EFFECT OF THE PROPOSED NAS FORTIFICATION POLICY FOR CEREAL GRAIN PRODUCTS ON THE NUTRITIONAL VALUE OF CORN MEAL MUSH

by

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TABLE OF CONTENTS

Page

INTRODUCTION 1

REVIEW OF LITERATURE 3

History of cereal enrichment 3

Corn products enrichment 4

Nutritional benefits of enrichment 5

Proposed fortification policy 5

Assessment of need for additional nutrients . . . 7

Iron 7

Distribution of iron in the body . . . 7

Measurement of iron status 8

Iron balance 8

Iron absorption 9

Vitamin A 11

Folic acid 11

Pyridoxine 12

Calcium 15

Magnesium 16

Zinc 17

MATERIALS AND METHODS 20

Enrichment and fortification of corn meal samples 20

Procedure for making and freeze-drying mush . . . 21

Diets and animal study 22

Hemoglobin determination 24

Hematocrit determination 24

Page

Statistical analysis 25
RESULTS AND DISCUSSION 26
Weight gain 26
Feed intake 31
Feed efficiency 33
Hemoglobin 33
Hematocrit 34
SUMMARY 35
ACKNOWLEDGMENTS 37
LITERATURE CITED 38
APPENDIX 44

INTRODUCTION

The concept of improvement of nutritional health of population groups by dietary means has developed during the last fifty years. Complementary relationships between plant proteins have been applied in nutrition programs in a variety of ways the world over. The iodization of table salt in 1924, the addition of vitamin A to margarine in 1939 and the fortification of milk with vitamin D in the 1930's marked the beginning of nutrient fortification of foods in the United States (1).

As a result of some studies concerning nutritional requirements (2, 3) and others that associated certain disease symptoms with vitamin deficiencies (4), efforts were initiated in the 1940's to meet the nutritional requirements of the American people. A nationwide program of enrichment of bread and wheat flour with some B-complex vitamins (niacin, riboflavin, and thiamine) and iron was endorsed in 1941 and became mandatory during World War II (5). Many states have continued this legislation and extended the enrichment to other cereal products. In 1971, in view of better nutritional knowledge and the many changes in food consumption patterns and food technology in the United States, a study Committee of the Food and Nutrition Board of the National Academy of Sciences (5) made a thorough review of the current enrichment programs for cereal grain products. They reported that there is evidence of potential

risk of deficiency of vitamin A, thiamine, riboflavin, niacin, pyridoxine, folacin, iron, calcium, magnesium, and zinc among significant segments of the U.S. population. They suggested that all of these 10 nutrients should be included in an expanded cereal-grain fortification program.

Problems in cereal enrichment include segregation of nutrients from carriers (6), production losses and storage instability (7), consumer acceptance due to changes in the flavor (8), and sometimes the baking characteristics (9), of the enriched product, and availability of the nutrients from the food carrier. The enrichment program in the past has been helpful in reducing the prevalence of vitamin B-deficiency diseases, but the iron level currently employed has had little effect on the prevalance of iron-deficiency anemia (10).

The purpose of this study was to compare the growth, feed intake, and hematological responses of rats fed corn meal mush that was: a) unenriched, b) enriched according to current standards with thiamine, riboflavin, niacin, and iron, c) fortified according to the NAS proposal with 3 additional vitamins (vitamin A, folacin, pyridoxine) and 3 additional mineral elements (calcium, zinc, and magnesium), along with thiamine, riboflavin, and niacin, but with iron remaining at the current level, d) fortified according to the NAS proposal with the 6 vitamins and 4 mineral elements with the recommended higher iron level.

REVIEW OF LITERATURE

History of Cereal Enrichment

Of the major classes of foods consumed in the United States, cereal products appear best to meet the guiding criteria for the selection of a food source to act as nutrient carriers. The 1965-66 Household Food Consumption Survey in the United States showed that about 26 percent of the calories consumed were provided by cereal products and that differences in extent of consumption due to regional and income differences were minimal (11).

In 1939 the Council on Foods of the American Medical Association encouraged the restorative addition of vitamins or minerals to general purpose foods (12). In response, thiamine, vitamin B-complex and non-fat dry milk were sometimes voluntarily added to bread.

In 1940, the Committee on Food and Nutrition (now known as the Foods and Nutrition Board) endorsed a program favoring the additions of thiamine, niacin, riboflavin, and iron to flour (12). However a standard of identity for enriched flour did not become effective until January 1, 1942. In 1943 this was amended to provide the standards which are currently in effect in the United States (13). The regulations provide the following nutrients per pound of wheat flour: riboflavin 1.2-1.5 mg, thiamine 2.0-2.5 mg, niacin 16-20 mg, and iron 13-16.5 mg. The inclusion of calcium and vitamin D was optional.

Between 1943 and 1946 the War Food Administration was made responsible for the handling of food emergencies created by World War II. This agency issued an order requiring enrichment of all white bread and rolls, made by commercial bakeries. By August, 1943, the Food and Drug Administration published a proposal that served as the basis for standards of identity for enriched bread (14). The following standards of identity for enriched bread became effective in August, 1952; a one-pound loaf of enriched bread must contain: 10-15 mg niacin, 1.1-1.8 mg thiamine, 0.7-1.6 mg riboflavin and 8.0-12.5 mg iron.

The enrichment program was extended to other cereal products. By January, 1943, the Food and Nutrition Board "favored" enrichment of corn meal and grits "in harmony with the enrichment program". By 1946, producers of pasta products such as macaroni, voluntarily requested permission to enrich their products (5). In 1958 the standards became effective for rice.

Corn Products Enrichment

A review of the extent of cultivation and utilization of corn food was presented by Senti and Schafer (15). The U.S. consumption of corn for food has doubled between 1955 and 1970. Products from both dry- and wet-milling of corn are used for food. The principal products of corn dry-milling are grits, meal, and flour which find various uses as "ready-to-eat" breakfast cereals, snack foods, pancakes, mush, muffins and corn bread.

Corn consumption is highest in the southern parts of the United States. By 1958, all degerminated corn meal and grits sold in Alabama, Georgia, Mississippi, North Carolina and South Carolina were required by law to be enriched (12) and in South Carolina and Alabama, it was mandatory to enrich both whole and degerminated corn meal. A survey conducted in 1957 by 12 southern states showed that all the states, except Florida and Virginia, had over 50 percent of their corn meal enriched (12). Today, almost all corn meal in the U.S. market is enriched.

Nutritional Benefits of Enrichment

Although it is difficult to assess thoroughly the success of the enrichment program, many of the manifestations associated with frank nutritional deficiencies that led to the development of the enrichment programs had disappeared by the early 1950's. This is evidenced by several studies and observations from various clinics. Jeans *et al*. (16) revealed the contribution of cereal enrichment to the diets of 400 pregnant women in Iowa. Bread alone was shown to have supplied from 15 to 31 percent of the thiamine, while cereal foods altogether provided from 40 to 50 percent. Had the cereal foods not been enriched the author speculated that the thiamine intake of the pregnant women would have been cut from 30 to 40 percent and many women would have been close to actual deficiency.

Proposed Fortification Policy

Circumstances which did not prevail when the present

program was initiated called for the need to review the current standard of cereal enrichment. There have been tremendous changes in the eating patterns of the U.S. population. Less bread is consumed while there is an increase in the consumption of other baked products such as cakes, doughnuts and crackers - items which are not normally enriched. The idea of "square meals" has given way to increased consumption of snack foods. Also increased weight consciousness, especially among adolescents and college women, has led to decreased food intake (5). New information concerning nutritional requirements of mineral elements, especially of trace elements, and the availability of improved techniques for evaluating the status of these nutrients in the individual have shown the current standard of enrichment to be inadequate.

