Bioassay for Vitamin A

The potency of vitamin A supplements can be determined by bioassay (21). Embree and co-workers (22) reviewed the various vitamin A bioassays and gave details for planning, performing, and statistically analyzing the results of 1) rat growth, 2) liver-storage, 3) vaginal-smear bioassays. They showed a strong preference for the liver-storage method. The liver-storage bioassay for vitamin A was originally devised by Guggenheim and Koch (23). It is based on the fact that substantial amounts (65-70% in the albino rat) of large doses of vitamin A are deposited in the liver and that the dose-response relationship is linear over a wide range (18, 21). Harris (24) concluded that in doing vitamin A bioassay tests one should 1) use a good, stable reference standard, 2) employ a method in which the criterion of response is specific for vitamin A, and 3) show the confidence limits as an integral part of the expression of biopotency. Ames, et al. (18) also noted that no bioassay is valid unless a suitable reference standard is employed.

In our work the bioassay technique had to be modified, since we were testing bioavailability of vitamin A from fortified flours used in diets. Thus, a test based on large dosages of vitamin A given over a short period of time was not appropriate. Our test was based on comparison of the amounts of vitamin A stored in the liver from dietary intakes from food fed over a period of one month.

Effect of Phytate and Cellulose on Iron Absorption

Widdowson and McCance (25) found evidence that the phytic acid in brown bread might interfere with the absorption of iron and they

suggested that, in spite of the large amount of iron in whole wheat, bread made from it may not be as good a source of iron as is generally supposed. McCance, et al. (26) also found that sodium phytate added to bread decreased the serum iron response by reducing the amount of iron absorbed. Sathe and Krishnamurthy (27) observed that in rats, phytate interfered with the utilization and storage of iron because of diminished absorption and concluded that with the increase in the amount of phytates in the diet, the absorption of iron correspondingly decreased. Hussain and Patwardhan (28) used two levels of phytate in diets fed to their human subjects and found a marked reduction in the retention of iron by all subjects when the diet contained the higher level of phytate. They concluded that the lowered retention was due to the incorporation of sodium phytate in the diet which interfered with the absorption of iron from the gastrointestinal tract. Peers (29) concluded that phytate can interfere with the assimilation of calcium and iron because those metals form phytates that are insoluble over a wide pH range.

Jenkins, et al. (30) found that consumption of breads made from unprocessed flour lowered serum levels of zinc, calcium, and iron and affected iron absorption. They suggested that it might be due in part to sequestration of minerals in the intestine by phytic acid in the bread, forming insoluble phytates, and, also, by binding of minerals by other constituents of dietary fiber, e.g. cellulose. Ismail-Beigi, et al. (31) found divalent metals bound to indigestible fiber components, such as cellulose, hemicellulose, and lignin, would be expected to remain unavailable for gastrointestinal absorption. Ismail-Beigi,

et al. (32) believed that a high level of cellulose could explain to a considerable extent the impaired utilization of dietary zinc, calcium, and magnesium among villagers in rural Iran. But Davies, et al. (33) found that phytate rather than fiber in bran was the major determinant to availability of zinc to rats. Hardinge (34) reported that the large fiber intakes of the pure vegetarians caused no alimentary disturbance in mineral utilization. Walker, Fox, and Irving (35) concluded that the iron retention was virtually the same from high and low phytate diets. Ranhotra, et al. (4) also found that increasing the level of phytate in the diet did not interfere substantially with the availability of iron occurring naturally in wheat. Reinhold, et al. (7) concluded that fiber largely determines the availability of divalent metals of bread for absorption by the intestine. Morris and Ellis (36) reported that, rather than the effect of phytate, the presence of food and fiber might just as well be the explanation for low absorption of iron in cereals by humans.

III. PROCEDURE

Experiment I: Availability of Nutrients and Growth of Rats Fed Flour Containing the NAS 1974 Proposed Nutrient Fortification Levels.

Materials

Animals. Forty-eight 25-day old male albino rats, obtained from the colony raised in the Department of Biochemistry, Small Animal Nutrition Laboratory, weight range 68-73 gm, were individually housed in wire-mesh stainless steel cages under controlled temperature conditions

for the four-week test period. The rats were randomly divided into six groups (8 rats per group) and each group was fed one of the test diets.

Diets. Composition of diets used is shown in Table III. Diets differed in type of flour used as follows:

(A) Diet HF - Contained hard-wheat bread flour with NAS 1974 proposed nutrient fortification levels, except for Fe, where the current fortification level of 16 mg/lb was used. The iron source was electrolytically reduced iron.

(B) Diet HS - Same as (A), except the iron source was $FeSO_4$.

(C) Diet HF* - Same as (A), except the iron fortification level was 40 mg/lb, as in the NAS 1974 proposal (2).

(D) Diet HS* - Same as (B), except the iron fortification level was 40 mg/lb, as in the NAS 1974 proposal (2).

(E) Diet 1941 - Used bread flour supplemented according to the NAS 1941 proposal (38), with 1000 IU vitamin A/lb added. Vitamin A source was USP Reference Standard.

(F) Diet Unsupplemented - Used unsupplemented bread flour with 1000 IU vitamin A/lb added. Vitamin A source was USP Reference Standard.

The flour used in each diet supplied about 30% of the total calories. Additional nutrients were added to all diets as needed to meet the requirements (37), except that iron, calcium, magnesium, zinc, and vitamin B_6 were added to only 90% of the requirement in order to lightly stress the rat by restricting those nutrients to be tested in the study, making it more likely to find measurable differences in response if

Table III

Diet Content of Experiment I

Ingredient	%
Flour[1]	33.2
Casein (vitamin-free)	13.5
Corn starch	27.8
Glucose (Dextrose)	9.4
Cellulose	2.0
Wesson oil	4.5
NaCl (Iodized)	0.2
$CaCO_3$	0.35
$Ca(H_2PO_4)_2H_2O$	1.07
Vitamin premix[2]	4.0
Mineral premix[3]	4.0

[1] Flour sources differed; see text, diets.

[2] See Appendix I for composition of vitamin premix.

[3] See Appendix II for composition of mineral premix.

there were differences in availabilities of these nutrients. Table IV shows the calculated nutrient contents of the diets.

Methods

Previous to the test period, the weanling rats were fed diet F with vitamin A for 5 days for adjustment.

Feed and water were given ad libitum for the 4-week test period, and the feed intakes and weight gains were measured and recorded weekly. At the end of the fourth week, animals were anesthetized; blood samples were withdrawn by heart puncture for hemoglobin and hematocrit determinations. Hepharin was used as an anti-coagulant. Plasma was obtained by centrifugation for use in the vitamin A, calcium, and magnesium analyses. Rats then were sacrificed, the livers were removed for liver vitamin A analysis and stored in a freezer until the analyses could be done. The tibias were removed to be used for bone ash analysis.