The Nationwide Survey of Food Intake of 14,500 adults and children (11) indicated that calcium, iron, vitamin A, ascorbic acid and riboflavin were the nutrients most often found to be below the recommended dietary allowances (RDA) for several groups. The survey also showed all males and females 9 years and over to have diets below the RDA for magnesium. Similarly the Ten-State Nutrition Survey of 1968-1970 indicated significant numbers of the population had intakes below the RDA for calcium, iron and vitamin A (17). Riboflavin appeared inadequate among Black and Spanish American ethnic groups - particularly at lower income levels.

Hence it was recommended in 1971 that the vitamin fortification of cereal products include vitamin A, pyridoxine, and

folic acid, while the mineral fortification would be extended to include calcium, zinc and magnesium. In addition, the level of iron was raised significantly (5).

Assessment of Need for Additional Nutrients

Iron. Iron enrichment of grain products has not resulted in significant benefits either because the iron in the compound used for fortification was unavailable or because the amount of iron added was insufficient (8). Therefore, in 1971 the Food and Nutrition Board recommended trebling the iron enrichment level.

Distribution of iron in the body. Iron represents about 0.004 percent of the body weight (18). The total amount varies from 3 to 5 gm. depending on age, sex, size, nutritional status and the amount of iron stored (18). Virtually all iron exists in combination with protein in transport forms, storage forms, enzymes, and respiratory compounds.

About 70 percent of the iron in the body is considered functional iron and the majority of this is found in the hemoglobin of the blood. Iron binds to porphyrin to form the heme fraction of the hemoglobin molecule. The remaining 30 percent is stored in the liver, spleen and bone marrow (18). Storage iron is present in the liver and spleen as ferritin, a soluble iron complex with a 20 percent iron content, or as hemosiderin, which is an insoluble iron-protein complex containing 35 percent iron. Both are capable of releasing stored iron. Iron is transported from the site of absorption or from the storage

site to the cells bound to a protein called transferrin (18).

Measurement of iron status. Iron stores in the liver have been used to assess nutritional status of this mineral. In iron deficiency the storage iron is the first to be depleted. This is reflected in the extent to which the transferrin is saturated (18). Several studies have employed the concentration of serum iron as a measurement of its deficiency (19, 20). Hemoglobin regeneration and hence formation of erythrocytes, is very sensitive to iron deficiency. Hemoglobin and hematocrit determinations have therefore been employed as a measurement of iron nutrition (19, 20). However, hemoglobin formation is only depressed after the iron storage is depleted.

Iron balance. The body does not possess any mechanism for iron excretion (18). Most iron is lost through bile and the exfoliated intestinal mucosa cells which form part of the unabsorbed fecal iron (21). Other channels of iron loss from the body include loss through exfoliated skin. Negligible amounts are lost through sweat and urine (21). Hemorrhage of any origin, including menstrual blood loss in females, raises iron requirements beyond these basal losses. Additional iron requirements are imposed by growth, especially during the first 20 years of human life and during the second and third trimesters in a pregnant woman (21).

Iron is absorbed only in response to need in normal subjects. Therefore, iron deficiency occurs only as a result of circumstances in which iron loss through one or more channels or increased growth requirements exceed the normal iron

absorption (21).

Iron absorption. Iron in food is present in form of heme or as ferric salts present in complex with other compounds in the food (22). Heme iron is absorbed directly into the mucosal cell of the upper intestinal tract (18). Within the cell, the iron is released from the porphyrin into the blood stream where it binds to transferrin. Nonheme iron, because of the complex form in which it occurs, is not as easily absorbed as heme iron. Furthermore, the ferric salts need to be reduced to the ferrous form prior to absorption. Iron is initially taken up by the brush border where it is passed into the mucosal cell. The fate of the iron within the mucosal cell is twofold (21). The iron can enter the plasma after which it binds to transferrin or the iron is deposited within the mucosal cell as ferritin. Such ferritin is not absorbed and is lost along with the mucosal cell when it is exfoliated.

The absorption of iron has been shown to depend on a variety of factors, ranging from the form of the iron in the food source to the type and composition of the diet. Heme iron is highly available, but contributes only 1 to 3 mg of iron per day in the average diet (18). Availability of nonheme iron is far less than that of heme iron, but its availability is improved in the presence of meat or ascorbic acid (21). Evidence from various studies show that protein quality of a food affects iron absorption (19, 20). Miller and Landes (23) observed that more hemoglobin was generated in anemic rats when starch was used as the dietary carbohydrate but glucose

was found detrimental to the utilization of iron.

Bioavailability of iron was shown to differ depending on the iron sources. Iron enrichment of most cereals is done by addition of ferrous sulfate, ferrous gluconate, reduced iron, ferric orthophosphate, or sodium iron pyrophosphate. Ferrous sulfate is the most available source and is the standard against which other iron sources are compared (24). Harrison _et al_. (8) observed that the finer particles of reduced iron were more than twice as available as the coarser fraction.

The effect of dietary calcium on the absorption of iron was studied by Apte and Venkatachalamp (25). They found that when the subjects had daily calcium intakes of 400 mg, 16.6 mg of daily dietary iron was insufficient to maintain a positive iron balance. Iron balance was achieved by increasing the calcium intake to 1000 mg per day.

The poor absorption of iron from cereals has been attributed to their considerable high content of phytic acid which complexes iron to form insoluble phytates (26). However, yeast fermentation was found to decrease phytate activity through the hydrolytic effect of the phytase produced by yeast (27).

The high incidence of iron-deficiency anemia in the United States is often attributed to low dietary iron (24), but results of some studies have contradicted this. Gershoff _et al_. (28) reported that iron fortification of grain products did not result in significant differences in hemoglobin levels of anemic elderly persons. In addition, daily administration

of ferrous sulfate to those persons with low hemoglobin was without effect.

Vitamin A. Vitamin A occurs in the body in several chemical forms: alcohol (retinol), aldehyde (retinaldehyde), acid (retinoic acid) and the precursor carotenoids, but certain forms are specific for certain physiological functions (29). The Ten-State Survey of 1968-70 (17) indicated that vitamin A status of some groups should be improved. Measurements of liver reserves or the retinol-binding proteins in the blood could be useful in evaluating vitamin A status (29). Vitamin A is essential to biochemical reactions necessary to preserve the health of epithelial cells (29).

In vitamin A-deficient animals the incidence of cancer has been shown to be higher than in animals receiving normal vitamin A intakes (30). In some cases the administration of toxic doses of vitamin A has caused a regression of the tumors (31). In severe vitamin A deficiency, abnormalities in both RNA metabolism (32) and protein synthesis (33) have been reported. However, hypervitaminosis A can result from overconsumption of the preformed vitamin (29). The RDA for vitamin A has been set at 1000 R.E. for men, 800 R.E. for women, 1000 R.E. during pregnancy, and 1200 R.E. during lactation (34).

Folic acid. The absorption of dietary folic acid is facilitated by specific enzymes and vitamin B_{12} in the gastrointestinal tract (35). The presence of ascorbic acid and NADPH are necessary to convert the absorbed folate to the biologically active form (35). The body stores folacin in the

liver, in the form of folate polyglutamates, but serum folate has been shown to be a good indication of folic acid status (36).

Folacin functions in all biological reactions involving the transfer of single-carbon units from one substance to another. The synthesis of nucleic acids depends on folic acid coenzymes, thus folacin exerts an indirect effect on essential protein compounds, including the red blood cells. The oxidation of phenylalanine to tyrosine and the decarboxylation of tyrosine require folacin. The formation of the porphyrin group of hemoglobin involves the participation of folacin coenzymes (35).

The current RDA for acid is 400 μg for adults in the U.S. (34). However, any circumstance, physiologic or pathologic, leading to increased rates of cell multiplication will result in higher requirements. Thus, pregnancy (especially during the third trimester and if multiple), lactation, early infancy and adolescence (37) are physiologic states particularly vulnerable to the development of deficiency. A number of substances interfere with normal absorption and utilization of folate; among these is alcohol (38). Folic acid deficiency is often due to drugs that interfere with absorption or with some aspect of folacin metabolism (39, 40). This greatly swells the number of the population at risk of deficiency. Herbert (41) showed that folate malabsorption occurs in gluten-induced enteropathy and in tropical sprue.

Pyridoxine. Pyridoxine, or vitamin B_6, is used to denote

several chemically related substances - pyridoxol, pyridoxal, and pyridoxamine. All of the three forms are capable of being metabolized into pyridoxal phosphate which functions as a coenzyme in various aspects of amino acid metabolism. These include the deamination, decarboxylation, and transamination of amino acids, the conversion of the amino acid tryptophan to the vitamin niacin, and the synthesis of all amino acids. Pyridoxal phosphate is required for the synthesis of δ-amino-levulinic acid, a precursor of heme (42).

Pyridoxine status is assessed by measuring excreted tryptophan metabolites, such as xanthurenic acid, after tryptophan load tests. Measurement of amino acid transferase activity in serum and red blood cells, and urinary excretion of pyridoxic acid (a metabolite of pyridoxine) have been used to indicate pyridoxine nutriture (43). Cinnamon and Beaton (44) have shown the measurement of erythrocyte transaminase activity to be a good method of pyridoxine assessment in man.

The requirement for pyridoxine is increased by high dietary protein (45). After evaluating evidence that pyridoxine requirement was protein dependent and relatively independent of caloric content of the diet, the National Research Council in 1968 recommended an intake of 2 mg per day, which they postulated would be adequate for the metabolism of 100 gm of protein (42).

It has been demonstrated that a marked increase in the excretion of tryptophan metabolites during pregnancy does occur, and that this can be prevented by administration of

pyridoxine (46). Several studies have shown that the administration of oral contraceptives in women resulted in abnormal levels of tryptophan metabolites, which could be prevented by pyridoxine supplementation (47, 48). It was suggested by Luhby *et al*. (48) that 30 mg of pyridoxine be taken daily in order to normalize tryptophan metabolism in users of oral contraceptives. This amount of pyridoxine cannot be supplied by ordinary diets. Daily intake of 10 mg of pyridoxine was found to normalize tryptophan metabolism in 75 percent of the subjects studied by Luhby *et al*. (48). Although it is questionable if oral contraceptives are harmful to women who do not receive pyridoxine supplements, there is room for concern: Baumblatt and Winston (49) reported that in a group of 58 women who complained of malaise during oral contraceptive administration, 18 had their problems completely resolved and there was considerable improvement in 26 women after receiving pyridoxine supplements. With millions of women in the U.S. taking oral contraceptive agents, there should be a concern to improve their pyridoxine status.

Several cases of drug antagonism of pyridoxine and genetic conditions in which abnormalities of pyridoxine metabolism occurs were discussed by Gershoff (43). He also suggested that pyridoxine requirement may be increased in the elderly.

The current enrichment program does not include addition of pyridoxine, but it is added to fortified cereal products by the processor, with the added nutrient declared on the label. It was shown that milling of cereals leads to pyridoxine losses as high

as 90 percent of the original values (50).

Calcium. Calcium is the most abundant mineral in the human body. It comprises 1.5 to 2.0 percent of the total adult weight (51) Of this 850 to 1400 gm of calcium in the adult body, 99 percent is present in bones (51). The 1 percent of the body's calcium outside of the bone functions in a number of essential processes and is found in extracellular fluid, soft tissue, and as a component of various membrane structures. The extra-skeletal calcium is involved in blood coagulation. It is necessary for muscle contractibility, for myocardial function, for normal neuromuscular irritability and is also an activator of a number of body enzymes (51). A significant decrease in serum ionized calcium results in tetany while an increase can cause cardiac or respiratory failure through an impairment of muscle function (52).

Calcium is actively absorbed against a concentration gradient by a process requiring energy. Vitamin D is required for this active transport, and a vitamin D-induced calcium-binding protein is thought to play this role (52).

Draper *et al*. (53) studied the effect of varying levels of dietary phosphorus, on calcium excretion in aging rats. Urinary excretion of calcium was depressed by increased dietary phosphorus, but high phosphorus intake promoted endogenous fecal calcium excretion. The overall effect was that high dietary phosphorus, up to a limit, increased calcium resorption from bones. The influence of high phosphorus intakes on bone metabolism could not be fully counteracted by

increasing calcium content of the diet. They attributed the counteraction failure to the fact that the efficiency of calcium absorption, unlike that of phosphorus, rapidly decreases as dietary calcium increases. Osteoporosis, a condition characterized by sufficient bone loss to cause disabling symptoms, is estimated to affect 14 million women in the United States (52). High intakes of calcium are generally found to produce rather large apparent calcium retentions but this retention is not demonstrable by radiologic methods (54).

Increased dietary protein was found to increase excretion of calcium (55). Varying protein intake from 0 to 90 gm N per day resulted in about 800% increase in calcium excretion, irrespective of calcium intake.

The RDA for calcium is 800 mg for adults (34). Calcium was an optional nutrient when the current enrichment for cereals was first legislated (13), and non-fat dry milk often has been added to bread. Fortification of cereals with calcium would therefore be necessary in view of the fact that addition of milk to bread has become less common.

Magnesium. In the adult the magnesium content of the body varies from 21 to 28 gm, of which 50 to 60 percent is concentrated in bone (56). About one-third of the magnesium is closely bound with phosphate, and the remainder is absorbed on the bone surface, from which it can be mobilized to maintain normal blood and tissue levels. In the soft tissue, magnesium is concentrated within the cell. In the blood it occurs primarily in the red blood cells. Magnesium levels in

the serum are maintained from 1 to 3 mg per 100 ml. In a deficiency the level in the red blood cells drops. In an excess the serum level rises (56).

Within the cell, magnesium plays a role as catalyst to numerous biological reactions, a major portion of which take place in the mitochondrion. It activates the production of ATP and all changes of ATP to ADP. Magnesium also influences protein synthesis by affecting the arrangement of the ribosomes and by facilitating the attachment of RNA to the ribosome. Although only 1 percent of magnesium occurs extracellularly, it is one of the minerals involved in promoting the conduction of nerve impulses as shown in rat studies (57).

Hypocalcuria, hypocalcemia and hypokalemia but normal serum sodium were observed in experimental magnesium deficiency in humans (58). Abnormal electrocardiograms were also observed in many of them. Low incidence of cardiovascular disease in the Orientals has been attributed to the high magnesium content of their diets (56).

Zinc. Zinc has been recognized as an essential for rat growth since 1934 (59). Since then, zinc deficiency has been recognized in farm animals and studied extensively in laboratory models (60).

The body contains from 2 to 3 gm of zinc, 73 percent of which is concentrated in the skeleton, although high concentrations are also found in the hair, skin, nails, and testes (61). In the blood 85 percent of the zinc occurs in the red blood cells, 4 percent in the leukocytes and platelets, and the remainder in the serum, where it is tightly bound to

protein (61).

Zinc is a stably-bound constituent of various enzymes involved in digestion and metabolism. Processes which are profoundly affected by experimental zinc deficiency include DNA metabolism, RNA metabolism, protein metabolism and mucopolysaccharide metabolism (62).

Apart from prompt growth arrest, an intolerance to parenteral glucose by zinc-deficient rats does occur, leading to the suggestion that zinc may in some way participate in the uptake of glucose by cells (60). Increased sensitivity to insulin has been found in zinc-deficient human subjects (63).

Dermatitis caused by zinc-deficiency was reported in rats (60) and in swine (64). The gross effect of the abnormalities in the chemistry of the skin as a result of zinc-deficiency in animals is impaired wound healing (65). The clinical equivalents of these animal abnormalities have been reported in persons with impaired healing of ulcers (66).