Hemoglobin content was determined by the AOAC method (39). Hematocrit value was determined by centrifugation of 1 ml blood in a hematocrit tube at approximately 3000 rpm for 30 minutes. The Kimble method (40) was used to determine serum vitamin A. Vitamin A content of liver was determined by alkaline hydrolysis, extraction with ether and hexane, and colorimetry, similar to the method for serum vitamin A determinations. Plasma calcium was determined by the Ferro-Ham method (41, 42). A micro-method was used for the plasma magnesium determination (43). Bone ash was determined by heating bones in a muffle furnace at 650°C after extraction and drying of the crushed bones (4, 44, 45).

Table IV A

Nutrient Content[1] per kg Diet, Experiment I

Nutrient	Amount/kg diet (g)	Nutrient	Amount/kg diet (mg)
Protein	146	Vitamin B_1	1.25
Fat	49.4	Vitamin B_2	2.5
Fiber	21.38	Niacin	15
Na	0.88	Folic acid	2
K	2.31	Vitamin B_{12}	0.05
P	4.15	Ca pantothenate	8
Choline Cl	0.5	Biotin	0.02
Methionine[2]	5.64	Vitamin C[3]	30
Lysine	10.2	Vitamin E	36
Vitamin A, IU	*	Vitamin K	0.05
Vitamin D, IU	1000	Mn	50
		Se	0.04
		Cu	5

[1] Nutrient contents shown in Table IV A were the same in all diets. Table IV B shows various nutrient contents in each diet. Values calculated from tables and nutrient data on flour, except vitamin A contents, are analytical values obtained on flour. Vitamin A standard (1000 IU/kg diet) was used in other two diets (diet 1941 and diet unsupplemented).

[2] Cystine also was in the ingredients.

[3] Vitamin C added to estimated 2/3 of human requirement, adjusted on weight basis.

Table IV A (Con't.)

* Diets HF, HS, HF* and HS* contained 3800 IU vitamin A/kg diet; diet 1941 and diet unsupplemented contained 1000 IU vitamin A/kg diet.

Table IV B

Nutrient Content[1] per Kg Diet, Experiment I

Nutrient	Diet			
	HF & HS	HF* & HS*	1941	Unsupplemented
Calcium, g	4.51	4.51	3.92	3.92
Magnesium	0.356	0.356	0.273	0.273
Zinc, mg	10.7	10.7	5.6	5.6
Fe,[2] mg	31.8	48.8	31.8	24.1
Vit. B_6, mg	6.3	6.3	4.91	4.91

[1] Used 90% of NRC requirement for rats (37) as a target level in diets HF and HS.

[2] In diets HF, HS, 1941 and unsupplemented, iron was supplied only by flour; in diets HF* and HS*, iron was supplied by flour and added iron to 40 mg of iron per lb. diet.

The data were tested for statistical significances by student's t-test (46).

Experiment II: Biopotency of the Stabilized Vitamin A Remaining in Flour Containing the NAS 1974 Proposed Nutrient Fortification, Stored Under Accelerated Conditions (40°C) for Six Months.

Materials

Animals. Forty 27-day old male albino rats, weight range 71-76 gm, obtained from the same source as those used in experiment I, were housed individually under the same environmental conditions as in experiment I for four weeks. Rats were randomly divided into four groups (10 rats per group) and each group was fed one of the test diets.

Diets. Diet compositions and nutrient contents are shown in Tables V and VI, respectively.

(A) Diet A - Contained 45% stored control flour; an all-purpose type flour to which supplements (2) were added only when the diet was mixed.[2]

(B) Diet B - Contained 45% experimental flour. Same type flour as diet (A), with fortification (2) added before flour was stored.

(C) Diet C - Contained 22.5% of same control flour as in diet (A).[3]

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[2] In diet (A), 3140 IU of vitamin A standard/kg diet was added to provide the same level of vitamin A as determined in the flour used in diet (B).

[3] In diet (C), 1570 IU of vitamin A standard/kg diet was added to provide the same level of vitamin A as determined in the flour used in diet (D).

Table V

Diet Composition (%) of Experiment II

Ingredient	Diets A & B	Diets C & D
Flour[1]	45	22.5
Casein (vit-free)	12.3	15.0
Corn starch	18	33
Wesson oil	4.85	5.0
Glucose	6.4	11.05
NaCl (Iodized)	2	2
Cellulose	1.9	1.9
$CaCO_3$	0.45	0.45
$Ca(H_2PO_4)_2H_2O$	1.1	1.0
Vitamin mix[2]	4	4
Mineral mix[3]	4	4

[1] For diets A & C used an all-purpose type flour made of 3 parts of H-40-6 (hard wheat flour stored at 40°C for 6 months) plus 1 part of S-40-6 (soft wheat flour stored at 40°C for 6 months). This flour had $CaSO_4$, ZnO, MgO and reduced iron fortification added. For diets B & D used an all-purpose type flour made of 3 parts of HF-40-6 plus 1 part of S-40-6 (flours contained the NAS 1974 proposed nutrient fortification level, iron source was reduced iron, and was stored 6 months at 40°C). This flour contained 3169 IU vitamin A/lb at the end storage period.

Table V (Continued)

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[2] For diets A & B used vitamin mix A and for diets C & D used vitamin mix B (see Appendix III for vitamin mix).

[3] For diets A & B used mineral mix A and for diets C & D used mineral mix B (see Appendix IV for mineral mix).

Table VI

Nutrient Content of the Diet in Experiment II

Nutrient	%	Nutrient	mg/kg diet
Protein	14.6	Thiamine	1.25
Fat	5.4	Riboflavin	2.5
Fiber	2.0	Niacin	15
Na	0.8	Folic acid	2
K	0.23	Vitamin B_{12}	0.05
P	0.4	Ca pantothenate	8
Ca	0.499	Vitamin B_6	6.4
Mg	0.04	Vitamin C[2]	30
Choline Cl	0.05	Vitamin E[3]	37
Methionine[1]	0.6	Mn	50
Lysine	1.0	Se	0.04
Vitamin A	*	Cu	5
Vitamin D, IU/kg	1000	Vitamin K	0.05
		Zn	12
		Fe[4]	32

[1] Cystine also was in the ingredients.

[2] Vitamin C added at estimated 2/3 of human requirement, adjusted to weight basis.

[3] For diets A and B, vitamin E was 36.7 mg/kg; for diets C and D, vitamin E was 36.5 mg/kg.

Table VI (Continued)

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[4] Used 90% of NRC requirement as a target level, for diets A & B was 31.56 mg/kg and for diets C & D was 31.22 mg/kg.

* Diets A & B contained 3140 IU vitamin A/kg, and diets C & D contained 1570 IU vitamin/kg. Vitamin A in diets A & C was supplied by adding vitamin A Standard, in diets B & D vitamin A was supplied by stored fortified flour.

(D) Diet D - Contained 22.5% of same experimental flour as in diet (B).

The diets were designed to meet the requirements of the growing rat (37), except for vitamin A and iron. Additional nutrients, except for vitamin A and iron, were added to the diets as needed to meet the requirements (Table VI). In the experimental diets (diets B and D), vitamin A at 3140 and 1575 IU per kg, respectively, was supplied solely by that remaining in the stored fortified flour. In the control diets (diets A and C) that were used for comparison with the experimental diets, vitamin A was added at equivalent levels from USP Vitamin A Reference Standard. Earlier studies had indicated that if the dietary vitamin A were available enough should be provided for growth and adequate serum vitamin A levels in rats receiving the experimental and the control diets (at both levels of Vitamin A) and there should be a markedly higher liver vitamin A storage in rats fed the experimental or control diets at the higher level of vitamin A. If, however, there was a significant decrease in vitamin A availability from the experimental diets, it would be reflected in less growth and serum vitamin A in rats fed the experimental diets containing the lower vitamin A level than in rats receiving the same amount of vitamin A from the control diet.

The iron level for all diets was 90% of the National Research Council (NRC) requirement; it was supplied from the flour used in the diet plus additional $FeSO_4$ added in order to reach the 90% level.

Methods

Rats were fed the preliminary diet [same as diet (B) except used a flour with the 1941 supplementation levels, plus $CaSO_4$, ZnO, and MgO, as in the flour used in diet (B)[4]] from the time of weaning for 5 days before rats were divided into four test groups and fed the experimental diets. The diet contained no vitamin A during the preliminary period.

The same methods were used to determine plasma vitamin A, liver vitamin A, hemoglobin contents and hematocrit values as in experiment I. Data were tested for statistical significance by student's t-test (46).

Experiment III: Relative Effects of Calcium Phytate and Two Levels of Cellulose on Utilization of Iron Supplied as Reduced Iron or Ferrous Sulfate by Rats Receiving Diets Containing Wheat Flour.

Materials

Animals. Sixty-four 25- and 26-day old male albino rats, obtained from the same source as those used in experiment I, weight range 66-76 gm, were housed under the same environmental conditions as in experiment I for 33 days. Rats were randomly divided into eight groups (8 rats per group) and each group was given one of the test diets.

Diets. Diet compositions and nutrient contents are shown in Tables VII and VIII, respectively.

(A) Diet A - A basal diet which contained 1% cellulose and $FeSO_4$ as the iron source.

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[4]Contained hard-wheat flour with the NAS 1974 proposed fortification level and stored at 40°C for 6 months.

Table VII

Diet Composition (%) of Experiment III

Ingredient	Diet			
	A & B	C & D	E & F	G & H
Flour (HRW)	60	56	51.2	57.2
Casein[1]	9.5	10	10.5	10
Glucose	15	14	12.8	14.3
Wesson oil	4.5	4.5	4.5	4.5
Cellulose mix[2]	1	5.5	11	1
NaCl (Iodized)	0.2	0.2	0.2	0.2
$CaCO_3$	0.7	0.7	0.7	0.7
$Ca(H_2PO_4)_2H_2O$	1.1	1.1	1.1	1.1
Vitamin mix[3]	4	4	4	4
Mineral mix[4]	4	4	4	4
Calcium phytate	--	--	--	3

[1] Diets A, B, C, and D contained vitamin-free casein, diets E, F, G, and H contained high-nitrogen casein.

[2] For diets A, C, E, and G, used cellulose mix A; for diets B, D, F, and H, used cellulose mix B (see Appendix VII).

[3] Vitamin mix, see Appendix V.

[4] For diets A, C, E, and G, used mineral mix A; for diets B, D, F, and H, used mineral mix B (see Appendix V).

Table VIII

Nutrient Content of Diet, Experiment III

Nutrient	%	Nutrient	mg/Kg
Protein	13.7	Thiamine	1.25
Fat	5.0	Riboflavin	2.5
Fiber	*	Niacin	15
Na	0.09	Vitamin B_6	7
K	0.24	Folic acid	2
P	0.4	Vitamin B_{12}	0.05
Ca	0.52	Ca pantothenate	8
Mg	0.04	Vitamin C[3]	30
Choline Cl	0.05	Vitamin E	36
Methionine[1]	0.64	Vitamin K	0.05
Lysine[2]	0.95	Zn	13
Vitamin A, IU/Kg	2000	Fe[4]	35
Vitamin D, IU/Kg	1000	Mn	50
		Se	0.04
		Cu	5

[1] Cystine also was in the ingredients.

[2] For diets A & B was 0.92%, and for diets E & F was 0.97%.

[3] Vitamin C added as estimated 2/3 of human requirement, adjusted to weight basis.

[4] Diets A, C, E, & G contained $FeSO_4$ as iron source; diets B, D, F, & H contained reduced iron as iron source.

* Fiber content for diets A & B was 1%, for diets C & D was 5%, for diets E & F was 10%, for G & H was 1%. Calcium phytate was added in G & H.

(B) Diet B - Same as diet (A), except the iron source was reduced iron.

(C) Diet C - Contained 5% cellulose, the iron source was $FeSO_4$.

(D) Diet D - Same as diet (C), except iron source was reduced iron.

(E) Diet E - Contained 10% cellulose, and $FeSO_4$ as the iron source.

(F) Diet F - Same as diet (E), except the iron source was reduced iron.

(G) Diet G - Contained 1% cellulose plus 3% calcium phytate, with $FeSO_4$ as the iron source.

(H) Diet H - Same as diet (G), except the iron source was reduced iron.

Methods

Weanling rats were given the preliminary diet [same as diet (A)] for 5 days before they were divided into test groups and fed the experimental diets for 33 days. Feed and water were given ad libitum; feed intakes and weight gains were measured and recorded weekly.

Hemoglobin contents and hematocrit values were determined by the same methods as in experiment I. The data were tested for statistical significance by student's t-test (46), analysis of variance (46), and by a correlation coefficient test.