Zinc deficiency syndrome in adolescent males in Egypt was described by Parsad *et al*. (67). The symptoms with the exception of dermatitis closely resembles experimental zinc deficiency in laboratory animals. The primary features are growth failure and hypogonadism. Confirmatory studies in Iran documented the syndrome in females (68) and also demonstrated zinc-responsive growth failure and delayed sexual maturation in school boys (69). Zinc deficiency in Egypt and Iran has been associated with a diet of wheat flour and little animal protein. A rat study supported the concept that phytate and

fiber in diets render zinc from foods of plant origin less available than those from animal origin (70).

That zinc deficiency does occur in the United States has been shown in pre-school children in Denver (71), by observation of low levels of zinc in hair, poor appetites, retarded growth and impaired taste acuity. Analysis of diets consumed by some children in the United States revealed intakes in the marginal to deficient range (72). Similar findings have also been reported by White (73) who analyzed the zinc content of weighed, self-selected diets of high school girls and college women. A majority of the diets contained less than the 15 mg recommended daily allowance for zinc and a significant number provided less than two-thirds of the allowance. In fact, approximately one-third of thirty diets (planned and prepared by professional nutritionists) for a metabolic study contained less than 10 mg zinc.

MATERIALS AND METHODS

Enrichment and Fortification of Corn Meal Samples

Degerminated yellow corn meal was obtained from the Department of Grain Science and Industry. Information on the nutrient contents is shown in Appendix 1. Four samples of the corn meal were prepared as follows:

A - unenriched

B - enriched according to current enrichment standard (13) to give 2.8 mg. thiamine, 1.75 mg riboflavin, 21.0 mg niacin and 13-16.5 mg iron per pound corn meal. This was prepared by mixing 0.7088 gm of "N-Richment-A type 41" mix (Appendix 2) with 10 pounds of the unenriched cornmeal.

C - fortified according to the proposed policy for cereal fortification (5) to give 2.9 mg thiamine, 1.8 mg. riboflavin, 24 mg niacin, 0.3 mg folic acid, 2 mg vitamin B_6, 1.3 R.E. vitamin A, 900 mg calcium, 200 mg magnesium, 10 mg zinc, but with 13-16.5 mg iron (current enrichment standard for iron). This was done in the Department of Grain Science and Industry by mixing 20 mg "vitamin premix no. 2077" (Appendix 3), 626.6 gm $CaSO_4$, 36.0 gm MgO and 1.8 gm ZnO with 200 pounds of unenriched cornmeal.

D - fortified according to the proposed policy for cereal fortification (5) as shown for sample C except that additional iron was added to give 40.0 mg iron per pound corn meal. This was achieved by mixing 235.4 mg electrolytic

iron with 10 pounds of the fortified cornmeal C.

Procedure for Making and Freeze-drying Mush

Mush was prepared from appropriate corn meal samples using the following recpie:

	gm.
Corn meal	306.0
salt, iodized	15.6
Cold water	448.0
Boiling water	1344.0

The cornmeal, salt and cold water were weighed out and mixed thoroughly in a stainless steel mixing bowl, using a plastic spoon. The mixture was gradually poured into the boiling water in a stainless steel sauce pan and stirred constantly. The mixture was returned to boil with continuous stirring. The heat was reduced, the sauce pan covered and cooking continued over low heat for 5 more minutes. Demineralized water was used in all the preparations. Using the same procedure, several batches were prepared until about 10 pounds of each sample had been cooked.

Each batch of cooled mush was immediately frozen in glass jars or stainless steel trays and then dried in a freeze dryer at ambient temperature of 65°F until the sample was uniformly fluffy and brittle. The products were ground in a roller mill and passed through a no. 20 mesh screen.

Diets and Animal Study

Each freeze-dried cornmeal mush was used to formulate the appropriate diet as indicated in Table 1. The diets were identified as A, B, C or D according to the type of corn meal mush used.

Thirty-two weanling Sprague-Dawley rats weighing between 45 and 67 gm were used. Eight rats were assigned to each of the four diets in such a way that the average weights of the four groups of rats were equal. Each group of rats was identified according to the diet consumed. The study lasted 28 days, with the exception of group C rats which were fed diet A (containing the unenriched cornmeal) for the first 7 days of study[a], after which the rats were fed diet C for 28 days. The first day on which the rats were fed diet C was considered as the zero day of study for these rats.

Each rat was weighed weekly and feed intake calculated. The cages were kept clean by removing wastes daily. Any spilled feed was weighed and the amount subtracted accordingly.

At the end of the study period, feed efficiency was calculated using the following formula:

$$\text{Feed Efficiency} = \frac{\text{Cumulative weight gain}}{\text{Cumulative feed intake}}$$

Blood samples were obtained for hemoglobin and hematocrit determination by amputating the tip of the tail of each rat.

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[a]Due to error.

TABLE 1

Composition of Experimental Diets[a]

Ingredients (gm/100 gm)	Diets			
	A	B	C	D
Corn meal mush A	81.2	-	-	-
Corn meal mush B	-	81.2	-	-
Corn meal mush C	-	-	81.2	-
Corn meal mush D	-	-	-	81.2
Vitamin-free Casein	10.0	10.0	10.0	10.0
DL-methionine	0.2	0.2	0.2	0.2
Corn oil	5.0	5.0	5.0	5.0
Vitamin mix[b]	2.2	2.2	2.2	2.2
Salt mix[c]	1.4	1.4	1.4	1.4

[a]As fed basis.

[b]Per kilogram vitamin mix: 100,000 USP units of vitamin D, 5 gm of alpha tocopherol, 45 gm of ascorbic acid, 5 gm of inositol, 75 gm of choline chloride, 2.25 gm menadione, 5 mg of P-aminobenzoic acid, 3 gm of calcium pantothenate, 0.02 gm of biotin, 1.35 mg of vitamin B_{12} and 859.6 gm of dextrose.

[c]Per 1.4 gm salt mix: 0.651 gm of sodium phosphate, 0.73 gm of potassium chloride, 15.4 mg of manganese sulfate ($MnSO_4 \cdot H_2O$), 1.3 mg of copper sulfate, and 0.1 mg potassium iodate.

Hemoglobin Determination

Hemoglobin content of blood samples from each rat was determined by the Hycel's Cyanmethemoglobin Certified Standard (74). A standard curve of hemoglobin concentration against absorbance was established preliminarily by diluting Hycel Cyanmethemoglobin Standard with Hycel Cyanmethemoglobin Reagent and measuring the absorbance of each dilution at 540 nm on the Bausch and Lomb Spectrophotometer. The hemoglobin concentration of each blood sample was measured by adding 0.2 ml whole blood (using a disposable micropipette) to 6 ml of Cyanmethemoglobin Reagent contained in a colorimeter tube. It stood for 10 minutes, after which the absorbance was read. Before each reading, the spectrophotometer was zeroed using 6 ml of the reagent as a blank. The corresponding hemoglobin concentration was then read off the standard curve. Two determinations of hemoglobin were made on each animal and the average calculated.

Hematocrit Determination

Heparinized capillary tubes were filled with blood to about 3/4 volume. One of the tube ends was then sealed with clay and the tubes centrifuged for 3 minutes in the Adams Autocrit Centrifuge (CT-2905). Percentage of packed cell volume was read from a hematocrit reading chart on the apparatus. Two hematocrit determinations were made on each animal and the average calculated.

Statistical Analysis

The data from weight gain, feed intake, feed efficiency, hemoglobin concentration, and hematocrit readings were subjected to analysis of variance. The means were separated by using Fischer's LSD with P=0.5 and P=0.01 when the F-test rejected the hypothesis of equal treatment means.