IV. RESULTS AND DISCUSSION

Experiment I

Table IX summarizes the results of experiment I. Animals fed each diet did not show visible signs of nutritional deficiency during the 4-week test period. Nutrient content of diets containing the NAS 1974 proposed fortification of flours (diets HF, HF*, HS, and HS*),

Table IX

Results Summary of Experiment I

Result	Diet HF	Diet HS	Diet HF*	Diet HS*	Diet 1941	Diet Unsupple.
Total weight gain, g	194±12[1]	194±15	205±14**	201±11**	186±14	190±18
Total feed intake, g	472±25	469±34	488±30**	505±15**	467±28	462±30
Liver weight, g	11.5±1.1	10.2±1.6	12.6±1.2	12.3±1.2	106.±1.2	10.7±1.3
Hemoglobin, gm/100 ml	12.4±0.5	13.3±0.9**	12.8±0.5	12.7±0.3	12.3±0.4	12.1±0.3
Hematocrit, %	40.1±1.8	39.8±3.5	38.2±2.5	37.7±1.9	38.3±2.2	35.5±3.0
Plasma vitamin A, µg/100 ml	43.7±12.6	45.7±8.3	52.6±10.5**	54.6±10.3**	37.9±10.1	28.4±5.4
Liver vitamin A, µg/gm liver	21.8±5.0**	22.6±3.3**	20.7±3.0**	24.0±3.9**	1.4±0.5	1.2±0.5
µg/whole liver	248±36**	259±40**	260±39**	292±40**	14±5	13±5
Plasma Mg, µg/100 ml	2.7±0.5	3.3±1.2	2.8±0.3	2.9±0.4	2.7±0.6	2.6±0.3
Bone ash, %	64.0±1.0	63.9±1.7	64.6±1.0	64.6±0.7	63.8±1.3	63.2±1.1

[1] Average ± Standard Deviation.

** When compared to diet 1941 or diet unsupplemented had significant difference (P = 0.05).

Table IV, was good. Most nutrients were provided either at or above the NRC requirement for rats (37) by supplementing the diets with the required nutrients. However, some nutrients (Ca, Mg, Zn, Fe and vitamin B_6) were fed at 90% of the NRC requirement levels to provide a slight nutrient stress for those nutrients. Even though in diets containing the 1941 fortified flour (diet 1941) and unfortified flour (diet unsupplemented) the levels of Ca, Mg, Zn, Fe and vitamin B_6 were below the levels of the former diets by the amount provided by supplemented flour, no measurable nutritional deficiencies were observed in rats receiving diets prepared from those flours.

Serum calcium values were all abnormal and not included in this report. The serum calcium analyses could not be repeated because of lack of sufficient serum.

Plasma magnesium values and percent bone ash were all in the normal ranges. There was no significant differences ($p < 0.05$) in plasma Mg and bone ash of rats fed any diet (Table IX).

Rats that received diets containing the NAS 1974 proposed fortified flour with 40 mg/lb of iron (diets HF* and HS*) had significantly higher ($p < 0.05$) total feed intakes, total weight gains, and plasma vitamin A values than those of any other groups. Higher iron intake apparently promoted better growth and development.

Rats receiving the diets containing the NAS 1974 proposed fortified flour (diets HF, HS, HF*, HS*) had significantly higher ($p < 0.05$) liver vitamin A content than those receiving 1941 supplemented or unsupplemented diets. That was expected because the quantity of fortified

flour used provided 190% of the rat's NRC vitamin A requirement without additional vitamin A supplementation, and the vitamin A level added to the 1941-supplemented and unsupplemented flour diets (diets 1941 and unsupplemented) was 1000 IU/kg diet, which provided only 50% of the NRC vitamin A requirement (37) (2000 IU/kg). It was necessary to provide some vitamin A in those latter diets to prevent poor rat growth and development from lack of vitamin A.

Hemoglobin contents were all within the normal range (12-17.5 gm/100 ml) and there were no significant differences among dietary groups ($p < 0.05$), except that rats fed the HS diet had significantly higher hemoglobin content than those fed diets with 1941 supplemented and unsupplemented flours. That finding is difficult to understand because iron intake was less than in rats getting HF* and HS* diets.

Rats receiving diets containing the NAS 1974 proposed fortified flour with 16 mg/lb of iron (diets HF and HS) had hematocrit values in the normal range (39-53%); other dietary groups had less than normal hematocrit values, but there was no significant difference in hematocrit values among those dietary groups. Increasing iron fortification to 40 mg/lb apparently did not improve the iron status in rats.

In general, fortification of flours according to the 1974 NAS proposal seems to promote better growth of rats than the 1941 fortification or non-fortification of flour, especially in those dietary groups that contained the higher level of iron (40 mg/lb). The fortification with Mg and Fe did not affect the Mg and Fe level in the blood of rats very much.

Results of experiment I indicate that it is nutritionally feasible to fortify wheat flour with vitamin A. Due to the lack of quantitative evidence from these animal studies as to the feasibility of fortification with the other nutrients proposed by NAS in 1974, further studies on those nutrients should be done in the future.

Experiment II

Table X summarizes the results of experiment II. There were no significant differences ($p < 0.05$) in total weight gains, total feed intakes and plasma vitamin A values among the four dietary groups; this was expected because the nutrient content of each diet (Table VI) was calculated to meet the NRC requirement for rats (37), except the iron level was supplied at 90% of the NRC requirement of iron for rats, and the vitamin A in diets C and D was about 3/4 of the rats' requirement. The few minor differences in Zn, vitamin E, and lysine contents of the diets were all above the NRC requirements (37).

Hemoglobin contents and hematocrit values of rats in all dietary groups were less than the normal range [hemoglobin, 12-17.5 gm/100 ml blood; hematocrit, 39-53% (47, 48)]. This might be due to the fact that the iron added to each diet was only about 90% of the NRC requirement of iron for rats (37). There were no significant differences ($p < 0.05$) in hemoglobin contents and hematocrit values when rats received the same level of vitamin A from stored or freshly prepared flour. There were no significant differences in hemoglobin content in rats among the four dietary groups, but rats receiving the lower level of vitamin A from the stored flour (diet D) had significantly higher

Table X

Results Summary of Experiment II

Result	Diet A	Diet B	Diet C	Diet D
Total weight gains, g	182±18[1]	194±17	191±9	194±16
Total feed intakes, g	397±45	434±31	424±26	428±16
Hematocrit, %	36.9±3.5	35.6±2.8[a]	38.2±1.8	38.9±2.0[a]
Hemoglobin, gm/100 ml	11.69±0.57	11.85±1.27	11.41±0.5	11.85±0.63
Plasma vitamin A, µg/100 ml	43.5±7.8	37.6±7.6	44.9±4.6	40.8±6.1
Liver weight, g	11.11±0.89[b]	10.88±1.31	12.25±1.0[b]	11.70±1.47
Liver vitamin A µg/gm liver	12.47±2.02[c]	12.61±2.43[d]	2.65±0.47[c]	2.35±0.53[d]
µg/whole liver	139±25[e]	135±25[f]	32±4[e,g]	26±4[f,g]

[1] Mean ± Standard Deviation

a,b,c,d,e,f,g: Those with same letters had significant difference ($P < 0.05$).