RESULTS AND DISCUSSION

Analyses of variance for weight gain, feed intake and feed efficiency are shown in Table 2. That of hemoglobin and hematocrit are presented in Table 5. Diets had a significant effect on each of the factors considered ($P<0.01$). Fischer's Least Significant Difference at 5% and 1% levels for weight gain, feed intake and feed efficiency is presented in Table 3, while that of hemoglobin and hematocrit is shown in Table 6. Table 4 shows data for mean total weight gain, feed intake and feed efficiency of the rats after the 28-day study. Values for the hemoglobin and hematocrit are shown in Table 7. Data for individual rats are presented in Appendix 4.

Weight Gain

Rats fed diet B (cornmeal enriched by current standard) gained slightly but not significantly more than those fed diet A (unenriched corn meal). Although diet B was enriched with niacin, riboflavin, thiamine and iron, lack of significant difference between this diet and the unenriched control diet A could be a manifestation of the deficiency of the other nutrients such as calcium, magnesium, zinc, vitamin A and folic acid not included in the diet B enrichment.

There was a highly significant difference ($P<0.01$) between rats fed diets B and C (fortified cornmeal with low iron level). Rats fed diet C gained more than those fed either diet

TABLE 2

Mean Squares and Significance[a] for Weight Gain, Feed Intake and Feed Efficiency of Rats After 28 Days on Experimental Diets

Source of Variation	Degrees of Freedom	Weight Gain	Feed Intake	Feed Efficiency
Diets	3	6474.71**	22189.87**	0.014467**
Rats:Diets	28	216.88	717.78	0.0009159
Total	31			

a** $P \leq 0.01$

TABLE 3

Fischer's Least Significant Difference at the 5% and 1% Levels for Mean Total Weight Gain, Feed Intake and Feed Efficiency

Sample Size	Significance Level					
	Weight Gain		Feed Intake		Feed Efficiency	
	5%	1%	5%	1%	5%	1%
8 & 8	15.08	20.34	27.44	37.01	0.031	0.111

TABLE 4

Significance[a] of Enrichment Level on Mean Total Weight Gain, Feed Intake and Feed Efficiency of Rats Fed Experimental Diets for 28 Days

Diet	Source of Diet	No. of Rats	Mean Total Weight Gain	Mean Total Feed Intake	Feed Efficiency
A	Unenriched corn meal	8	61.9[a]	211.1[a]	0.293[a]
B	Corn meal enriched at current standard	8	70.4[a]	242.8[b]	0.289[a]
C	Corn meal fortified according to proposed standard, but current low iron level	8	104.0[b]	306.5[c]	0.335[b]
D	Corn meal fortified according to proposed standard	8	122.5[c]	332.8[c]	0.380[c]

[a]Diets with a common letter in a column are not significantly different ($P>0.05$).

TABLE 5

Mean Squares and Significance[a] for Hemoglobin and Hematocrit of Rats After 28 Days on Experimental Diets

Source of Variation	Degrees of Freedom	Hemoglobin	Hematocrit
Diets	3	17.963**	223.86**
Rats:Diets	27	0.551	7.29
Total	30		

[a]**P<0.01

TABLE 6

Fischer's Least Significant Difference at the 5% and 1% Levels for Hemoglobin and Hematocrit

Sample Size	Significance Level			
	Hemoglobin		Hematocrit	
	5%	1%	5%	1%
8 & 8	0.76	1.03	2.77	3.74
7 & 8	0.79	1.07	2.87	3.87

TABLE 7

Significance[a] of Enrichment Level on Hemoglobin and Hematocrit of Rats After 28 Days on Experimental Diets

Diet	Source of Diet	No. of Rats	Hemoglobin (gm/dl)	Hematocrit (%)
A	Unenriched corn meal	8	12.46[a]	41.4[a]
B	Corm meal enriched at current standard	7[d]	15.94[b]	52.9[b]
C	Corn meal fortified according to proposed standard, but current low iron level	8	15.21[bc]	52.6[b]
D	Corn meal fortified according to proposed standard	8	15.04[c]	48.3[c]

[a]Diets with a common letter in a column are not significantly different ($P>0.05$).

[d]One rat died during blood sampling.

A or B. This improvement in the performance of rats fed diet C over those fed diets A and B could be attributed to the inclusion in diet C of the nutrients left out in the diet B enrichment, since these added nutrients have been described as essential for optimum performance of rats. Rats fed diet C were on the deficient unenriched corn meal for a week and tended to grow rapidly as a "catch-up mechanism" during the first week on the fortified corn meal (Fig. 1).

Rats fed diet D (cornmeal fortified according to proposed standard) gained more ($P<0.05$) than those fed diet C. Diet D contained more iron than diet C. This iron fortification might explain the improvement in weight gain. The results of the growth study tend to support the proposed standard of fortification.

Feed Intake

Rats fed diet B had higher total feed intake than those fed diet A ($P<0.05$). This is not surprising, since it is expected that rats will consume more of an adequate diet than one that is subadequate. Diet B in this study contained a higher level of niacin, riboflavin, thiamine and iron than diet A.

Total feed intake was greater for rats fed diet C than those fed diets A ($P<0.01$) and B ($P<0.05$). Rats fed diet D ate significantly more feed than those fed diets A or B ($P<0.01$). Feed intake of rats fed diets C and D were not significantly different ($P<0.05$).

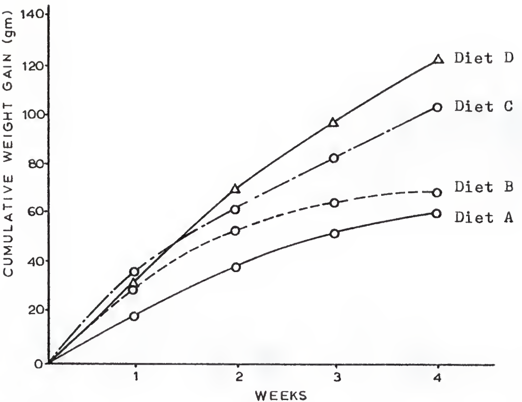

Fig. 1. Growth curve showing cumulative weight gain in gms against weeks.

Feed Efficiency

Feed efficiency was not significantly different for rats fed diets A and B. This result could be because the effect of enrichment of the B diet with some nutrients is masked by the absence of other essential nutrients. Rats fed diets C and D (proposed fortification standards) had significantly higher feed efficiencies than rats fed diets A or B. Moreover, diet D (fortified corn meal with the higher level of iron) resulted in more efficient feed utilization than diet C (fortified corn meal with the lower level of iron).

Hemoglobin

Rats fed control diet A had lower hemoglobin concentration than any of the other rats ($P<0.05$). There was no significant difference between hemoglobin concentration of rats fed diets B and C. This is expected, since iron levels in these two diets were similar.

Although diet D contained a higher level of iron than diet C, there was no significant difference between the hemoglobin concentration of rats fed these diets. Rats fed diet B had a higher hemoglobin level than those fed diet D. This depression in hemoglobin level of the high iron diet D could be due to excess iron dose which could result in decreased percentage iron absorption in the gut (18). This could also suggest that the requirement for dietary iron does not exceed that in diets B or C. It has been suggested that excess of any one nutrient is just as bad as deficiency of that nutrient

(75). By adapting the high iron level recommended in the proposed policy for fortification, the risk of iron overload may be increased. The adverse effect of iron overload has been described by Finch (21).

Hematocrit

Hematocrit is a measure of the total cellular fraction of the blood. Since iron is involved in erythropoiesis as part of heme (18), hematocrit value (% Packed Cell Volume) has been used as a measure of iron availability (19, 20).

As observed with the hemoglobin results, the hematocrit values for rats fed control diet A were lower than for those fed the other diets ($P<0.01$). Rats consuming diets B and C did not differ significantly in hematocrit value. This supports the earlier assertion that similar levels of iron enrichment in diets B and C were responsible for the lack of difference between the hemoglobin concentration of rats consuming these diets. Rats consuming diet D had lower hematocrit values than those consuming either diets B or C ($P<0.01$). This further supports the earlier suggestion that superfortification of diet D with iron could result in decreased utilization of iron. Therefore it is questionable whether the higher level of iron fortification should be endorsed.