($p < 0.05$) hematocrit values than those receiving the lower level of vitamin A from freshly prepared flour (diet B).

There were no significant differences ($p < 0.05$) in vitamin A content per gram of liver when rats receiving the same level of vitamin A from stored or freshly prepared flour, but there were significant differences ($p < 0.05$) in vitamin A content per whole liver when rats received the diets containing the lower level of vitamin A (diets C & D). Although these results indicate a statistical difference based on vitamin A content per whole liver, the values were in the same range and probably of little nutritional significance.

As expected, the rats receiving the diets containing the higher level of vitamin A had significantly higher ($p < 0.05$) liver vitamin A contents than those receiving the diets containing the lower level of vitamin A.

By a chemical method based on use of the maleic anhydride reagent to estimate the relative biopotency of vitamin A remaining in fortified flour stored for 6 months at 45°C, it was found that relative biopotency was 95% or more.[5]

Since the results of the determinations of vitamin A in plasma and vitamin A stored in livers showed there were no differences in plasma vitamin A contents and liver vitamin A content per gram of liver when rats received the same level of vitamin A from the diet, whether from diets made of stored or freshly prepared flour, it is reasonable

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[5]Data obtained from technician's analyses in this laboratory.

to conclude that the vitamin A remaining in stored flour (40°C for 6 months) had vitamin A biopotency similar to that of the vitamin A standard added to the control flour. Thus, results of the rat biological test are in agreement with the chemical test of relative biopotency. In general, storage had a comparatively small effect on the relative biopotency of vitamin A that was added to the flour as a stabilized vitamin A product, even though total vitamin A decreased about 30%[6] during storage. Parrish and Aquilar (19, 20) also found little or no change in relative biopotency of vitamin A in stored concentrates and supplements. Experiment II indicated that it is feasible to fortify wheat flour with vitamin A.

Experiment III

Table XI summarizes the results of experiment III. During the experimental period, animals fed each diet did not show any abnormal characteristics. In those groups receiving $FeSO_4$ as the iron source (diets A,C,E,G), the rats fed the diet containing 10% cellulose had significantly higher ($p < 0.05$) total feed intake (638 ± 31 gm) than those receiving the diet containing 5% cellulose (594 ± 17 gm) or 1% cellulose (586 ± 33 gm). In the groups receiving reduced Fe as the iron source (diets B,D,F,H), the rats fed the diets containing 5% cellulose or 3% calcium phytate had significantly higher ($p < 0.05$) total feed intake than those fed the diet containing 1% cellulose (basal diet), and rats fed the diet containing 10% cellulose had significantly higher feed

[6] All vitamin A data in this report are based on analytical values.

Table XI

Results Summary of Experiment III

Result	Diet							
	A	B	C	D	E	F	G	H
Total weight gains, g	239 ± 17^{lac}	216 ± 25^{ade}	236 ± 18^{b}	219 ± 20^{df}	239 ± 16^{bc}	244 ± 22^{f}	225 ± 19	232 ± 25^{e}
Total feed intakes, g	586 ± 33^{a}	530 ± 49^{ab}	594 ± 17	572 ± 27^{c}	638 ± 31	643 ± 59^{bc}	584 ± 25	594 ± 53
Hemoglobin, gm/100ml	12.3 ± 0.2^{a}	$91. \pm 0.7^{abcd}$	12.3 ± 0.4^{g}	11.1 ± 0.5^{beg}	12.5 ± 0.5^{h}	11.4 ± 0.7^{cfh}	12.3 ± 0.6	12.3 ± 1.0^{def}
Hematocrit, %	46.9 ± 2.6^{a}	36.5 ± 3.0^{abcd}	45.4 ± 2.1^{g}	41.7 ± 1.4^{beg}	45.5 ± 1.7^{h}	42.8 ± 2.0^{cfh}	45.6 ± 1.4	45.6 ± 0.8^{def}

[1] Mean $\pm$ Standard Deviation.

a,b,c,d,e,f,g,h: Those with same letters had significant difference at $P < 0.05$.

intake than those fed the diet containing 5% cellulose. When the cellulose content of the diet was increased, the rats tended to eat more, probably in order to supply their appetite for energy.

There were no significant differences between groups in total weight gain when the rats received diets containing $FeSO_4$ as the iron source, but in those groups receiving reduced iron, rats fed the diet containing 10% cellulose had significantly higher total weight gain (244 $\pm$ 20 gm) than those fed the diet containing 5% cellulose (219 $\pm$ 16 gm) or 1% cellulose (216 $\pm$ 25 gm). In each group, rats fed the diets with $FeSO_4$ or reduced iron as the iron source, at each level of cellulose or calcium phytate, had no significant differences in total weight gains and feed intakes. Except for the basal group (1% cellulose), the rats fed the diet with $FeSO_4$ as the iron source had significantly higher total feed intake and total weight gain than those fed the diet with reduced iron as the iron source. However, differences in body weight are not a satisfactory measurement of iron utilization, since depression of body weight occurs only when the animals are severely anemic (49).

The rats fed diets with $FeSO_4$ as the iron source had significantly higher hemoglobin contents and hematocrit values than those fed diets with reduced iron[7] as the iron source, except for those fed the diet containing 3% calcium phytate. Fritz, et al. (50) listed the Relative Biological Values (RBV) of iron sources based on chick and rat assays. If RBV for $FeSO_4$ (anhydrous) was assigned a value of 100%, that for

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[7]Electrolytically reduced iron.

reduced iron was 37% (range 8-66%). Harrison, et al. (51) listed the RBV of electrolytic iron (v.s. $FeSO_4$ = 100%) as 29-75%. Also in agreement with our results, others have reported the availability of iron from $FeSO_4$ (anhydrous or hydrous) tends to be higher than that of reduced iron (hydrogen reduced or electrolytically prepared) (14, 52).

Rats receiving diets containing $FeSO_4$ as an iron source had no significant differences among groups in hemoglobin contents and hematocrit values. In rat groups fed diets containing reduced iron, those receiving 3% calcium phytate in the diet had significantly higher ($p < 0.05$) hemoglobin contents and hematocrit values than the other groups.

Rats fed diets containing 5% cellulose or 10% cellulose with reduced iron as the iron source had significantly higher hemoglobin contents and hematocrit values than those fed the basal diet with 1% cellulose. Increasing the cellulose level in the diet seemed to have had little, if any, effect on iron availability from $FeSO_4$, but increased the availability from reduced iron. Fritz, et al. (50) reported that addition of 10% cellulose to the diet had little effect on availability of iron from ferrous sulfate, but it did improve the utilization of iron furnished by ferric orthophosphate. They thought inclusion of the cellulose may have slowed the rate of passage of food through the intestinal tract and thereby increased the period of gut exposure to the iron supplement. However, McCance, et al. (53) reported an increasing relationship between fiber intake and transit time. Our results showed that increasing cellulose levels had little or no effect on iron availability

from $FeSO_4$ and perhaps tended to improve the utilization of iron from the reduced iron source.

The rats fed 3% calcium phytate diets had the same mean hemoglobin (12.3 gm/100 ml blood) and hematocrit values (45.6%) regardless of the source of iron. Thus, adding 3% calcium phytate to the diet apparently had no effect on iron availability from the two iron sources. The lack of inhibition due to phytate may be explained by the presence of intestinal phytase activity in rats (4, 54, 55, 56). Some studies (4, 57) indicated that added sodium phytate had slight or no depressing effects on utilization of iron by laboratory rats. Also, in our work, the calcium phytate might have been resistant to enzymatic digestive change and, thus, being inert, had little or no effect. The mechanism of how phytase activity might relate to iron absorption in either humans or rats is not clear at the present time (36).

The correlation coefficient data (Table XII) showed that hemoglobin contents and hematocrit values are highly correlated ($r = 0.888$), as might be expected.

In summary, it seems that increased cellulose levels and added calcium phytate did not affect the iron absorption; on the contrary, cellulose levels had a positive relationship to reduced iron availability, but had no effect on the $FeSO_4$ availability. More experiments also should be done in the future to clarify the mechanism of these relationships.

Table XII

Correlation Coefficient Data for Experiment III

	Intake	Weight	Hemoglobin	Hematocrit
Intake	1.000	0.724	0.391	0.309
Weight	0.724	1.000	0.202	0.196
Hemoglobin	0.391	0.202	1.000	0.888
Hematocrit	0.309	0.196	0.888	1.000

V. SUMMARY

Animal studies were carried out to evaluate certain nutritional qualities of flour prepared according to the NAS 1974 fortification proposal.

In experiment I, diets containing flour with the NAS 1974 proposed nutrient fortification levels promoted more growth in rats than diets containing flour prepared with 1941 NAS nutrient fortification levels or unsupplemented flour, but flour with the new fortification did not materially affect Mg and Fe status of rats. Experiment I seems to show the nutritional feasibility of vitamin A fortification to wheat flour.

Results of experiment II showed that storing the NAS 1974 proposed fortified flour at 40°C for 6 months had comparatively small effect on relative biopotency of vitamin A remaining in the flour, even though about 30% of the total vitamin A was lost during storage. Relative biopotency by chemical tests of the vitamin A remaining in the stored flour was 95% or more. Experiment II also indicated that it is feasible to fortify wheat flour with vitamin A.

Results of experiment III showed that there was no deleterious effect on iron utilization in flour fortified with either $FeSO_4$ or reduced iron as the iron sources, when cellulose levels in the diet were increased to 5% or 10%. Increasing the cellulose level increased availability of reduced iron to rats, as measured by hemoglobin contents and hematocrit values. Calcium phytate, 3%, in the diet also had no deleterious effect on iron utilization. Further experiments should be done to clarify the mechanisms of these relationships.

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LITERATURE CITED

1. Cort, W. M., Borenstein, B., Harley, J. H., Osader, M. & Scheiner, J. (1976) Nutrient stability of fortified cereal products. Food Technology. 30, (April), 52-62.
2. Neshein, R. O., Owen, G. M., Stokstad, E. L. R. & Tannenbaum, S. R. (1974) Proposed fortification policy for cereal-grain products. ISBN-0-309-02232-0. Nat. Acad. Sci. Washington, D.C.
3. Association of Official Analytical Chemists. (1975) Official Methods of Analysis. 12th Ed., Washington, D.C. 43.008-43.013.
4. Ranhotra, G. S., Loewe, R. J. & Puyat, L. V. (1974) Effect of dietary phytic acid on the availability of iron and phosphorus. Cereal Chem. 51, 323-329.
5. Cowan, J. W., Esfahani, M., Salji, J. P. & Nahaptiam, A. (1967) Nutritive value of middle-eastern foodstuff. III.---Physiological availability of iron in selected foods common to the middle east. J. Sci. Food Ag. 18, 227-231.
6. Callender, S. T. & Warner, G. T. (1970) Iron absorption from brown bread. Lancet 1, 546-547.
7. Reinhold, J. G., Ismail-Beigi, F. & Faradji, B. (1975) Fiber vs. phytate as determinant to the availability of calcium, zinc, and iron of bread stuff. Nutr. Rep. Int. 12, 75-85.
8. Estwood, M. A. (1974) Dietary fiber in human nutrition. J. Sci. Fd. Ag. 25, 1523-1527.
9. Trowell, H. (1972) Ischemic heart disease and dietary fiber. Am. J. Clin. Nutr. 25, 926-932.

10. Cummings, J. H. (1973) Progress report: dietary fibre. Gut 14, 69-81.

11. Sebrell, W. J., Jr. (1966) Enrichment: good gift of yesterday. Cereal Sci. Today 11, 228-230.

12. Ranum, P. M. & Loewe, R. J. (1978) Iron enrichment of cereals. Baker's Digest 52, 14-20.

13. Hallberg, L., Björn-Rasmussen, E., Garby, L., Pleehachinda, R. & Suwanik, R. (1978) Iron absorption from south-east asian diets and the effect of iron fortification. Am. J. Clin. Nutr. 31, 1403-1408.

14. Pla, G. W. & Fritz, J. C. (1970) Availability of iron. J. Assoc. Off. Anal. Chem. 53, 791-797.

15. Enrichment: remembrance of things past. (1966) Cereal Sci. Today 11, 258-264.

16. Ten-state nutrition survey (1968-1970). U. S. Dept. Health, Educ. and Welfare.

17. Ames, S. R. & Lehman, R. W. (1960) Estimation of the biological potency of vitamin A sources from their maleic values. J. Assoc. Off. Anal. Chem. 43, 21-25.

18. Ames, S. R. (1966) Methods for evaluating vitamin A isomers. J. Assoc. Off. Anal. Chem. 49, 1071-1078.

19. Parrish, D. B. & Aguilar, D. (1970) Estimation of vitamin A biopotency in liquid feed supplements. J. Assoc. Off. Anal. Chem. 53, 1151-1154.

20. Parrish, D. B. & Aguilar, D. (1971) Estimation of vitamin A biopotency in dry supplements and premixes. J. Assoc. Off. Anal. Chem. 54, 18-20.