SUMMARY

The nutritional effects of the current and proposed policies for cereal fortification were compared using Sprague-Dawley weanling male rats. A control diet A was prepared using unenriched corn meal. Diet B contained corn meal enriched by current standard. Diet C was prepared from corn meal fortified according to the proposed policy, except that it was lower in iron. The iron level was similar to that of corn meal sample B. Diet D contained corn meal fortified to meet all recommendations for the proposed standard, including iron.

Rats were fed for 28 days, after which weight gain, feed intake, hemoglobin concentration and hematocrit were measured. Feed efficiency was also calculated.

Rats fed control diet A ate significantly less feed but were not significantly different in weight gain and feed efficiency in comparison to rats fed diet B. Weight gain and feed efficiency were greater for diet C than for diet B. Diet D produced the highest weight gain and feed efficiency. Feed intake of rats fed diet C was higher than that of rats fed diet B, but there was no significant difference between feed intakes of rats fed diets C and D.

Hemoglobin concentration was higher for rats fed diet B than for those fed diets A or D. Hemoglobin concentrations of rats fed diets B or C were not significantly different. Also there was no significant difference in the hemoglobin

concentration of rats fed diets C or D. Rats fed diet A had the lowest hemoglobin values.

Hematocrit values for rats fed diet A were significantly lower than those of rats fed diets B, C, or D. Hematocrit values for rats fed diet D were significantly lower than for rats fed either diets B or C. There was no significant difference between hematocrit values of rats fed diets B and C.

Enrichment of cornmeal to the proposed fortification standard resulted in improvement in growth, feed intake and feed efficiency over the corn meal enriched according to the current standard. The higher iron fortification according to the proposed standard of fortification did not result in increased iron utilization as measured by hemoglobin generation and hematocrit value. It is, therefore, questionable whether the higher level of iron should be endorsed.

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LITERATURE CITED

1. TODHUNTER, E.N. Historical landmarks in nutrition. In: Present Knowledge in Nutrition (4th ed.). p. 552. The Nutrition Foundation, Inc.: New York, Washington (1976).

2. WILLIAMS, R.D., MASON, H.L., and WILDER, R.M. The minimum daily requirement of thiamine of man. J. Nutr. 25: 71 (1943).

3. FOLTZ, E.E., BARBDRKA, C.J., and IVY, A.C. The level of vitamin B-complex in the diet at which detectable symptoms of deficiency occur in man. Gastroenterology 2: 323 (1944).

4. SPIES, T.D., VILTER, R.W., and ASHE, W.F. Pellagra, beriberi and riboflavin deficiency in human beings. J. Am. Med. Assoc. 113: 931 (1939).

5. FOOD AND NUTRITION BOARD, NATIONAL RESEARCH COUNCIL. Proposed fortification policy for cereal-grain products. Nat. Acad. Sci.: Washington, D.C. (1974).

6. ANDERSON, R.A., VOJNOVICH, C., and BOOKWALTER, G.N. Iron enrichment of dry-milled corn products. Cereal Chem. 53: 937 (1975).

7. STEELE, C.J. Cereal fortification-technological problems. Cereal Foods World 21: 538 (1976).

8. HARRISON, B.N., PLA, G.W., CLARK, G.A., and FRITZ, J.C. Selection of iron sources for cereal enrichment. Cereal Chem. 53: 78 (1976).

9. RANHOTRA, G.S., LOEWE, R.J., LEHMANN, T.A., and HEPBURN, F.N. Effect of various magnesium sources on bread-making characteristics of wheat flour. J. Food. Sci. 41: 952 (1976).

10. GOLDSMITH, G.A. Clinical nutritional problems in the United States today. Nutr. Rev. 23: 1 (1965).

11. CONSUMER AND FOOD ECONOMICS RESEARCH DIVISION, AGRICULTURAL RESEARCH SERVICE. Food and nutrient intake of individuals of the United States, Spring 1965. Household Food Consumption Survey 1965-66, Rep. No. 11. USDA, Washington, D.C. (1972).

12. THE COMMITTEE ON CEREALS. Cereal enrichment in perspective. p. 1. NAS-NRC, Washington, 25, D.C. (1958).

13. FEDERAL REGISTER 8: 9115 (1943).

14. FEDERAL REGISTER 8: 10780 (1943).

15. SENTI, F.R., and SCHAEFER, W.C. Corn, its importance in food, feed and industrial uses. Cereal Sci. Today 17: 352 (1972).

16. JEANS, P.C., SMITH, M.B., and STEARNS, G. Dietary habits of pregnant women of low income in a rural state. J. Am. Dietet. Assoc. 28: 27 (1952).

17. FIRST HEALTH AND NUTRITION EXAMINATION SURVEY. Dietary intake and biochemical findings. U.S. Dept. of Health, Educ. and Welfare: Rockville, Maryland (1974).

18. GUTHRIE, H.A. Iron. In: Introductory Nutrition. (3rd ed.). p. 145-160. C.V. Mosby Company; St. Louis (1975).

19. MILLER, J. Utilization of iron from enriched wheat bread by normal and enemic rats. Cereal Chem. 53: 33 (1976).

20. MILLER, J. Metabolism of iron by rats fed wheat diets supplemented with lysine and methionine. Nutr. Rep. Int. 10: 1 (1974).

21. FINCH, C.A. Iron. In: Present Knowledge in Nutrition (4th ed.) p. 280-288). The Nutrition Foundation, Inc.: New York, Washington (1976).

22. BJORN-RASMUSSEN, E., HALLBERG, L., ISAKSSON, B., and ARVIDSSON, B. Food iron absorption in man. Applications of the two-pool extrinsic tag method to measure heme and nonheme iron absorption from the whole diet. J. Clin. Invest. 53: 247 (1974).

23. MILLER, J., and LANDES, D.R. Effects of starch, sucrose, and glucose on iron absorption by anemic rats. Nutr. Rep. Int. 14: 7 (1976).

24. DAVIDSON, J.T., and RUSSO, M.E. Iron fortification in breakfast cereal. Cereals Foods World 20 : 534 (1976).

25. APTE, S.V., and VENKATACHALAMPS, P.S. The influence of dietary calcium on absorption of iron. Ind. J. Med. Res. 52: 213 (1964).

26. HAGHSHENASS, M., MAHLOUDJI, M., REINHOLD, J.G., and MOHAMMEDI, N. Iron-deficiency anemia in an Iranian population associated with high intake of iron. Am. J. Clin. Nutr. 25: 1143 (1972).

27. REINHOLD, J.G. Phytate destruction by yeast fermentation in whole wheat meals. J. Am. Dietet. Assoc. 66: 38 (1975).

28. GERSHOFF, S.N., BRUSIS, O.A., NINO, H.V., and HUBER, A.M. The effects of iron fortification on moderately anemic people. Am. J. Clin. Nutr. 30: 226 (1977).

29. GUTHRIE, H.A. Vitamin A. In: Introductory Nutrition. (3rd ed.) p. 202-218. C.V. Mosby Company; St. Louis (1975).

30. ROGERS, A.E., HERNDON, B.J., and NEWBERNE, P.M. Induction by dimethylhydrazine of intestinal carcinoma in normal rats and rats fed high or low levels of vitamin A. Cancer Res. 33: 1003 (1973).

31. SAFFIOTTI, U., MONTESANA, R., SELLAKUMAR, A.R. and BORG, S.A. Experimental cancer of the lung. Inhibition by vitamin A of the induction of tracheobronchial squamous metaplasia and squamous cell tumours. Cancer 20: 857 (1967).