21. Ames, S. R. & Harris, P. L. (1956) Slope-ratio liver storage bioassay for vitamin A. Anal. Chem. 28, 874-878.

22. Embree, N. D., Ames, S. R., Lehman, R. W. & Harris, P. L. (1957) Methods of Biochem. Anal. 4, 43-98.

23. Guggenhein, K. & Koch, W. (1944) A liver storage test for the assessment of vitamin A. Biochem. J. 38, 256-260.

24. Harris, P. L. (1960) Bioassay of vitamin A compounds. Vitamins and Hormones 18, 341-370.

25. Widdowson, E. M. & McCance, R. A. (1942) Iron exchanges of adults on white and brown bread diet. Lancet 2, 588-591.

26. McCance, R. A., Edgecombe, C. N. & Widdowson, E. M. (1943) Phytic acid and iron absorption. Lancet 2, 126-128.

27. Sathe, V. & Krishnamurthy, K. (1953) Phytic acid and absorption of iron. Ind. J. Med. Res. 41, 453-457.

28. Hussain, R. & Patwardhan, V. N. (1959) The influence of phytate on the absorption of iron. Ind. J. Med. Res. 47, 676-682.

29. Peers, F. G. (1953) The phytase of wheat. Biochem. J. 53, 102-110.

30. Jenkins, D. J. A., Hill, M. S. & Cummings, J. H. (1975) Effects of wheat fiber on blood lipids, fecal steroid excretion and serum iron. Am. J. Clin. Nutr. 28, 1408-1411.

31. Ismail-Beigi, F., Faraji, B. & Reinhold, J. G. (1977) Binding of Zn and iron to wheat bread, wheat bran and their component. Am. J. Clin. Nutr. 30, 1721-1725.

32. Ismail-Beigi, F., Reinhold, J. G., Faraji, B. & Abadi, P. (1977) Effect of cellulose added to diets of low and high fiber content upon the metabolism of calcium, magnesium, zinc and phosphorus by man. J. Nutr. 107, 510-518.

33. Davis, N. T., Histic, V. & Flett, A. A. (1977) Phytate rather than fiber in bran as the major determinant of Zn availability of rats. Nutr. Rep. Int. 15, 207-214.

34. Hardinge, M. G. H. (1978) Plant fiber and human health. In: Topics in dietary fiber research, pp. 117-126, (Spiller, G. A., ed.), Plenum Press, New York.

35. Walker, A. R. P., Fox, F. W. & Irving, J. T. (1948) The effect of bread rich in phytate phosphorus on the metabolism of certain mineral salts with special reference to calcium. Biochem. J. 42, 452-462.

36. Morris, E. R. & Ellis, R. (1976) Isolation of monoferric phytate from wheat bran and its biological value as an iron source to the rats. J. Nutr. 106, 753-760.

37. National Academy of Science-National Research Council. Nutrient Requirements of Laboratory Animals (1972) ISBN-309-02028-X, p. 64.

38. Federal Register (1943) Food and Drug Administration 8, 9170, (No. 131).

39. Official Methods of Analysis, Association of Official Analytical Chemists, 12th ed., Washington, D.C. 43.189.

40. Kimble, M. S. (1939) The photocolorimetric determination of vitamin A and carotene in human plasma. J. Lab. Clin. Med. 24, 1055-1065.

41. Ferro, P. V. & Ham, A. B. (1957) A simple spectrophotometric method for the determination of calcium. Am. J. Clin. Path. 28, 208-217.

42. Ferro, P. V. & Ham, A. B. (1957) A simple spectrophotometric method for the determination of calcium. II. A semi-micro method with reduced precipitation time. Am. J. Clin. Path. 28, 689-693.

43. Natelson, S. (1961) Microtechniques of Clinical Chemistry, 2nd ed., p. 292-293, Thomas, C. C., Springfield, Illinois.

44. Ranhotra, G. S., Loewe, R. J. & Puyat, L. V. (1977) Bioavailability and functionality (breadmaking) of zinc in various organic and inorganic sources. Cereal Chem. 54, 496-502.

45. Ranhotra, G. S., Loewe, R. J. & Puyat, L. V. (1976) Bioavailability of magnesium from wheat flour and various organic and inorganic salts. Cereal Chem. 53, 770-776.

46. Snedecor, G. W. & Cochran, W. G. (1967) Statistical Methods, 6th ed., Iowa State University Press, Ames, Iowa.

47. Spector, W. S. (1956) Handbook of biological data. p. 275. W. B. Saunders Co., Philadelphia.

48. Albriton, E. C. (1952) Standard values in blood. pp. 42-43. W. B. Saunders Co., Philadelphia.

49. Amine, E. K., Neff, R. & Hegsted, D. M. (1972) Biological estimation of available iron using chicks and rats. Ag. Food Chem. 20, 240-251.

50. Fritz, J. C., Pla, G. W., Roberts, T., Boehne, J. W. & Hove, E. L. (1970) Biological availability in animals of iron from common dietary sources. Ag. Food Chem. 18, 647-651.

51. Harrison, B. N., Pla, G. W., Clark, G. A. & Fritz, J. G. (1976) Selection of iron sources for cereal enrichment. Cereal Chem. 53, 78-84.

52. McCance, R. A., Prior, K. M. & Widdowson, E. M. (1953) A radiological study of the rate of passage of brown and white bread through the digestive tract of man. Brit. J. Nutr. 7, 98-104.

53. Rees, J. M. & Monsen, E. R. (1973) Absorption of fortification iron by the rat: Comparison of type and level of iron incorporated into mixed grain cereal. Ag. Food Chem. 21, 913-915.

54. Bitar, K. & Reinhold, J. G. (1972) Phytase and alkaline phosphatase activities in intestinal mucosas of rat, chicken, calf and man. Biochim. Biophys. Acta 268, 442-452.

55. Patwardhan, V. N. (1937) The occurrence of phytin-splitting enzyme in the intestines of albino rats. Biochem. J. 31, 560-564.

56. Pileggi, V. J. (1959) Distribution of phytase in the rat. Arch. Biochem. Biophys. 80, 1-8.

57. Cowan, J. W., Esfahani, M. Salji, J. P. & Azzam, S. A. (1966) Effect of phytate on iron absorption in the rat. J. Nutr. 90, 423-427.

APPENDIX I

Vitamin premix[1] of experiment I

Ingredient[2]	Unit	Amount of ingredient/40 gm premix[3]
Methionine	gm	1.4
Vitamin B_1	mg	1.25
Vitamin B_2	mg	2.5
Niacin	mg	15
Vitamin B_6	mg	4.78
Folic acid	mg	2
Vitamin B_{12}	mg	10
Ca pantothenate	mg	8
Biotin	mg	0.02
Choline Cl	gm	0.5
Vitamin C	mg	30
Vitamin D	gm	0.067
Vitamin E	gm	0.112
Vitamin K (Menadione)	mg	0.05

[1] Added 1,000 IU USP vitamin Reference Standard per 40 g of premix to make "Vitamin Premix plus vitamin A"

[2] All the ingredients were from pure source, except vitamin D used was 1500 IU vitamin D_3/g, and vitamin E used was a 25% concentrate tocopherol acetate.