32. KAUFMAN, D.G., BAKER, M.S., SMITH, J.M., HENDERSON, W.R., HARRIS, C.C., SPORN, M.B., and SAFFIOTT, U. RNA metabolism in tracheal epithelium: Alteration in hamsters deficient in vitamin A. Science 177: 1105 (1972).

33. DELUCA, L., LITTLE, E.P., and WOLF, G. Vitamin A and protein synthesis by rat intestinal mucosa. J. Biol. Chem. 244: 701 (1969).

34. FOOD AND NUTRITION BOARD. Recommended Dietary Allowances (8th ed.). Nat. Acad. Sci.: Washington, D.C. (1974).

35. GUTHRIE, H.A. Folic acid. In: Introductory nutrition. (3rd ed.) p. 280-287. C.V. Mosby Company: St. Louis (1975).

36. HERBERT, V., LARRABEE, A.R., and BUCHANAN, J.M. Studies on the identification of a folate compound of human serum. J. Clin. Invest. 41: 1134 (1962).

37. DANIEL, W.A., JR., GAINES, E.G., and BENNET, D.L. Dietary intakes and plasma concentrations of folate in healthy adolescents. Am. J. Clin. Nutr. 28: 363 (1975).

38. HALSTED, C.H., GRIGGS, R.C., and HARRIS, J.W. The effect of alcoholism on the absorption of folic acid (H^3-PGA) evaluated by plasma levels and urine excretion. J. Lab. Clin. Med. 69: 116 (1967).

39. GERSON, C.D., HEPNER, G.W., BROWN, N., COHEN, N., HERBERT, V., and JANDWITZ, H.D. Inhibition by diphenyhydantoin of folic acid absorption in man. Gastroenterology 63: 246 (1972).

40. JAFFE, N., FREI, E., III, TRAGGIS, D., and BISHOP, Y. Adjuvant methotexate and citrovorum-factor treatment of osteogenic sarcoma. New Eng. J. Med. 291: 1944 (1974).

41. HERBERT, V. The five possible causes of all nutrient deficiency: Illustrated by deficiencies of vitamin B_{12} and folic acid. Am. J. Clin. Nutr. 26: 77 (1973).

42. GUTHRIE, H.A. Pyridoxine. In: Introductory Nutrition (3rd ed.) p. 272-277. C.V. Mosby Company; St. Louis (1975).

43. GERSHOFF, S.N. Vitamin B_6. In: Present Knowledge in Nutrition (4th ed.) p. 149. The Nutrition Foundation, Inc.: New York, Washington (1976).

44. CINNAMON, A.D. and BEATON, J.R. Biochemical assessment of vitamin B_6 status in man. Am. J. Clin. Nutr. 23: 696 (1970).

45. GERSHOFF, S.N. Effect of dietary levels of macronutrients on vitamin requirements. Fed. Proc. 23: 1077 (1964).

46. WACHSTEIN, M. Evidence for a relative vitamin B_6 deficiency in pregnancy and some disease states. Vitamin Hormones 22: 705 (1964).

47. PRICE, J.M., THORNTON, M.J., and MUELLER, L.M. Tryptophan metabolism in women using steroid hormones for ovulation control. Am. J. Clin. Nutr. 20: 452 (1967).

48. LUHBY, A.L., BRIN, M., GORDON, M., DAVIS, P., MURPHY, M., and SPIEGEL, H. Vitamin B_6 metabolism in users of oral contraceptive agents. I. Abnormal urinary xanthurenic acid excretion and its correction of pyridoxine. Am. J. Clin. Nutr. 24: 684 (1971).

49. BAUMBLATT, M.J., and WINSTON, F. Pyridoxine and the pill. Lancet 1: 832 (1970).

50. POLANSKY, M.M., MURPHY, E.W. and TOEPFER, E.W. Components of vitamin B_6 in grains and cereal products. J. Ass. Agric. Chem. 47: 750 (1964).

51. GUTHRIE, H.A. Calcium. In: Introductory Nutrition. (3rd ed.) p. 113-131. C.V. Mobby Company: St. Louis (1975).

52. GOODHART, R.S. and SHILS, M. Modern nutrition in health and disease (5th ed.) p. 268-286. Lea and Febiger, Philadelphia (1973).

53. DRAPER, H.H., SIE, T.L., and BERGAN, J.G. Osteoporosis in aging rats induced by high phosphorous diets. J. Nutr. 102: 1133 (1972).

54. NORDIN, B.E.C. Calcium balance and calcium requirement in spinal osteoporosis. Am. J. Clin. Nutr. 10: 384 (1962).

55. MARGEN, S., CHU, J.Y., KAUFMANN, N.A., and CALLOWAY, D.H. Studies in calcium metabolism. I. The calciuretic effect of dietary protein. Am. J. Clin. Nutr. 27: 584 (1974).

56. GUTHRIE, H.A. Magnesium. In: Introductory Nutrition. (3rd ed.) p. 140-143. C.V. Mosby Company; St. Louis (1975).

57. KRUSE, H.D., ORENT, E.R., and MCCOLLUM, E.V. Studies on magnesium deficiency in animals. I. Symptomatology resulting from magnesium deprivation. J. Biol. Chem. 96: 519 (1932).

58. SHILS, M.E. Experimental human magnesium depletion. I. Clinical observations and blood chemistry alterations. Am. J. Clin. Nutr. 15: 133 (1964).

59. TODD, W.R., ELVEHJEM, C.A., and HART, E.B. Zinc in the nutrition of the rat. Am. J. Physiol. 107: 146 (1934).

60. MILLS, C.F., QUARTERMAN, J., CHESTERS, J.K., WILLIAMS, R.B., and DALGARNO, A.C. Metabolic role of zinc. Am. J. Clin. Nutr. 22: 1240 (1969).

61. GUTHRIE, H.A. Zinc. In: Introductory nutrition. (3rd ed.) p. 171-173. C.V. Mosby Company, St. Louis (1975).

62. SANDSTEAD, H.H. Zinc. In: Present Knowledge in Nutrition (4th ed.) p. 291. The Nutrition Foundation, Inc.: New York, Washington (1976).

63. SANDSTEAD, H.H., PRASAD, A.S., SCHULERT, A.R., FARID, Z., MIALE, A., JR., BASSILLY, S., and DARBY, W.J. Human zinc deficiency, endocrine manifestations and response to treatment. Am. J. Clin. Nutr. 20: 422 (1967).

64. TUCKER, H.F., and SALMON, W.D. Parakeratosis or zinc deficiency disease in the pig. Proc. Soc. Exp. Biol. Med. 88: 613 (1955).

65. SANDSTEAD, H.H., LANIER, V.C., JR., SHEPHARD, G.H., and GILLESPIE, D.D. Zinc and wound healing. Effects of zinc deficiency and zinc supplementation. Am. J. Clin. Nutr. 23: 514 (1970).

66. SERJEANT, G.R., GALLOWAY, R.E., and GUERI, M.C. Oral zinc sulfate in sickle-cell ulcers. Lancet 11: 891 (1970).

67. PRASAD, A.S., MIALE, A., JR., FARID, Z., SANDSTEAD, H.H., and SCHULERT, A.R. Zinc metabolism in patients with the syndrome of iron deficiency anemia, hepatosplenomegaly, dwarfism, and hypogonadism. J. Lab. Clin. Med. 61: 537 (1963).

68. HALSTED, J.A., RONAGHY, H.A., ABADI, P., HAGHSHENASS, M., AMIRHAKEMI, G.H., BARAKAT, R.M., and REINHOLD, J.G. Zinc deficiency in man. The Shiraz experiment. Am. J. Med. 53: 277 (1972).

69. RONAGHY, H.S., REINHOLD, J.G., MAHLOUDJI, M., GHAUMI, P., SPIREYFOX, M.R., and HALSTEAD, J.A. Zinc supplementation of malnourished school boys in Iran. Increased growth and other effects. Am. J. Clin. Nutr. 27: 112 (1974).

70. O'DELL, B.L., BURPO, C.E. and SAVAGE, J.E. Evaluation of zinc availability in foodstuffs of plant and animal origin. J. Nutr. 102: 653 (1972).

71. HAMBIDGE, K.M., HAMBIDGE, C., JACOBS, M., and BAUM, J.D. Low levels of zinc in hair, anorexia, poor growth, and hypogeusia in children. Pediat. Res. 6: 868 (1972).

72. SANDSTEAD, H.H. Zinc nutrition in the United States. Am. J. Clin. Nutr. 26: 1251 (1973).

73. WHITE, H.S. Zinc content and the zinc-to-calorie ratio of weighed diets. J. Am. Dietet. Ass. 68: 243 (1976).

74. HYCEL, INC. P.O. Box 36329, Houston, Texas (Revised, September, 1975).

75. TODHUNTER, E.N. Historical landmarks in nutrition. In: Present Knowledge in Nutrition (4th ed.) p. 551. The Nutrition Foundation, Inc.: New York, Washington, (1976).

APPENDIX

APPENDIX la: PROXIMATE ANALYSIS OF UNENRICHED DEGERMINATED CORN MEAL

	KSU[a] %	Quaker[b] %
Moisture	12.1	10.7
Protein	8.0	8.3
Fat	-	1.6
Carbohydrate	-	78.2
Fiber	-	0.6
Ash	-	0.6
Calories	-	3.6 Cal/g

[a]KSU - analyses made by Dr. David Wetzel in Dept. of Grain Science and Industry.

[b]Quaker - values supplied by Dr. R.O. Neisheim of the Quaker Oats Co., Barrington, Ill.

APPENDIX 1b: MINERAL AND VITAMIN COMPOSITION OF UNENRICHED DEGERMINATED CORN MEAL

	KSU[a] mg/lb	Quaker[b] mg/lb
Thiamine	0.55	0.63
Riboflavin	0.36	0.23
Niacin	6.89	4.49
Vitamin B_6	1.32	1.59
Biotin	-	0.018
Folic acid	-	0.241
Vitamin E	-	9.1 I.U.
Calcium	81.4	22.7
Iron	10.0	5.0
Magnesium	167.7	186
Zinc	3.40	4.5
Copper	-	0.36
Sodium	-	9.1
Potassium	-	859

[a]KSU - analyses made by Dr. David Wetzel in Dept. of Grain Science and Industry.

[b]Quaker - values supplied by Dr. R.O. Neisheim of the Quaker Oats Co., Barrington, Ill.

APPENDIX 2: COMPOSITION OF 1 OZ. OF "N-RICHMENT-A-TYPE 41"[a]

	mg/oz. mix[b]
Niacin	8400
Thiamine mononitrate	1088=1120 mg Thiamine hydrochloride
Riboflavin	700
Electrolytic iron	4800

[a]Recommended to use 0.25 oz./100 lb. cereal product.

[b]0.25 oz./100 lb. product gives 2.8 mg. thiamine, 1.75 mg. riboflavin, 21.0 mg. niacin, and 12.0 mg. iron per pound product.

APPENDIX 3: COMPOSITION OF VITAMIN PREMIX # 2077

	gm
Thiamine mononitrate	2.57
Riboflavin	1.80
Niacinamide	21.0
Reduced iron	11.46
Pyridoxine HCl	2.0
Folic acid	0.26
Vitamin A Palmitate	20.0
Tricalcium phosphate	3.0
Cornstarch q.s.	100

APPENDIX 4: TOTAL WEIGHT GAIN, FEED INTAKE, FEED EFFICIENCY, HEMOGLOBIN, AND HEMATOCRIT OF RATS AFTER 28 DAYS ON EXPERIMENTAL DIETS[a]

Diet A Rat No.	Weight Gain (gm)	Feed Intake (gm)	Feed Efficiency	Hemoglobin (gm/100 m)	Hematocrit (%)
1	58	191	0.30	13.1	41.5
2	60	268	0.22	11.0	39.5
3	74	250	0.30	12.6	41.5
4	62	225	0.28	12.1	40.5
5	64	210	0.30	13.5	44.5
6	53	202	0.26	12.7	40.0
7	66	200	0.33	12.7	44.0
8	58	199	0.29	12.0	40.0
Diet B Rat No.					
1	78	272	0.29	16.0	53.0
2[b]	86	266	0.32	-	-
3	64	243	0.26	17.3	58.0
4	77	254	0.30	15.8	51.5
5	77	239	0.32	15.4	48.0
6	61	221	0.28	16.3	57.5
7	66	234	0.28	14.5	51.0
8	54	213	0.25	16.2	52.0

APPENDIX 4: (CONTINUED)

Diet C Rat No.	Weight Gain (gm)	Feed Intake (gm)	Feed Efficiency	Hemoblobin (gm/100 m)	Hematocrit (%)
1	120	328	0.37	15.1	53.0
2	103	310	0.33	15.3	52.0
3	137	365	0.38	14.8	50.5
4	58	253	0.23	16.2	56.5
5	86	268	0.32	14.4	52.0
6	117	311	0.38	16.0	53.5
7	107	319	0.34	15.2	51.5
8	104	298	0.35	14.9	52.0
Diet D					
Rat No.					
1	136	364	0.37	15.5	52.0
2	109	278	0.39	16.5	54.0
3	122	337	0.36	15.4	47.0
4	121	332	0.36	14.7	43.5
5	118	296	0.40	14.7	47.0
6	114	303	0.38	14.5	47.0
7	115	317	0.36	14.7	47.0
8	145	355	0.41	14.4	49.0

[a]A - unenriched corn meal.
B - cornmeal enriched by current standard.
C - corn meal fortified according to proposed fortification standard, but with lower iron.
D - corn meal fortified according to proposed fortification standard with the high iron level.

[b]Died during blood sampling.

EFFECT OF THE PROPOSED NAS
FORTIFICATION POLICY FOR CEREAL
GRAIN PRODUCTS ON THE NUTRITIONAL
VALUE OF CORN MEAL MUSH

by

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ABSTRACT

The nutritional effects of the current and proposed policies for cereal fortification were compared using Sprague-Dawley weanling male rats. A control diet A was prepared using unenriched corn meal. Diet B contained corn meal enriched by current standard. Diet C was prepared from corn meal fortified according to the proposed policy, except that it was lower in iron. The iron level was similar to that of corn meal sample B. Diet D contained corn meal fortified to meet all recommendations for the proposed standard, including iron.

Rats were fed for 28 days, after which weight gain, feed intake, hemoglobin concentration and hematocrit were measured. Feed efficiency was also calculated.

Rats fed control diet A ate significantly less feed but were not significantly different in weight gain and feed efficiency in comparison to rats fed diet B. Weight gain and feed efficiency were greater for diet C than for diet B. Diet D produced the highest weight gain and feed efficiency. Feed intake of rats fed diet C was higher than that of rats fed diet B, but there was no significant difference between feed intakes of rats fed diets C and D.

Hemoglobin concentration was higher for rats fed diet B than for those fed diets A or D. Hemoglobin concentrations of rats fed diets B or C were not significantly different. Also there was no significant difference in the hemoglobin

concentration of rats fed diets C or D. Rats fed diet A had the lowest hemoglobin values.

Hematocrit values for rats fed diet A were significantly lower than those of rats fed diets B, C, or D. Hematocrit values for rats fed diet D were significantly lower than for rats fed either diets B or C. There was no significant difference between hematocrit values of rats fed diets B and C.

Enrichment of corn meal to the proposed fortification standard resulted in improvement in growth, feed intake and feed efficiency over the corn meal enriched according to the current standard. The higher iron fortification according to the proposed standard of fortification did not result in increased iron utilization as measured by hemoglobin generation and hematocrit value. It is, therefore, questionable whether the higher level of iron should be endorsed.