[3] Used 40 gm premix per Kg diet. Added 3 : 1 of starch and glucose as carriers to make total 40 g mix.

APPENDIX II

Mineral mix[1] of experiment I

Ingredient	Source	Amount of ingredient per 40 g mix[2]
K	KCl	1.9 g
Mg	MgO	0.17 g
Zn	ZnO	1 mg
Mn	$MnSO_4 \cdot H_2O$	50 mg
Cu	$CuSO_4$	5 mg
Se	$NaSeO_3 \cdot 5H_2O$	0.04 mg

[1] For mineral mix HF* (for diet HF*), added 0.13 g reduced iron/300 g mineral mix.

For mineral mix HS* (for diet HS*), added 0.35 g $FeSO_4$/300 g mineral mix.

[2] Used 40 g of mix per Kg diet. Added 3 : 1 of starch and glucose as carrier to make total 40 g of mix.

APPENDIX III

Vitamin Mix[1] of experiment II

Ingredient[2]	Amount used per 40 g mix[3]
Thiamin	1.25 mg
Riboflavin	2.5 mg
Niacin	15 mg
Folic acid	2.0 mg
Vitamin B_6	4.78 mg
Vitamin B_{12}	0.05 mg
Biotin	0.02 mg
Ca pantothenate	8.0 mg
Vitamin C	30 mg
Vitamin D	1000 IU
Vitamin E	28 mg
Vitamin K	0.05 mg
Choline Cl	0.5 g

[1] In vitamin mix A, added 2.0 g of methionine per 40 g mix.
In mineral mix B, added 1.6 g of methionine per 40 g mix.

[2] All ingredients were from same source as experiment I (Appendix I).

[3] Used 40 g mix per Kg diet. Added starch and glucose (3 : 1) as carriers to total 40 g mix.

APPENDIX IV

Mineral mix of experiment II

Ingredient	Source	Unit	Amount of ingredient/Kg diet[1] A	B
K	KCl	g	1.8	2.0
Mg	MgO	g	0.17	0.26
Zn	ZnO	mg	1.0	5.4
Fe	$FeSO_4$	mg	14	20
Mn	$MnSO_4 \cdot H_2O$	mg	50	50
Cu	$CuSO_4$	mg	5	5
Se	$NaSeO_3 \cdot 5H_2O$	mg	0.4	0.4

[1] Used 40 g mix per Kg diet. Added 3 : 1 of starch and glucose as carrier to make total 40 g mix.

APPENDIX V

Vitamin mix of experiment III

Ingredient*	Amount used/ Kg diet or 40 g mix[1]
Vitamin A	2000 IU
Thiamine HCl	1.25 mg
Vitamin B_1	2.5 mg
Niacin	15 mg
Vitamin B_6	4.3 mg
Vitamin B_{12}	0.005 mg
Folic acid	2 mg
Biotin	0.02 mg
Choline Cl	0.5 g
Ca pantothenate	8 mg
Vitamin C	30 mg
Vitamin D	1000 IU
Vitamin E	28 mg
Vitamin K (Menadione)	0.05 mg
Methionine	2.5 g
Lysine	1.0 g

* All the ingredients were from the same sources as in experiment I (Appendix I).

[1] Added 3 : 1 of starch and glucose as carriers to make total 40 g mix.

APPENDIX VI

Mineral mix of experiment III

Ingredient	Source	Amount of ingredient/Kg diet
K	KCl	1.6 g
Mg	MgO	0.3 g
Zn	ZnO	8 mg
Mn	$MnSO_4 \cdot H_2O$	50 mg
Cu	$CuSO_4$	5 mg
Se	$NaSeO_3 \cdot 5H_2O$	0.04 mg
Fe	*	29 mg

[1] Used 40 g of mix per Kg diet. Added 3 : 1 of starch and glucose as carrier to make total 40 g mix.

* In mineral mix A used $FeSO_4$ as iron source, in mineral mix B used reduced iron as iron source.

APPENDIX VII

Cellulose mix[1] of experiment III

Ingredient	Unit	Amount of ingredient
α - Cellulose	g	100
Fe[2]	mg	1

[1] Added iron to make cellulose with same iron content as flour.

[2] In cellulose mix A used $FeSO_4$ as iron source, in mineral mix B used reduced iron as iron source.

THREE STUDIES OF NUTRITIONAL QUALITIES OF FORTIFIED FLOUR

by

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AN ABSTRACT OF A MASTER'S THESIS

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Animal studies were carried out to evaluate certain nutritional qualities of flour prepared according to the NAS 1974 fortification proposal. Experiment I was to test whether use of diets containing flour with the proposed fortification levels fed to growing rats would result in measurable changes in animal performance, such as growth and increased levels of the nutrients used for fortification in blood and tissues. Experiment II was to test whether there was a decrease in the relative biopotency of the stabilized vitamin A used for fortification that remained in flour stored at 40°C for 6 months. Experiment III was to determine whether increasing the level of cellulose or adding calcium phytate in flour-based diets fed to rats affected utilization of supplemental iron, $FeSO_4$ or reduced iron, used in the fortification mix.

In experiment I, diets containing flour with the NAS 1974 proposed nutrients fortification levels promoted more growth in rats than diets containing flour prepared with 1941 NAS nutrient fortification levels or unsupplemented flour, but flour with the new fortification did not materially affect Mg and Fe status of rats. Experiment I seems to show the nutritional feasibility of vitamin A fortification to wheat flour. Due to the lack of quantitative evidence from these animal studies as to the feasibility of fortification with the other nutrients proposed by NAS in 1974, further studies on those nutrients should be done in the future.

Results of experiment II showed that storing the NAS 1974 proposed fortified flour at 40°C for 6 months had comparatively small

effect on relative biopotency of vitamin A remaining in the flour, even though about 30 percent of the total vitamin A was lost during storage; relative biopotency by chemical tests of that remaining vitamin A was 95 percent or more. Experiment II also indicated that it is feasible to fortify wheat flour with vitamin A.

Results of experiment III showed that there was no deleterious effect on iron utilization in flour fortified with either $FeSO_4$ or reduced iron as the iron source, when cellulose levels in the diet were increased to five percent or ten percent. Increasing the cellulose level increased availability of reduced iron to rats, as measured by hemoglobin contents and hematocrit values. Calcium phytate, three percent, in the diet also had no deleterious effect on iron utilization. Further experiments should be done to clarify the mechanisms of these relationships.